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Original article

Evaluation of the Possible Effect of Methotrexate, Fluoxetine and Moringa Oleifera in Rat Model of Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic, relapsing inflammatory and autoimmune multisystem disease that affects the joints. Methotrexate (MTX) is considered by many rheumatologists to be the most important and useful disease-modifying antirheumatic drugs (DMARDs). Fluoxetine is a selective serotonin reuptake inhibitor that has been widely used for the treatment of depression due to its safe profile. Moringa Oleifera (MO) is known for its nutritional and medicinal uses. Various parts of these plant act as cardiac and circulatory stimulants. **Aim of work:** The present study was designed to evaluate the possible effect of methotrexate, Fluoxetine and Moringa Oleifera on rat model of RA. **Materials and methods:** Rats were classified into: Group I: control normal group. Group II: was not treated (diseased group). Group III: was treated with methotrexate (MTX) (0. 6 mg/kg/week/by oral gavage). Group IV: was treated with fluoxetine (20 mg/kg/day by oral gavage) Group V: was treated with Moringa Oleifera extract (200 mg/kg/day by oral gavage). Treated groups received drugs for 4 weeks. **Results**: Treated groups showed significant improvement in all parameters (rheumatoid factor (RF), tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), reduced glutathione (GSH), anti-cyclic citrullinated peptide (anti-CCP), arthritis score).and improvement of the histopathology of the joint. A significant reduction in the score was seen in the treated group at the end of 3rd and 4th weeks. With best results in Moringa Oleifera group in comparison with other treated groups.**Conclusion:** Methotrexate, fluoxetine and Moringa Oleifera extract groups showed significant improvement of parameters of RA.

Key words: Rheumatoid arthritis, methotrexate, fluoxetine, Moringa Oleifera, adjuvant-induced arthritis.

Introduction:

Rheumatologic diseases are the most prevalent diseases worldwide; they are one of the main causes of disability and morbidity all over the world with greatly bad impact on the quality of life. (1).

They are characterized by the presence of long-standing inflammation of the joints resulting in symmetric polyarthritis and synovial membrane hypertrophy with progressive joint damage, bone and cartilage destruction and also deformity. The disease is systemic, leading to extra-articular manifestations (EAM) (2).

The pathogenesis of the rheumatoid joint involves Synovial hyperplasia results from a marked increase in macrophage and fibroblast-like synoviocytes. Locally expressed degradative enzymes, including metalloproteinase and proteases, digest the extracellular matrix and destroy the articular structures. (3).

Cytokines, particularly IL-1 and IL-17, tumor necrosis factor-α (TNF-α) and reactive oxygen intermediates affect chondrocytes that undergo apoptosis. This results in cartilage degradation and joint-space narrowing on radiography (4).

Systemic involvement may be explained by the systemic activities of cytokines released from the inflamed synovium. (5).

The main classes of drugs are currently used: analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, as well as biologic and non-biologic disease-modifying antirheumatic drugs (DMARDs). (6).

Methotrexate (MTX) is the recommended first-line DMARD used in the treatment of RA. This is due to its low cost and many patients achieve disease control with monotherapy. However, about 30% of patients develop inadequate treatment response and many patients stop MTX due to toxicity.(7).

Part of MTX anti-inflammatory action is due to its folate antagonist Methotrexate up taken into the cells by folate transporter 1 (FOLT, also known as RFC1). Within the cell, MTX is polyglutamated in a reversible reaction by folylpolyglutamate synthase. Polyglutamation of methotrexate increasing inhibition of dihydrofolate reductase (DHFR), thymidylate synthetase and 5-aminoimidazole- 4-carboxamide ribonucleotide transformylase.(8).

The most common adverse effects include: gastrointestinal side effects (nausea, vomiting, abdominal pain) followed by stomatitis (oral ulcers), liver function abnormalities, bone marrow suppression, and alopecia. (9).

Neuropsychiatric manifestations are quite common in RA, including depression, cognitive dysfunction,

behavior changes, spinal cord compression and peripheral nerve involvement.(10).

The current trend of medical treatment of rheumatoid arthritis seeks for new drugs with more efficacies and less side effects since methotrexate, a standard diseased modified anti-rheumatoid drug, causes many adverse effects and toxicities. (11).

Fluoxetine is a selective serotonin reuptake inhibitor that has been widely used to enhance the neurotransmission of serotonin in the central nervous system and has emerged as the drug of choice for the treatment of depression due to is safer profile and fewer side effects. (12).

Also there are important functions of fluoxetine related to the central nervous system e.g. neuroprotection; anti-inflammatory properties, antioxidant properties and anti-apoptotic properties, with greater neuron survival and a reduction in apoptosis mediators and also oxidative substances, such as superoxide dismutase and hydrogen peroxide. (13).

Moringa Oleifera (MO) is known for its nutritional and numerous medicinal uses. Various parts of these plant act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiulcer, antispasmodic, diuretic, antihypertensive, analgesic, antioxidant, antimicrobial, and anti-inflammatory effect.(14).

Aim of work:

These observations provided a rationale for testing the effect of monotherapy with fluoxetine and MO extract in comparison with MTX monotherapy on adjuvant induced arthritis (AIA).

Materials & methods:

A. Animals:

The study is carried out on 30 Adult male albino rats obtained from Experimental Animal Breeding Farm, Helwan-Cairo) weighing between 150- 200 g (at the beginning of the study), were used for in-vivo experiments. They were acclimatized for one week and were caged (6 rat/ cage) in fully ventilated room at room temperature in the pharmacology department, Benha Faculty of Medicine. Rats were fed a standard chow with water. This study was approved from ethical committee of Benha Faculty of Medicine.

B. Drugs and chemicals:

Complete Freund's adjuvant (CFA) (Sigma-Aldrich Chemical Company), Methotrexate (Minapharm., Egypt), fluoxitine (Amoun pharmaceutical Co, Egypt)., Moringa olifera (powder) (National Research Centre, Giza, Egypt), Formalin, solution, neutral 10% formaline (El Gomhoria Pharmaceutical Chemical Co., ARE),Urethane , Ethyl carbamat, white crystals (Sigma Chemical Co., USA), Hematoxylin and eosin (E.Merk,

Darmastadt.,)[Germany], Rheumatoid factor kits (Abnova corporation, Taipei city ,Taiwan).,CRP kits (Thermoscientific, USA), Tumor necrosis factor alpha (TNF-α) kits (USA & Canada | R&D Systems, Inc), Reduced Glutathione kits (Biodiagnostic Co., Giza, Egypt), anticyclic citrullinated peptide (anti-CCP)kits (Alpha diagnostic international,Texas,USA)

C. Induction of RA:

Complete Freund's Adjuvant Arthritis was induced by S.C injection of 0.4 ml of compelete Freund's adjuvant in the right hind limb for 12 day in three doses (one dose every four days).(11).

D. Experimental design:

Group (1): Non-arthritic untreated normal control group :

This group received a standard chow and tap water with no medication.

Group (2): Untreated rats with complete freund's adjuvant arthritis group:

This group was injected with complete freund's adjuvant to induce rheumatoid arthritis.

Group (3): Rheumatoid arthritis (RA) methotrexate treated group:

This group received a standard chow and tap water with methotrexate at a dose (0. 6 mg/kg/week/by oral gavage) (15) for 4 weeks after induction of arthritis with CFA.

Group (4): Rheumatoid arthritis (RA) rats, fluoxetine treated group:

This group treated with fluoxetine (20 mg/kg/day in saline by oral gavage) (16) for four weeks after after induction of arthritis with CFA.

Group (5): Rheumatoid arthritis (RA) rats, Moringa oleifera treated group:

This group treated with (200 mg/kg/day by oral gavage of methanolic extract of M. oleifera) (17) for four weeks after induction of arthritis with CFA**.**

The treated groups received drugs for 4 weeks. Dose selection was based on previously published studies and pilot experiments.

At the end of study period, blood samples were collected from the retro-orbital venous plexus of rats using microcapillary tubes. (18).

The blood samples (each=2ml) were allowed to clot at room temperature, centrifuged at 3000 rotation/minute and the sera were separated. Samples were stored at -20 Ć in dark containers for measurement of rheumatoid factor (RF), C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α) , reduced glutathione (GSH)and anti-cyclic citrullinated peptide (anti-CCP).

Rats were euthanized at the end of the study and hind paws were removed. They were embedded in paraffin after fixing in formalin solution (10% neutral buffered).

Sections were cut in various slices having thickness of 6 um and examined under microscope after staining with hematoxylin-eosin for perivascular inflammatory cell infiltrate in synovium, morphological changes including synovial cell hyperplasia and proliferation, villous hyperplasia, inflammatory cells infilterations and dilated blood vessels.(19).

E. Assessment of arthritis:

a- Rats were scored for arthritis (arthritis index) by a set visual criterion at the end of each week of experiment according to the following criteria: No change = 0, Erythema = 1, Mild swelling = 2, Gross swelling = 3, Gross swelling and deformity = 4. (20).

b- Microscopic examination of sections of paw joint stained by H&E stain.

F. Biochemical assays:

a) RF was determined by ELISA technique (21).
b) Serum TNF-α was measured by ELISA (22).
c) Serum CRP was determined using an enzyme-linked immunosorbent assay (23).
d) Serum GSH was determined by using a colorimetric method (24).
e) Serum anti-cyclic citrullinated peptide (anti-CCP) was determined using an enzyme-linked immunosorbent assay.(25).

Statistical analysis:

The clinical data were recorded on a report form. These data were tabulated and analysed using the computer program SPSS (Statistical package for social science) version 26 to obtain descriptive statistics were calculated for the data in the form of mean and standard deviation for quantitative data.

In the statistical comparison between the different groups, the significance of difference was tested using ANOVA test Used to compare mean of more than two groups of quantitative data.

A P value <0.05 was considered statistically significant (*) while >0.05 statistically insignificant P value <0.01 was considered highly significant (**) in all analyses.

Results:

Injection of (CFA) resulted in significant increase in level of RF, CRP, TNF-α and anti-CCP antibodies compared to control normal group.

There is a significant progressive increase in arthritic score of diseased group every week without treatment compared to the score at 1st day before adjuvant injection. While there was significant decrease in GSH level compared to control normal group. Joints obtained from diseased group showed proliferated blood vessels,

inflammatory infiltrate and pannus formation.

Regarding, the treated groups there was significant improvement in serum RF, TNF-α, CRP, GSH and anti-CCP antibodies compared to diseased group. MTX showed better results than fluoxetine group, also MO showed the best results in all treated groups in the following parameters serum RF, CRP and anti-CCP antibodies. While fluoxetine showed more decrease in TNF-α and increase in GSH more than MTX group but the best improvement is also in MO group.

Table (1): The effect of treatment with MTX, fluoxetine and MO on (serum RF, TNF-α, CRP, GSH and anti-CCP antibodies) on experimentally induced rheumatoid arthritis, in rats:

	Control group		**Diseased group**		**Methotrexate group**		**Fluoxetine group**		**Moringa Oleifera group**	
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
RF (IU/ml)	1.46	0.16	9.62**a**	0.70	3.94 **ab**	0.38	5.14 **abc**	0.23	2.91 **abcd**	0.19
CRP (ng/ml)	1.64	0.11	8.63**a**	0.54	4.74 **ab**	0.76	5.9 **abc**	0.32	3.67 **abcd**	0.38
TNF-α (pg/ml)	16.67	0.50	87.53**a**	0.54	45.84 **ab**	0.78	38.64 **abc**	0.98	29.16 **abcd**	0.64
Reduced Glutathione (µg/ml)	21.47	0.87	6.97**a**	0.34	11.63 **ab**	0.66	14.8 **abc**	0.76	24.15 **abcd**	1.04
Anti-CCP (U/ml)	3.71	1.19	29.05**a**	0.35	14.3 **ab**	1.57	18.85 **abc**	0.54	10.12 **abcd**	1.04

a: significant versus G1 at p<0.05 **b: significant versus G2 at p<0.05**

c: significant versus G3 at p<0.05 **d: significant versus G4 at p<0.05**

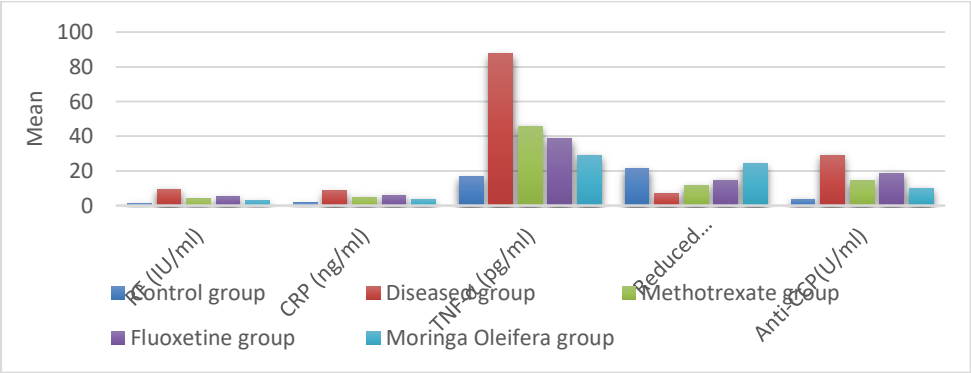

Figure (1): The effect of treatment with MTX fluoxetine and MO, on (serum RF, TNF-α, CRP, GSH and anti-CCP antibodies) on experimentally induced rheumatoid arthritis, in rat

Table (2): The effect of treatment with fluoxetine and methotrexate, on arthritic score at different times, on experimentally induced rheumatoid arthritis in rats:

	G1		G2		G3		G4		G5	
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
Arteritis score 0	0.0	0.0	3.0	0.0	3.0	0.0	3.0	0.0	3.0	0.0
Arteritis score 1st w	0.0	0.0	4.0 **a**	0.0	3.33 **ab**	0.52	3.5 **ab**	0.55	3.0 **ab**	0.0
Arteritis score 2nd w	0.0	0.0	4.0 **a**	0.0	3.0 **ab**	0.63	3.33 **ab**	0.52	2.5 **abd**	0.55
Arteritis score 3rd w	0.0	0.0	4.0 **a**	0.0	2.83 **ab**	0.75	3.0 **abc**	0.63	2.33 **abcd**	0.52
Arteritis score 4th w	0.0	0.0	4.0 **a**	0.0	2.10 **ab**	0.63	2.67 **abc**	0.52	1.5 **abcd**	0.55

a: significant versus G1at p<0.05 b: significant versus G2 at p<0.05 c: significant versus G3 at p<0.05 d: significant versus G4 at p<0.05

Histopathological changes:

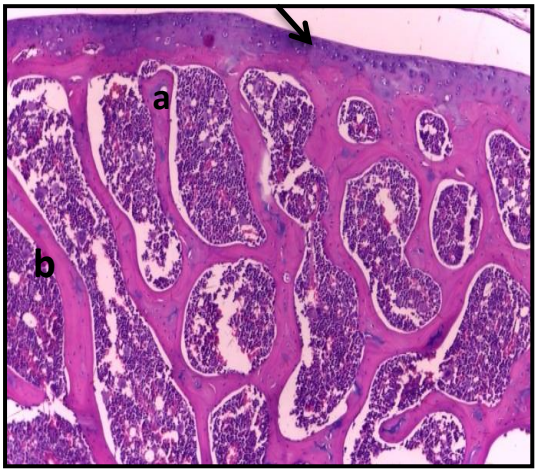

Figure (2): Photomicrograph of a cut section in normal rat joint shows (black arrow) flattened synovial membrane (a) cartilage associated with (b) bone marrow with no inflammatory cellular infiltrate (H&E x 100).

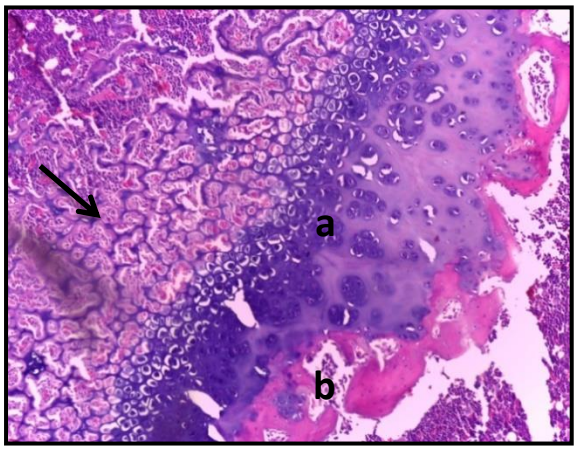

Figure (3): Photomicrograph of a cut section in rat joint of diseased group shows inflammatory infiltrate and proliferated blood vesseles (black arrow),synovial hyperplasia(a),pannus formation(b) (H&E x 100).

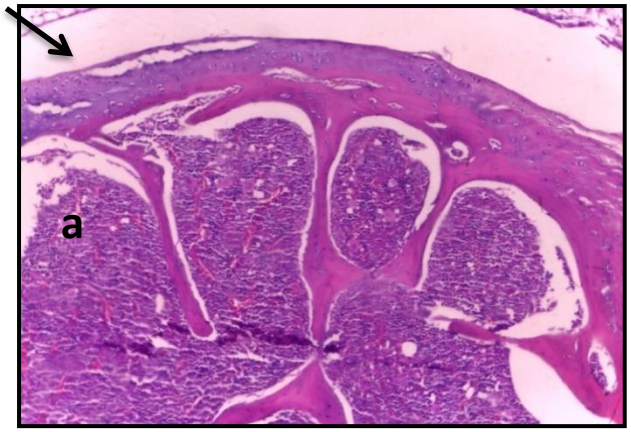

Figure (4): Photomicrograph of a cut section in rat joint of MTX treated group shows improvement of the inflammatory response (a) and flattening of synovial membrane(black arrow). (H&E x100).

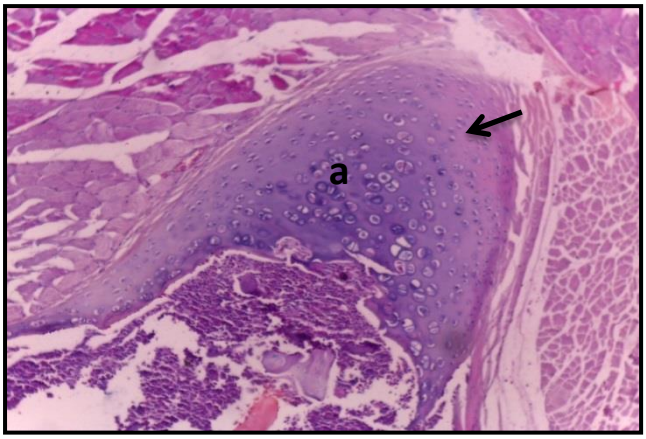

Figure (5): Photomicrograph of a cut section in rat joint of fluoxetine treated group showed (black arrow) multilayered synovial membrane, (a) mild improvement of inflammatory infiltrate (H&E x100).

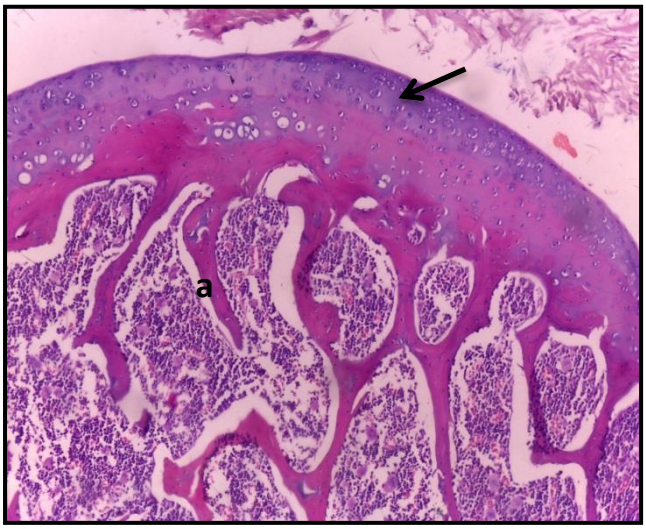

Figure (6): Photomicrograph of a cut section in rat joint of Moringa oleifera treated group shows (black arrow) marked flattening of superficial synovial layer (a) and marked improvement of inflammatory infiltrate (H&E x 100).

Regarding arthritis score, a significant reduction in the score was seen in treated groups at the end of 3rd and 4th weeks compared to diseased group, MO group was better than fluoxetine and MTX groups.

Histopathology revealed improvement of the joint as regard synovial hyperplasia, cartilage degeneration and inflammatory cell infiltration. This improvement was observed in all treated groups with best result in MO group

Discussion:

The present work was designed to evaluate the effect of Methotrexate, Fluoxetine and Moringa Oleifera extract for 4 weeks, on rheumatoid factor (RF, tumor necrosis factor- α (TNF-α), C-reactive protein (CRP), reduced glutathione (GSH), anti-cyclic citrullinated peptide (anti-CCP) and arthritis score and histopathological changes of joint, on RA induced experimentally in rats by S.C. injection of complete Freund's adjuvant in right hind limb.

Complete Freund's adjuvant (CFA)-induced arthritis is considered a scientifically standard experimental procedure for the induction of chronic immune-pathological RA in laboratory animals with similar cellular immunity response and pathological mechanism as in the human. (26).

The present study showed that S.C. injection of (CFA) resulted in increase in level of RF, CRP, TNF-α, arthritic score, decrease in level of GSH. This finding is similar to the observations of other study proved that injection of CFA led to significant elevation of serum RF, serum TNF-α and decrease blood GSH level compared to normal control group (27).

These results are in agreement with the study that reported that injection of complete Freund's adjuvant leads to edematous inflammation, increased vascularity owing to vasodilation, marked inflammatory cell infiltration compared to normal control group (28).

CFA showed that serum rheumatoid factor and serum CRP level were significantly increased in CFA rats compared with normal control group which is in consistence with our study (29).

The results in this study also are parallel with the study that tested the effect of galantamine on adjuvant-induced arthritis in rats. The adjuvant arthritis model showed the anti-CCP level increased in the untreated adjuvant arthritic rats relative to the healthy control group. (30).

The cytokines are involved in the pathogenesis of RA. In particular, tumor necrosis factor alpha (TNFα) has been

suggested as one of the most potent cytokines associated with RA. (31).

TNF-α induces activation of leukocyte and endothelial and synoviocyte activation and survival, cytokine and chemokine amplification, angiogenesis and nociceptor activation. The blockade of TNF significantly decreases the production of other pro-inflammatory cytokines and chemokines, as IL-1, IL-6, IL-8, or GMCSF (32).

Reduced glutathione (GSH) is the most abundant intracellular small-molecule thiol and is essential for maintaining the thiol status of various molecules. GSH has many biological roles, including protection against reactive oxygen and nitrogen species (ROS/NOS), which are reduced by two GSH molecules forming oxidized glutathione (GSSG) in the process (33).

Methotrexate significantly improved all the tested parameters of rheumatoid arthritis, arthritic score and histopathology of the joints. The result of this study is in agreement with the observations of other study that reported that MTX treatment after induction of RA by CFA, induced a highly significant decrease in serum RF, serum TNF-α, serum CRP and induced a highly significant increase in blood GSH level compared with diseased group.(34).

Also, in line with these results another study reported that the paw edema, RF and anti-CCP antibody were significantly reduced with MTX treated group (35).

In addition, our results showed that a significant reduction in paw diameters in MTX-treated was that observed in arthritic group and histopathology of joints of MTX-treated group showed reduction in vascular proliferation, destruction of cartilage, synovial membrane, and sub periosteal region (36).

For the arthritic score another study who aimed to compare the safety and effectiveness of MTX administrated his study is in agreement with our results as MTX administration decreased the arthritis score significantly (37)

The anti-inflammatory actions of MTX are also due to the participation of adenosine. Adenosine is an endogenous anti-inflammatory factor in arthritis. MTX acts as a 5-aminoimidazole-4-carboxamide ribonucleotide suppressor and increases adenosine levels. Adenosine suppresses neutrophil migration to areas of inflammation, promotes the differentiation of macrophages and also inhibits the production of interleukin-1 or leukotriene B4 (38).

Some researchers have postulated that MTX treatment decrease of the severity of arthritis by down-regulation of pro-inflammatory TNF-α, IL-6 and IL-17A cytokine expression (39).

Regarding treatment with fluoxetine, it showed significant improvement of RF, TNF-α, CRP, GSH and anti-CCP antibodies, compared to diseased. For arthritic score, this group showed improvement of the score at the end of 3rd and 4th weeks, compared to diseased group.

The effect of fluoxetine on serum level of TNF-α and arthritis score was studied and showed significant improve in arthritis score and significant decrease in TNF-α level compared to non-treated group as approved in our study.(40).

Fluoxetine inhibiting effect on cytokine secretion can be explained by the study that reported that fluoxetine counteracting depressive symptoms by inhibiting the reuptake of serotonin and thus, augments serotonin concentration. The relatively high extracellular serotonin levels can inhibit the secretion of cytokines (41).

Also a possible mechanism by which fluoxetine inhibit endosomal Toll like receptor (TLR) such as TLR 8 which plays an important role in the production of TNF-α.(40).

Fluoxetine effect on serum CRP was significantly decreased as shown by the study of the effect of fluoxetine on inflammatory markers; it showed significant reduction in serum CRP level, and this run in consistence with the current work (42).

IL-6 is also the main pro-inflammatory cytokine that induce synthesis of type 1 acute phase proteins such as CRP, elevated levels of stress leads to activation that triggers an NF-KB–dependent cascade of pro-inflammatory events that contribute to increases in CRP (43).

It also proved that fluoxetine decrease expression of nuclear factor NF-κB , this explain the reduction of the release of a number of pro-inflammatory and cytotoxic factors such as TNF-α, IL-1ß, nitric oxide, and reactive oxygen radicals. It also suggested that fluoxetine inhibits the mRNA for these cytokines (as well as for IL-6) (44).

Our study is in line with the study that showed fluoxetine has increased level of GSH significantly as approved in our study (45).

Effect of fluoxetine on GSH is explained by restoring the affected GSH pathways with Fluoxetine treatment may relate to neuroprotection, as the antioxidative effects of fluoxetine are thought to be mediated by increases in serotonin levels (46).

And it has been reported that fluoxetine suppressing T cell proliferation and inhibit interferon- (IFN) production in whole blood cultures. These also explain the decreased cytokines level in our present study (47).

In addition fluoxetine may also inhibit the response of antigen presenting cells these can explain the decreased levels of auto-antibodies as RF and Anti-CCP antibodies (48).

According to paw edema and arthritis score fluoxetine showed significant reduction in edema and improvement of arthritis score especially at 3rd and 4th weeks and this is in line with y the anti-inflammatory and immunomodulatory effects of fluoxetine in rat models.(49).

Histopathological examination to fluoxetine group showed decreased inflammatory infiltration, bone and cartilage destruction which is in line with study that approved the inhibitory effect of fluoxetine on inflammation and bone loss in rats. And this is due to the anti-inflammatory effect of fluoxetine (50 & 51).

On the other hand another study showed that10 mg/kg of fluoxetine the same dose in our study showed a small reduction in the clinical score and a slower decrease in paw swelling but at the higher dose (25 mg/kg), fluoxetine profoundly halted disease progression, with no further elevation in the clinical score or paw swelling (40).

Regarding treatment with Moringa oleifera extract, it showed highly significant improvement of RF, TNF-α, CRP, GSH and anti-CCP, compared to diseased, normal control group and other treated groups. For arthritic score, this group showed significant improvement of the score at the end of 3rd and 4th weeks, compared to diseased group and other treated groups.

The result of this study showed that MO group has highly significant decrease in TNF- α and this is supported by a study which studied anti-inflammatory effect of MO extract, it showed *MO* reduce TNF-α significantly and this may explained by β-sitosterol present in MO which is a compound with potent activity against inflammation, whose mechanism of action includes reducing the production of TNF-α (52).

MO also used in treatment of asthma and associated allergic diseases; these studies showed significant decrease in TNF-α, IL-6, these findings also indicate that the possible mechanism of action may be associated with a reduction in cytokine production/release (53).

Lipopolysaccharide (LPS) can bind to TLR and activate the NF-κB signaling pathway. The activation of these cascades and transcription factors subsequently results in the releasing of pro-inflammatory cytokines by macrophages and circulating monocytes, resulting in a transient immune activation, which is characterized by elevated levels of TNF-α, IL-1β, and IL-6, MO extract strongly

inhibit the LPS-induced expression of IL-6 and TNF-α during inflammation.(54).

In addition, our results are in consistence with the study that showed the effect of MO on vascular oxidative stress in hypertensive rats, it showed that significant decrease in CRP in MO treated group. (55).

IL-6 is an important mediator of the inflammatory response as it participates in the development and differentiation of B- and T-cells, as well as the activation of acute phase proteins as CRP, MO extract inhibit mRNA expression of IL-6and thus consciously leads to decreased CRP level.(56).

Also the study of *the antioxidant, anti-inflammatory and anti-arthritic effect of M. oleifera* showed in histopathology minimal inflammation, no pannus formation and erosion of epithelial cells. Also decrease in paw edema of Moringa treated rats as approved in our study. (57)

M.O extract shows significant protection against lymphocytic infiltration, bone destruction and cartilage erosion and this is in line with our study. Also significant reduction in RF and TNF-α, and this is supported by the study of the protective effect of ethanolic extract of seeds of MO in arthritic rats (58).

B cells isolated from RA synovium can secrete RF and anti-CCP antibodies, indicating that the autoantibody is produced locally in the joint (59).

Moringa seed extract has the ability to attenuate the chronic immune-mediated inflammatory responses typical of certain diseases such as asthma and RA and this explain the decrease in parameters of rheumatoid arthritis such as RF and anti-CCP antibodies (60).

MO extract showed significant increase in GSH and this is supported by the study of the protective effect of MO extract seeds against diabetic nephropathy in rats (61).

The significant increase in GSH is also supported by studying the effect of MO seed extract on induced testicular toxicity in rats (62).

These significant increase in GSH may explained by Glutathione Reductase (GR) is essential in maintaining adequate GSH level by facilitating the regeneration of GSH from oxidized glutathione (GSSG).The protective effect of MO was also reflected in the induction of GR activity by MO extract.(63).

Cysteine and methionine rich proteins that are present in high amounts in MO seeds .Beside this, MO, which is also rich with other potent antioxidant s like vitamin C, vitamin E and B-carotene.(64)

Conclusion

Methotrexate, Fluoxetine and Moringa Oleifera had improved adjuvant arthritis. Moringa Oleifera extract can be used as

new treatment in cases of rheumatoid arthritis thus can decrease MTX dose to avoid its side effects. Also, fluoxetine can be used in in treatment of rheumatoid arthritis to avoid possible side effect of high doses of methotrexate also it has beneficial effect in cases of depression associated with rheumatoid arthritis.

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