

## Original Article

# Effect of sitagliptin and methotrexate on rheumatoid arthritis induced experimentally in rats.

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## ABSTRACT

**Background:** Methotrexate (MXT) is the corner stone in treatment of rheumatoid arthritis. Although methotrexate is usually administrated in low weakly dose in patients with rheumatoid arthritis discontinuation rates are as high as 16% due to adverse effects. Hepatotoxicity is the most common side effect after long term course of methotrexate.

**Aim of the study:** This work aimed to investigate the potential effect of sitagliptin alone and in combination with different doses of methotrexate on rheumatoid arthritis (RA) induced experimentally in rats. Moreover, we investigated the benefits of sitagliptin co-administration with methotrexate not only on RA progression, but also on organs commonly injured on using methotrexate alone as a disease-modifying anti-rheumatic drug such as the liver.

**Methods:** 42 male adult Sprague Dawley rats divided into seven equal groups: Normal control group, None treated Freund's adjuvant induced rheumatoid arthritis, High dose MXT treated rheumatoid arthritis (1 mg /kg /3 days i.p.) over 4 weeks, Sitagliptin treated rheumatoid arthritis (10 mg /kg / day p.o) for 4 weeks, high dose MXT+ sitagliptin treated arthritis, 1/2 dose MXT+ sitagliptin treated arthritis, 1/4 dose MXT + sitagliptin treated arthritis. Serological parameters namely, cyclic citrullinated peptide antibodies (Anti-CCP), matrix metalloproteinase -3(MMP-3), cartilage oligometric matrix protein (COMP) and TNF- $\alpha$  level were measured. Liver tissue and hind paw joint were subjected to histopathological examination.

**Results:** Best degree of improvement was obtained with high dose MXT+ sitagliptin. It is significantly better than that of either drug alone. Half dose of MXT /sitagliptin combination was proved to be equally effective to the full dose of methotrexate alone regarding all tested parameters.

**Conclusion:** concomitant administration of sitagliptin augmented immunosuppressive effect and ameliorated hepatotoxicity of methotrexate in Freund's adjuvant model of rheumatoid arthritis.

**Key Words:** Methotrexate; rheumatoid arthritis; sitagliptin; rats.

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## 1. INTRODUCTION

Methotrexate (MXT) is the corner stone in treatment of rheumatoid arthritis. It is a potent inhibitor of cell division and protein synthesis through inhibition of de novo synthesis of thymine required for DNA synthesis (Goodsell 1999). Such effect is mediated by inhibition of folate activating enzyme namely dihydrofolatereductase (DHFR) (Hu et al., 2016).

For the treatment of rheumatoid arthritis, inhibition of DHFR is not thought to be the main mechanism, but rather multiple mechanisms appear to be involved, including the inhibition of enzymes involved in purine metabolism, leading to accumulation of adenosine; inhibition of T cell activation and suppression of intercellular adhesion molecule expression by T cells; selective down-regulation of B cells; increasing CD95

sensitivity of activated T cells; and inhibition of methyl transferase activity, leading to deactivation of enzyme activity relevant to immune system function (Wessels et al., 2008). Another mechanism of MTX is the inhibition of the binding of interleukin 1-beta to its cell surface receptor (Brody et al., 1993). Although methotrexate is usually administrated in low weakly dose in patients with rheumatoid arthritis discontinuation rates are as high as 16% due to adverse effects (Cetinkaya et al., 2006). Although methotrexate for autoimmune diseases is taken in lower doses than it is for cancer, side effects such as hair loss, nausea, headaches, and skin pigmentation are still common (Colebatch 2011). Moreover, hepatotoxicity is the most common side effect after long term course of methotrexate (Goodsell 1999). Therefore, new therapeutic protocols are highly

needed in order to reduce dose and hence side effects of methotrexate.

This work aimed to investigate the potential effect of sitagliptin alone and in combination with different doses of methotrexate on rheumatoid arthritis (RA) induced experimentally in rats. Moreover, we investigated the benefits of sitagliptin co-administration with methotrexate not only on RA progression, but also on organs commonly injured on using methotrexate alone as a disease-modifying anti-rheumatic drug such as the liver.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals

Complete Freund's adjuvant (CFA) was obtained from Sigma-Aldrich Chemical Company (St. Louis, Mo., USA). Januvia tablets (highly water soluble sitagliptin phosphate monohydrate) was dissolved in normal saline, MTX was supplied by the United Company for Distribution (Cairo, Egypt) and was dissolved in saline obtained from EL-Gomhouria Co. Enzyme-linked immunosorbent assay (ELISA) kits of serum anti CCP, MMP-3, COMP and TNF- $\alpha$  were purchased from Clini Lab Company (Cairo, Egypt).

### 3. Experimental design

The use of sitagliptin in treatment of rheumatoid arthritis stems from the fact that the drug has anti-inflammatory effect immune-modulator role (Raptopoulou et al., 2007) probably through modulation of CD26 metabolism (Makdissi et al., 2012). The dose of sitagliptin (10 mg /kg / day p.o) is similar to anti hyperglycemic one used by (Mohamed et al., 2013) in diabetic rats. It is 10% lower than human dose (Pajet and Barns 1964).

Three doses of methotrexate were used the largest one (1 mg /kg /3 days i.p.) is similar to experimental immunosuppressive dose in rats (Ahmed et al., 2015). It was equivalent to lowest dose of corrected human immunosuppressive one. The other doses were 1/2 and 1/4 of human dose. The latter 2 doses were given in combination with sitagliptin to highlight the possible augmenting effect.

The tested parameters regarding efficacy of tested drugs in treatment of rheumatoid arthritis include macroscopic measurement of thickness of hind paw by paw edema meter. Microscopic examination of longitudinal sections of hind paw joints for signs of inflammation as well as serological tests including serum levels of cyclic citrullinated peptide antibodies (anti-CCP), matrix metalloproteinase -3 (MMP-3), cartilage oligomeric matrix protein (COMP) and serum tumor necrosis factor alpha TNF- $\alpha$ . Moreover, the hepatotoxic effect

of tested drugs was assessed by histopathological study of liver sections.

### 3.1. Animal grouping

To achieve the above mentioned goals, 42 male adult Sprague Dawley rats weighing 100-110 g were obtained from Experimental Animal Breeding Farm, Helwan-Cairo). They were caged 6 per cage in well ventilated place at room temperature in animal house at department of pharmacology Benha faculty of medicine. They allowed free water and standard food (pellets specific for rat feeding obtained from animal breeding farm) for 7 days for acclimatization. During the whole period of experiment, animals were treated humanically according to the protocol of handling of experimental animals of Benha faculty of medicine. Then, animals were divided into 7 weight matched groups of 6 animals each.

**Group I:** normal control group. They received saline orally by stomach gavage.

**Group II:** none treated Freund's adjuvant induced rheumatoid arthritis: 0.1 ml of sterile paraffin oil diluted complete Freund's adjuvant was injected intradermally in the rat tail (Dardick et al., 1986).

**Group III:** high dose MXT treated Freund's adjuvant induced rheumatoid arthritis: experimental rheumatoid arthritis was induced as in group II. All animals were received methotrexate (1 mg /kg /3 days i.p.) over 4 weeks starting from day seventeen from Freund's adjuvant injection.

**Group IV:** sitagliptin treated Freund's adjuvant induced rheumatoid arthritis: experimental rheumatoid arthritis was induced as in group II. All animals were received sitagliptin (10 mg /kg / day p.o) for 4 weeks starting from day seventeen from Freund's adjuvant injection.

**Group V:** high dose MXT+ sitagliptin treated Freund's adjuvant induced arthritis: experimental rheumatoid arthritis was induced as in group II. All animals were received methotrexate and sitagliptin in the same manner as group III and IV.

**Group VI:** low dose MXT + sitagliptin treated Freund's adjuvant induced arthritis: all animals of this group received drugs in the same manner as group V except methotrexate was administrated in 1/2 dose.

**Group VII:** very low dose MXT + sitagliptin treated Freund's adjuvant induced arthritis: all animals of this group received drugs in the same manner as group V except MXT was administrated in 1/4 dose.

The signs of arthritis were detected by inspection of hind paw of all animals for macroscopic signs of inflammatory arthritis namely redness, ankle swelling, paw deformity and ankylosis every 3 days of

Freund's adjuvant injection. The severity of arthritis was assessed by special score (Alonzi et al., 1998).

After 28 days of drug administration (24 hours of last dose of tested drugs) blood samples were obtained from retro orbital plexus of all animals. All blood samples were incubated at 37°C for one hour then centrifuged at 3000 round/minute for ten minutes. Clear upper plasma layer was collected, kept at -80°C till used within 24 hours for determination of serological parameters namely, cyclic citrullinated peptide antibodies (anti-CCP), matrix metalloproteinase -3 (MMP-3), cartilage oligomeric matrix proteins (COMP) and serum TNF-a.

All animals were then sacrificed by decapitation and dissected for liver and hind paw joint. Two g liver samples were obtained from each animal and preserved in 10% formaldehyde till used for histopathological study. Hind paw samples were preserved in 10% formaldehyde for 2 days and decalcified by formic acid. All samples were then embedded in paraffin. Three Longitudinal sections of 4 mm thickness were taken from different parts of joint specimens and liver. All samples were stained by H & E (Cetinkaya et al., 2006).

### 3.2 Methods:

#### 3.2.1 Induction of experimental rheumatoid arthritis by complete Freund's adjuvant:

Complete Freund's adjuvant is solution of antigenemulsified in mineral oil used as an immunopotentiator (booster). It is composed of inactivated and dried mycobacteria (usually *M. tuberculosis*), whereas the incomplete form (IFA or FIA) lacks the mycobacterial components (hence just the water in oil emulsion). It is named after Jules T. Freund. The basis of using Freund's adjuvant in experimental induction of rheumatoid arthritis is related to its effectiveness in stimulating cell-mediated immunity and leads to potentiation T helper cells that lead to the production of certain immunoglobulins and effector T cells. This agent was obtained as powder and dissolved in sterile paraffin oil to make a final concentration of 1mg/ml.

#### 3.2.2 Assessment of arthritis:

Severity of arthritis was assessed by:

- a- Inspection of animals every day for signs of onset of arthritis (redness, swelling in hind paw and wrist joints).
- b- Scoring the severity of arthritis: each paw was scored according to the following criteria: 0, normal; 1, mild redness and swelling of ankle or wrist joints; 2, moderate redness and swelling of ankle or wrist joints; 3, severe redness and swelling of the entire paw including digits; and 4,

paws with deformity or ankylosis (Alonzi et al., 1998). The maximum score for a single paw was 4 and for a single rat was 16; arthritis scores for all four paws of each rat were summed as arthritis index. In a given group, the mean arthritis score for each group was calculated as the mean of total arthritis scores of all rats within the group. Arthritis index was conducted under blinded conditions.

- c- Measurement of paw thickness at the end of experiment after scarification of all animals and separation of hind paw using paw edema meter which depends on the volume of fluid raised in one limb of u tube after immersing the hind paw in another limb of the u tube.
- d- Microscopic examination of longitudinal sections of paw joint stained by H&E stain.

#### 3.2.3 Seriological tests:

- a- Serum cyclic citrullinated peptide antibodies were measured by ELISA as described by (Kim et al., 2006).
- b- Serum TNF-a was measured by ELISA using method of (Brouckaert et al., 1993).
- c- Serum metalloproteinase was measured by ELISA using method of (Haro et al., 2000).
- d- Serum cartilage matrix protein was measured by ELISA using method of (Paulsson and Heinegard 1981).

### 4. Statistical analysis:

Data were expressed as mean values  $\pm$  SD and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. With post HOC test to compare measures of two independent groups of quantitative data. The level  $P \leq 0.05$  was considered the cut-off value for significance. Mathematical calculation was performed using SPSS computer program version 18 windows 7.

### 5. RESULTS

Administration of complete Freund's adjuvant induced arthritis in rats. The onset of arthritis was after 16 days in most of tested animals. None treated group show progression of arthritis till the end of experiment. A full blown picture of rheumatoid like experimental arthritis was manifested by significant elevation of hind paw thickness, and arthritic score serum cyclic citrullinated peptide antibodies (anti CCP), metalloproteinase enzyme (MMP-3), serum cartilage oligomeric matrix protein (COMP) and TNF-a compared with normal control group.

Administration of either of tested drugs singly or in combination resulted in partial improvement of

the above mentioned parameters. The results of drug administration were statistically significant compared with both non treated Freund's adjuvant induced arthritis and normal control.

Best degree of improvement was obtained with (1 mg /kg methotrexate and 10 mg sitagliptin combination which is the largest dose of methotrexate used in this experiment. It is significantly better than that of either drug alone.

Half dose of methotrexate /sitagliptin combination was proved to be equally effective as the

full dose of methotrexate alone regards all tested parameters.

Comparing either tested drug alone revealed that methotrexate alone produced statistically significant better results than sitagliptin alone regards to reduction of paw thickness, serum anti-cyclic citrullinated peptide antibodies (anti-CCP), matrix metalloproteinase-3 (MMP-3), cartilage oligomeric matrix protein (COMP) and TNF- $\alpha$  level

**Table 1: Effect of administration of different doses of methotrexate and sitagliptin either singly or in combination on average ( Mean  $\pm$  SD) paw thickness (mm) , arthritic index , serum anti-cyclic citrullinated peptide antibodies (anti-CCP  $\mu$ g/ml ) , matrix metalloproteinase -3(MMP-3 pg/ml ) , cartilage oligomeric matrix protein (COMP ng /ml )and TNF- $\alpha$ (pg/ml ) in male adult albino rats (n=6).**

Variables	Hind paws thickness(mm)	Arthritic index	Serum anti-CCP level( $\mu$ /ml)	Serum MMP-3 level(pg/ml)	Serum COMP level(ng/ml)	Serum TNF- $\alpha$ level(pg/ml)
Normal control	1.96 $\pm$ 0.055	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	35.4 $\pm$ 1.01	8.8 $\pm$ 0.58	11.5 $\pm$ 0.92
Non treated arthritis	5.29 $\pm$ 0.33 <sup>a</sup>	10.4 $\pm$ 0.22 <sup>a</sup>	46.6 $\pm$ 0.37 <sup>a</sup>	121.3 $\pm$ 1.2 <sup>a</sup>	39.4 $\pm$ 1.4 <sup>a</sup>	71.1 $\pm$ 1.8 <sup>a</sup>
Full dose MXT treated arthritis	3.11 $\pm$ 0.16 <sup>a,b</sup>	3.87 $\pm$ 0.16 <sup>a,b</sup>	20.2 $\pm$ 0.33 <sup>a,b</sup>	61.3 $\pm$ 0.95 <sup>a,b</sup>	17.1 $\pm$ 0.91 <sup>a,b</sup>	21.4 $\pm$ 0.71 <sup>a,b</sup>
Sitagliptin treated arthritis	4.22 $\pm$ 0.52 <sup>a,b,c</sup>	2.39 $\pm$ 0.013 <sup>a,b,c</sup>	27.3 $\pm$ 0.56 <sup>a,b,c</sup>	72 $\pm$ 1.7 <sup>a,b,c</sup>	19.2 $\pm$ 0.54 <sup>a,b,c</sup>	26.7 $\pm$ 0.98 <sup>a,b,c</sup>
Full dose MXT+sitag treated arthritis	2.57 $\pm$ 0.15 <sup>a,b,c,d</sup>	1.15 $\pm$ 0.17 <sup>a,b,c,d</sup>	15.9 $\pm$ 0.37 <sup>a,b,c,d</sup>	46.6 $\pm$ 1.3 <sup>a,b,c,d</sup>	13.2 $\pm$ 0.89 <sup>a,b,c,d</sup>	16.9 $\pm$ 1.2 <sup>a,b,c,d</sup>
1/2 dose MXT+sitaglip treated arthritis	3.08 $\pm$ 0.095 <sup>a,b,d,e</sup>	3.059 $\pm$ 0.43 <sup>a,b,d,e</sup>	20.9 $\pm$ 1.22 <sup>a,b,d,e</sup>	63.7 $\pm$ 1.2 <sup>a,b,d,e</sup>	16.1 $\pm$ 0.96 <sup>a,b,d,e</sup>	21.3 $\pm$ 1.1 <sup>a,b,d,e</sup>
1/4dose MXT+sitaglip treated arthritis	3.71 $\pm$ 0.15 <sup>a,b,c,d,e,f</sup>	7.14 $\pm$ 0.23 <sup>a,b,c,d,e,f</sup>	31.4 $\pm$ 0.26 <sup>a,b,c,d,e,f</sup>	81.1 $\pm$ 1 <sup>a,b,c,d,e,f</sup>	22.2 $\pm$ 1.2 <sup>a,b,c,d,e,f</sup>	35.2 $\pm$ 1.6 <sup>a,b,c,d,e,f</sup>

a: Significant compared with normal control group at P < 0.05

b: Significant compared with non- treated freund's adjuvant induced arthritis group at P < 0.05

c: Significant compared with full dose MXT treated freund's adjuvant induced arthritis group at P < 0.05

d: Significant compared with sitagliptin alone treated freund's adjuvant induced arthritis group at P < 0.05

e: Significant compared with full dose MXT + sitagliptin treated freund's adjuvant induced arthritis group at P < 0.05.

f: Significant compared with 1/2 dose MXT+ sitagliptin treated freund's adjuvant induced arthritis group at P < 0.05.



Figure1: Representative examples of rat hind paws.

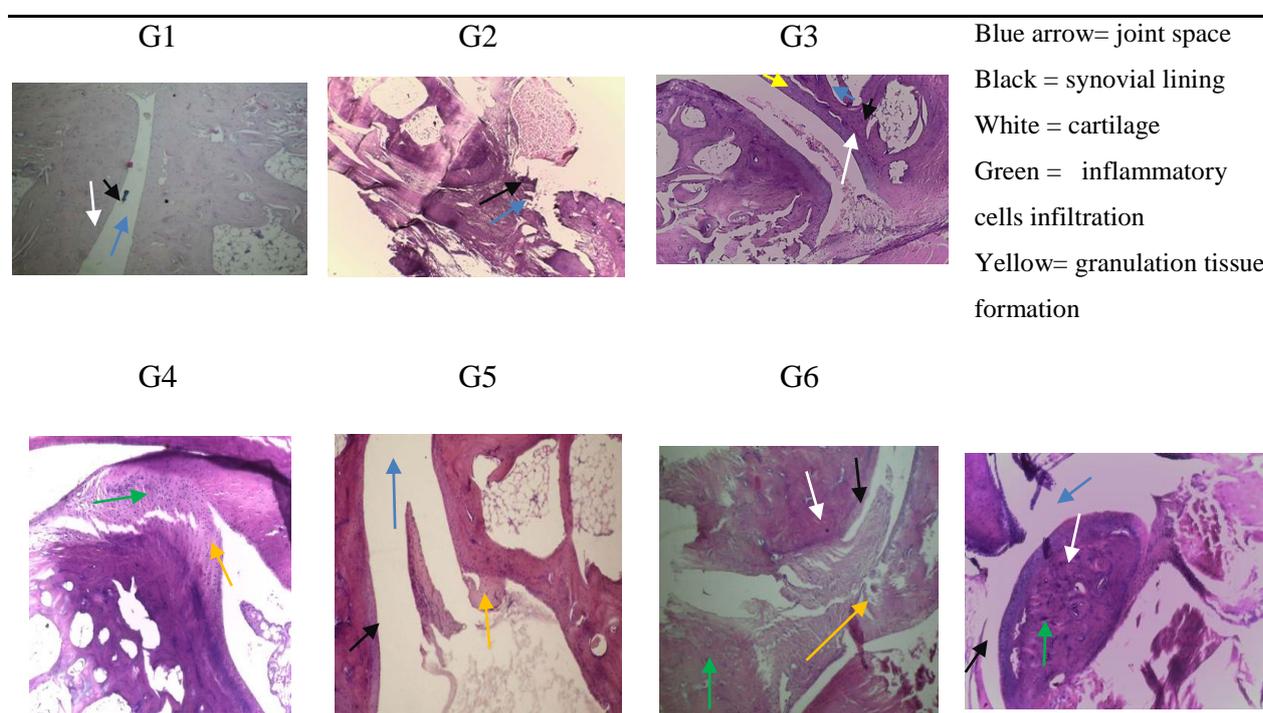
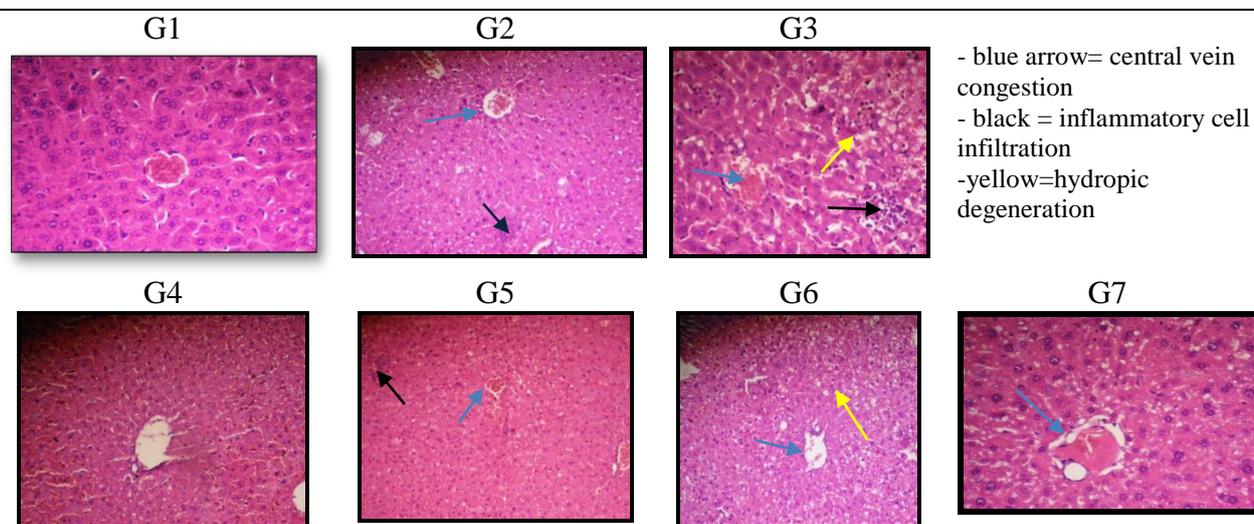


Figure 2. Photomicrographs of articular cartilages of different sections (H & E x 20). Normal control rat section (G1). Arthritis control rat section (G2) shows disrupted articular surface (white arrow) with severe cartilage degeneration and synovial hyperplasia (black arrow). MXT-treated arthritic rat section (G3) shows improvement as regard synovial hyperplasia, cartilage degeneration, inflammatory cell infiltration and granulation tissue formation. Sitagliptin treated arthritic section (G4) shows improvement but less than G3. High dose MXT +sitagliptin treated arthritic section (G5) shows a high degree of improvement, the joint seemed to be normal with minimal inflammation. (G6) & (G7) show variable degrees of improvement.



**Figure3: Photomicrographs of rat liver tissue of different sections (H&E X 20). Rat liver sections in control (G1) and sitagliptin (G4) groups show normal liver architecture. Rat liver sections in arthritic group (G2) shows mild inflammatory cell infiltration. In MTX group (G3) liver sections showed severe loss of liver architecture as disturbance of the hepatocytes radially arranged cords, marked degeneration with inflammatory cell infiltration and congestion in central veins. Rat liver sections in co-treated MTX with sitagliptin groups (G5, 6, 7) show a good degree of improvement in the form of few vacuolated hepatocytes, mild inflammation and mild congestion in central veins.**

## 6. DISCUSSION

In this work, an experimental model of rheumatoid arthritis was adopted. This model proved to be very similar to natural disease as evidenced not only by observed signs of joint deformity and functional disability but also through elevation of specific biomarkers of rheumatoid arthritis which distinguish the disease from other causes of arthritis (Nishimura et al., 2007). The most specific of them is anti-cyclic citrullinated peptide antibody. Nishimura et al., (2007) demonstrated that autoantibodies from RA patients react with a series of different citrullinated antigens.

It is postulated that unknown triggering agents probably viral, mycoplasma or mycobacteria infections or smoking produce initial inflammation in genetically susceptible patient resulting in enzymatic conversion of arginine amino acid residues into citrulline residues in connective tissue proteins such as vimentin, by a process called citrullination. If their shapes are significantly altered, the proteins may be seen as antigens by the immune system, thereby generating an immune response (Raptopoulou et al., 2007). This triggers macrophage to produce inflammatory mediators such as TNF- $\alpha$ , IL-1, IL-15 and IL-6, IL-17 which induce destructive effects in affected joints leading to release of multiple joint components in plasma such as metalloproteinase and cartilage matrix proteins (Chiu and Ritchlin 2017).

Moreover, liver histopathology showed mild inflammatory infiltration which may represent

extension of autoimmune inflammatory process to extra-articular tissues. This is supported by (Selmi et al., 2011) who showed frequent deterioration of liver functions in patients with rheumatoid arthritis.

Methotrexate significantly improved all tested parameters of rheumatoid arthritis. This may be attributed to inhibition of immune cell proliferation as a result of antimetabolite cytotoxic effect as well as previously explained specific immunosuppressive action such as specific down regulation of B lymphocytes, adenosine accumulation, decrease expression of adhesive molecules, increasing CD 95 sensitivity of activated T cells. On the contrary, liver histopathology showed marked pathological changes namely loss of hepatic architecture, marked degeneration, inflammatory cell infiltration and congestion of central vein. This work confirms the clinical study of (Sotoudehmanesh et al., 2010) that showed elevated liver enzymes in rheumatic patients treated by methotrexate. This effect may be attributed to tetrahydrofolate reductase inhibitory effect of methotrexate which inhibits release of methionine from s-homocysteine. Methionine deficiency may induce oxidative stress via decrease natural antioxidant such as glutathione. In addition, homocysteine accumulate in hepatic stellate cells produces pro-inflammatory cytokine activation manifested by inflammatory cell infiltration and liver fibrosis (Pandit et al., 2012). It also induced lipid peroxidation through sensitization of liver cell to oxidative stress (Cetinkaya et al., 2006). Congestion

of central vein observed in this study may be due to mechanical pressure of inflammatory infiltrate.

Sitagliptin significantly improved all markers of rheumatoid arthritis. Nevertheless, it was less effective than methotrexate and did not cause complete remission as evidenced by significant elevation of tested markers compared with normal values. Contrary to methotrexate, no detectable hepatotoxic effect of sitagliptin. The anti-inflammatory effect of sitagliptin is in line with (Pathak et al., 2010) who showed that dipeptidyl peptidase IV inhibitors have therapeutic potential for treatment of inflammatory diseases. Nader et al., (2012) Reported that treatment with sitagliptin significantly decreases serum TNF- $\alpha$ . Moreover, Wronkowitz, et al., (2014) showed that DDP IV enzyme induces inflammation through NF $\kappa$ B activation and increase expression of cytokine and nitric oxide synthase. The above mentioned changes were inhibited by sitagliptin administration as suggested by (Hu et al., 2016).

The mechanism of anti-inflammatory effect of sitagliptin may be explained by the fact that dipeptidyl peptidase IV enzyme forms a complex with adenosine deaminase complexing protein forming CD26 which cleaves X-proline dipeptides such as chemokines, neuropeptides, and peptide hormones; this may exert potent pro-inflammatory effect through proteolytic activation of cytokines and increasing lymphocyte proliferation (Matteucci and Giampietro 2009). This complex is expressed on cell surface of many cell types including immune cells. On human T cells, CD26 can deliver a potent co-stimulatory T-cell activation signal (Morimoto and Schlossman 1998). Sitagliptin exerts anti-inflammatory and immunosuppressant effect through inhibiting dipeptidylpeptidase IV moiety of CD26 complex. Moreover, anti-inflammatory effect of dipeptidyl peptidase IV inhibitors may be more powerful in proline rich proteins such as cartilage and joint proteins (Matteucci and Giampietro 2009).

The above mentioned immunosuppressant effect of dipeptidyl peptidase IV inhibitors may explain the fact that sitagliptin administration is associated with increased incidence of many types of infections including respiratory and urinary tract (Pathak et al., 2010).

On the contrary, several clinical studies showed increased incidence of arthralgia after long term intake of dipeptidyl peptidase IV inhibitors in diabetic. This controversial results may be explained by the fact that diabetes mellitus has been associated with autoimmune disorders (such as RA) (Lu et al., 2014). This correlation seems to be related to the high levels of inflammatory mediators such as C-reactive protein

(CRP), interleukin-6 and tumor necrosis factor- $\alpha$ , suggesting a role of diabetes in the immunopathogenesis of RA (Goldberg 2009). Moreover, Aroor et al., (2013) proved that immune system is mal adapted in type II diabetes. Abo-Haded et al., (2017) emphasized the role of genetics in pathogenesis of dipeptidyl peptidase IV inhibitor induced joint disorders in diabetic patients.

Concomitant administration of sitagliptin and methotrexate in full dose was proved to be superior over the use of either drugs separately. Moreover; it ameliorated methotrexate induced hepatotoxic pathological changes. This may be attributed to the antioxidant effect of sitagliptin which is in conformation with (Abo-Haded et al., 2017) who showed that of sitagliptin has a considerable hepatoprotective effect with marked alleviation of oxidative stress markers. Liliana et al., (2010) showed that chronic sitagliptin treatment corrected the glycemic dysmetabolism, hypertriglyceridemia, inflammation, and hypertension, reduced the severity of the histopathological lesions of pancreatic endocrine and exocrine tissues, together with a favorable redox status.

Taking above mentioned reports together, one may conclude that anti-inflammatory effect of sitagliptin is independent on its antihyperglycemic effect. It is manifested in diabetic and non-diabetic individual due to inhibition of dipeptidyl peptidase mediated cytokine activation. In conclusion, concomitant administration of sitagliptin augmented immunosuppressive effect and ameliorated hepatotoxicity of methotrexate in Freund's adjuvant model of rheumatoid arthritis. Combination of sitagliptin in anti-hyperglycemic dose with half immunosuppressant dose of methotrexate have modest equally effective anti-rheumatic action with minimal hepatotoxic effect. It is highly recommended to investigate the effect of such combination on other side effects of methotrexate such as bone marrow suppression and infertility.

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