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A POSSIBLE AMELIORATIVE ROLE OF CURCUMIN IN DECLINING THE SIDE EFFECT OF METHOTREXATE IN ADJUVANT INDUCED ARTHRITIS

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ABSTRACT

Our study aimed to identify the role of curcumin in overcoming the toxicity of methotrexate on liver in adjuvant-induced arthritis. Rats were divided into eight groups. (Control), without treatment. (MTX), was injected (IP) with methotrexate in a dose of 0.75 mg/kg b.w./week for 20 days. (CUR), was treated with a daily oral dose of curcumin (150 mg/kg b.w./day) for 20 days. (MTX + CUR), was treated with methotrexate and curcumin as the same previous corresponding dose and period. (AA), was injected with Complete Freund's adjuvant for arthritis induction. (AA/MTX), arthritis induction then treated with methotrexate as the same previous dose and period. (AA/CUR), arthritis induction then treated with curcumin as the same previous dose and period. (AA/MTX +CUR), arthritis induction then treated with both methotrexate and curcumin as the same previous dose and period. Results showed significant improvement in body weight, liver weights, WBCS count, RBCS count, and HB level in rats treated with either MTX alone or with CUR. The combination of MTX + CUR revealed notable activities in a decreased ANA level. Generally, MTX + CUR administration exerted restorative effects on the liver structure and functional damages**.**

KEYWORDS: Arthritis, Methotrexate, Curcumin.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that leads to articular cartilage and bone destruction. Approximately 3% of the worldwide population are affected

by this disease. It causes not only physical discomfort and pain, but also increased risk of work disability as arthritis usually starts in people aged between 30 and 40 years.[1] Although the cause of rheumatoid arthritis is unknown, autoimmunity plays an important role in both its chronicity and progression.[2] RA is characterized by a chronic inflammation of synovium, leading to progressive joint destruction. It causes pain, stiffness, swelling of the joints, restricts the range of motion, decreases strength, and affects the quality of life.[3,4]

The histopathologic appearance of synovium in RA is characterized by greatly hypertrophied (8-10 cells thick) of synovial lining or intimal layer. Primary cell populations in this layer are fibroblasts and macrophages. However, the subintimal area is heavily infiltrated with inflammatory cells, including T and B lymphocytes, macrophages, mast cells, and mononuclear cells that differentiate into multinucleated osteoclasts. The intense cellular infiltrate is accompanied by new blood vessel growth (angiogenesis). This hypertrophied synovium (also called pannus) invades and erodes contiguous cartilage and bone. Moreover, the synovial cavity became effusive (large collections of fluid filtrates of plasma) and exudative (high protein content). The synovial fluid is highly inflammatory. However, unlike the rheumatoid synovial tissue in which the infiltrating cells are lymphocytes and macrophages but not neutrophils, in synovial fluid the predominant cell is the neutrophil.[5]

RA patients are treated with three general classes of drugs such as disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory agents (NSAIDs), and corticosteroids to reduce joint inflammation and pain, maximize joint function, and prevent joint destruction and deformity.[6]

Methotrexate (MTX) is considered the first-line DMARD agent for most patients with RA. No other DMARD enjoys the popularity of methotrexate for the treatment of RA.[7] It has a relatively rapid onset of action at therapeutic doses (6-8 weeks), good efficacy, ease of administration, relatively low cost and effective in reducing the signs and symptoms of RA.[8]

MTX continues to play an important role in the chemotherapy of human malignancies, including childhood acute lymphocytic leukemia, lymphoma, osteosarcoma, head and neck cancer, lung cancer and breast cancer. It is also effective in many other forms of inflammatory arthritis including psoriatic arthritis, juvenile chronic arthritis, psoriasis and systemic lupus erythematosus.[8]

MTX is commonly known to inhibit the folate pathway by competitively inhibiting several important enzymes including: dihydrofolate reductase (DHFR), thymidylate synthase (TS), glycinamide ribonucleotide transformylase (GART), and aminoimidazole carboxamide ribonucleotide transformylase (AICART). This inhibition leads to reduced or blocked TS and de novo pyrimidine and purine synthesis, which are needed for DNA synthesis.[9]

The usage of MTX is associated with severe adverse effects, including gastrointestinal bleeding, cardiovascular complications and hepatotoxicity where as methotrexate is associated with mild hepatitis, fatty changes, liver fibrosis and cirrhosis. Also it considered risk of cancer (lymphoma), and Interstitial pneumonitis.[8]

Owing to the side effects and the high cost of conventionally used anti-inflammatory drugs, patients with arthritis are increasingly using complementary and alternative medicine (CAM) modalities of treatment.[10]

Curcumin (CUR) (1, 7-bis (4-hydroxy-3- methoxyphenyl)-1, heptadiene-3,5-dione), also known as diferuloylmethane, is an active compound extracted from the rhizomes of turmeric (*Curcuma longa*). It is a curry spice with a brilliant yellow colour that is native to Southeast Asia, primarily in India. It has many curcuminoids including curcumin, demethoxycurcumin and bisdemethoxycurcumin (77%, 17% and 3%, respectively).[11]

CUR is an antioxidant, which means it can efficiently reduce the level of reactive oxygen species (ROS), weaken redox signaling and reduce inflammation.[12] Also, recent studies demonstrated that curcumin has several biological and pharmacological effects, including antiinflammation, anticarcinogenic, hepatoprotective, thrombosuppressive, antiarthritic and modulation of multiple signalling pathways. Furthermore, it is a natural remedy for the prevention and treatment of many disorders such as skin disease, chronic kidney disease, diabetes, allergy, asthma, cardiovascular diseases, neurodegenerative diseases, pancreatitis, inflammatory bowel disease and rheumatoid arthritis.[13]

Pourhabibi-Zarandi *et al.*, 2021[14] assess all studies regarding the efficacy of curcumin on rheumatoid arthritis and reported different mechanisms such as inhibition of mitogen-activated protein kinase family, extracellular signal-regulated protein kinase, activator protein-1 and nuclear factor kappa B. However, Flynn *et al.*, 2011 and Somasundaram *et al.*,

2014[15,16] used the therapeutic effect of curcumin supplementation in modulating the expression of NF-κB transcription factor involved in inflammation in the joints.

The goal of this study was to determine the ameliorative role of Curcumin on initiating side effects of Methotrexate, and explore the potential mechanisms of anti-inflammatory activity of Curcumin on macrophage expression, activation and cytokines secretion.

2. MATERIALS AND METHODS

2.1.Chemicals

Complete Freund's adjuvant (CFA), Methotrexate, and Curcumin (>95%) were purchased from Sigma Chemical Company (St. Louis, U.S.A).

2.2. Experimental animals

Eighty young adult male albino rats were used in the present investigation. They were obtained from the animal house of the National Research Center, Cairo, Egypt. Their weights ranged between 150-180 gm. All animal procedures were performed in accordance with the guidelines for the care and use of experimental animals established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH) protocol. All animals were in perfectly good condition and health in order to avoid any other intruding factor upon the experiment. They received food and water ad libitum with fresh supplies presented daily. All animals were allowed to acclimatize for a period of one week before the commencement of the experiment. All animal investigates received approval from the animal care committee, National Research Center (registration number: 13/165).

2.3. Induction of Rhumatoid arthritis

The arthritis model was induced by injected with 0.1 ml of Complete Freund's adjuvant into the right hand paw. To increase the sevirety of arthirits, a booster injection was administrated in the same manner on day 5 according to by.[17]

2.4. Experimental design

After acclimatization period, animals were randomly distributed into eight groups of 10 rats each as following:-

1. Group one served as a control group (C), and left without treatment.

2. Group two, Methotrexate group (MTX), was injected intraperitoneally (IP) with Methotrexate in a dose of 0.75 mg/kg b.w./week for 20 days [18].
3. Group three, Curcumin group (CUR), was treated with a daily oral dose of Curcumin (150 mg/kg b.w./day) for 20 days (16).
4. Group four, both Methotrexate and Curcumin group (MTX + CUR), was treated with Methotrexate accompanied with Curcumin as the same previous corresponding doses, periods and route of administration.
5. Group five, Adjuvant arthritis (AA) group, was injected with Complete Freund's adjuvant for arthritis induction and kept without treatment.
6. Group six, (AA/MTX), was injected with adjuvant for arthritis induction then treated with Methotrexate as the same previous doses, periods and route of administration.
7. Group seven, (AA/CUR), was injected with adjuvant for arthritis induction then treated with Curcumin as the same previous doses, periods and route of administration.
8. Group eight, (AA/MTX +CUR), was injected with adjuvant for arthritis induction then treated with both Methotrexate and Curcumin as the same previous doses, periods and route of administration.

After the end of the experimental period, all experimental rats were sacrificed under ether anesthesia and the liver tissue were dissected and fixed in formalin for histopathological investigation. Furthermore, Blood samples were collected into ethylenediaminetetraacetic acid (EDTA) containing tubes to be used in the determination of complete blood count. Blood samples were collected in clean, dry tubes and allowed to clot, then centrifuged at 14000g for 10 min for serum extraction to be used in the estimation of antinuclear autoantibodies (ANA).

2.5. Morphological studies

2.5.1. Determination of total body weight, absolute and relative liver weight

2.5.1.1. Total body weight

Animals of control and treated groups were weighed prior to the time of treatment by using a sensitive electronic balance, weekly and again prior to sacrifice.

The differences between the initial and final weights were calculated to determine percentage of change in body weight within the whole period of the experiment, thus:

Percentage of change in body weight =

$$\frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

2.5.1.2.Liver and relative liver weight

At the time of sacrifice, rats from control and treated groups were weighed and sacrificed. The thoracic cavity was opened and the lungs were rapidly removed, blotted with a piece of filter paper and weighed. The lung weight was calculated relative to the total body weight, thus:

$$\text{Relative lung weights} = \frac{\text{Lung weight}}{\text{Total body weight}} \times 100$$

2.5.2. Determination of autoantibody

Antinuclear antibodies (ANA) level was performed by the enzyme linked immunoassay technique utilizing Rat Anti-nuclear antibody ELISA Kit for quantitative detection of Rat ANA in serum, catalogue number: MBS269217, MyBioSource Company.

2.6. Histological investigation

For histological examinations, joints from animals of the control and experimental groups were fixed in 10% neutral buffered formalin, then disqualified for 18 days in 10% formic acid. Dissected organ specimens of spleen and liver were placed separately in 10% buffered formalin after washing with 10% formol saline solution. Dehydration of fixed soft tissues was carried out using ascending grades of ethyl alcohol, and then cleared with xylene. Infiltration with paraffin wax at 60°C was followed by embedding in paraffin. Paraffin blocks were cut at 6 microns thickness using a Cambridge rocking microtome and affixed to slides.For general histological examination, sections were stained as a routine in: Harris's alum haematoxylin and eosin.[19]

3. Statistical analysis

Statistical analysis was performed by using of the statistical package for social science (SPSS, Chicago, IL) version 17 statistical software. All data were analyzed by one-way ANOVA analysis. Differences were considered significant at $p < 0.05$. Data were expressed as the mean ± SE.

4. RESULTS AND DISCUSSION

4.1. Determination of total body weight

It is obvious from figure (1) that The mean body weight control, MTX, CUR, and MTX+CUR groups showed continuous increase of body weights with the lapse of time reaching 188.0 gm, 156.66 gm, 169.33 gm, and 153.66 respectively at the end of the study. On the other hand, the average body weights of AA group recorded a very highly significant decrease ($p<0.001$) as compared to the control group.

At the same time, the average body weights of animals treated with methotrexate in AA/MTX group showed decreases in body weight reaching 140.66 gm in week 6 compared to 175.33 gm in week1. But, treatment of animals with Curcumin in the AA/CUR group showed increases in body weight reaching 159 gm at the end of experiment.

Moreover, the data showed a very highly significant increase in the body weight gain in treated group with both methotrexate and Curcumin (AA/MTX +CUR) compared to the beginning of the expeirment.

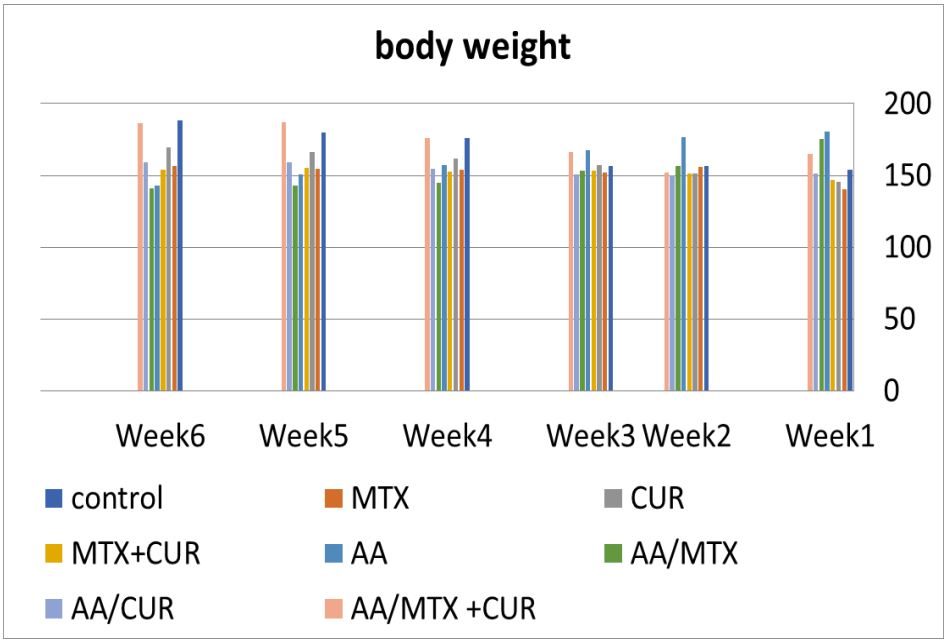

Figure 1: Averages of growth rates in Control and Experimental groups.

4.2. Determination of absolute liver weight and relative lung weight

4.2.1 Absolute liver weight

Averages of absolute liver weight control and experimental groups with their standard errors were recorded after 6 weeks from the beginning of the treatment. The data obtained are

shown in in (Fig. 2). It was observed that there is a significant decrease in absolute liver weights of AA group compared to control group.

It was definite that the most improved results are displayed in AA/MTX +CUR group which demonstrated results near to control group. (Fig.2).

4.3. Relative liver weights

Determination of liver weights relative to body weights is shown graphically presented in (Fig.2).

After 6 weeks from the beginning of the treatment, there was a significant increase in relative liver weights in AA group when compared to control group. On the contrary, after 6 weeks from the beginning of the treatment, results from AA/MTX, AA/CUR and AA/MTX+CUR were ameliorated by a decrease in relative liver weights.

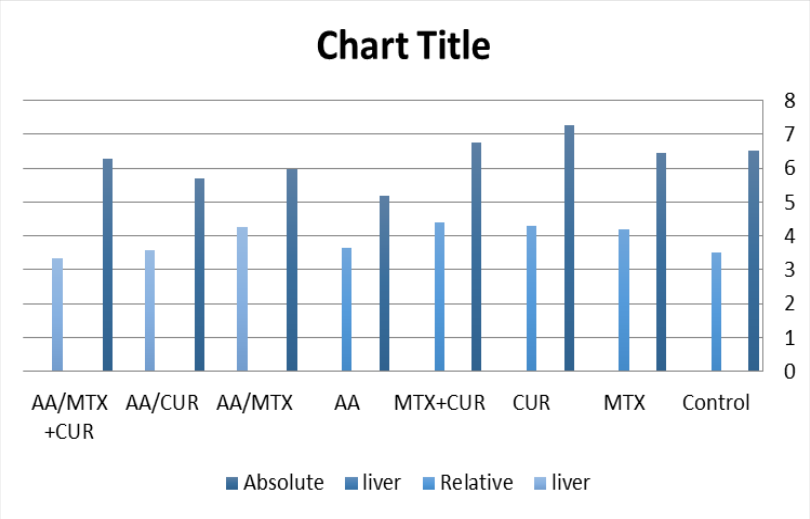

Figure 2: The mean values of the absolute and relative liver weight (g.) of Control and Experimental groups.

4.4. Hematological analysis

4.4.1. Total leucocytic count

A high WBC count of 24.75 was considered a positive sign of induction of arthritis in AA group in comparison to normal WBC count of 9.2 in control group *(P<0.001)*. Also, AA/MTX treated group showed a significant increase *(P<0.001)* in WBC count when compared to control group. On the other hand, WBC count significantly decreased *(P<0.001)*

in treated groups (AA/MTX, AA/CUR and AA/ MTX + CUR when compared to AA group.(Fig.3).

4.4.2. Total Red Blood Cell Count (RBC's)

From the results graphically presented in (Fig.3) the control group showed normal RBCs count throughout the whole experimental period. Otherwise, AA, AA/MTX and AA/ MTX + CUR were significantly decreased *(P<0.001)* in RBCs count comparing to Control group.

4.4.3. Evaluation of Hemoglobin Content (Hb)

The data obtained for Hb are graphically presented in (Fig. 3). The results showed that both AA and AA/MTX were significantly decreased (P<0.001) in heamoglobin content in compared to control group.

4.4.4. Evaluation of platlets counts

From the results presented in (Fig.3) The control group showed normal platelets count throughout the whole experimental period. Notably, both MTX and AA groups were decrease in platelets count relating to control group.

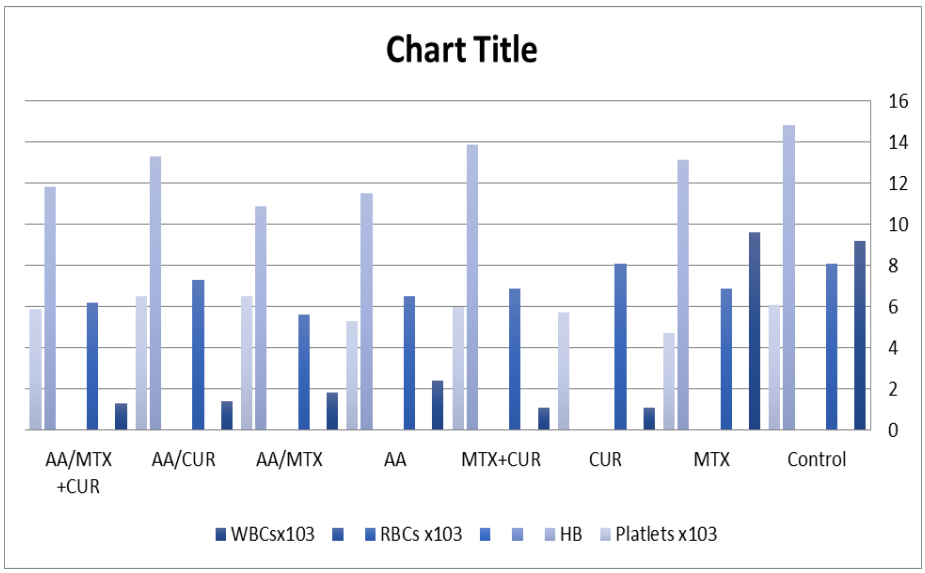

Figure 3: The mean values Total Leucocytic Count, Total Red Blood Cell Count, Hemoglobin Content (Hb), and Platlets counts of control and experimental groups.

4.4.5. Evaluation of Packed Cell Volume (PCV) and mean corpuscular volume (MCV)

Figure4 recorded that control groups showed similar values to normal PCV and MCV.

Reductions in PCV percentage were speculated in AA, AA/MTX, AA/CUR and AA/MTX+CUR groups recorded 34.20, 30.78, 35.58 and 30.92 percentage, respectively.

4.4.6. Evaluation of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)

Figure (4) recorded that control groups showed similar values to normal MCH pg and MCHC percentage.

Markedly, MTX group showed decreased in both MCH and MCHC in compared to control group. On the other hand, AA/MTX+CUR group revealed significantly decreased (P<0.001) in MCHC that recording 38.25 percentage in compared to 35.40 in AA group.

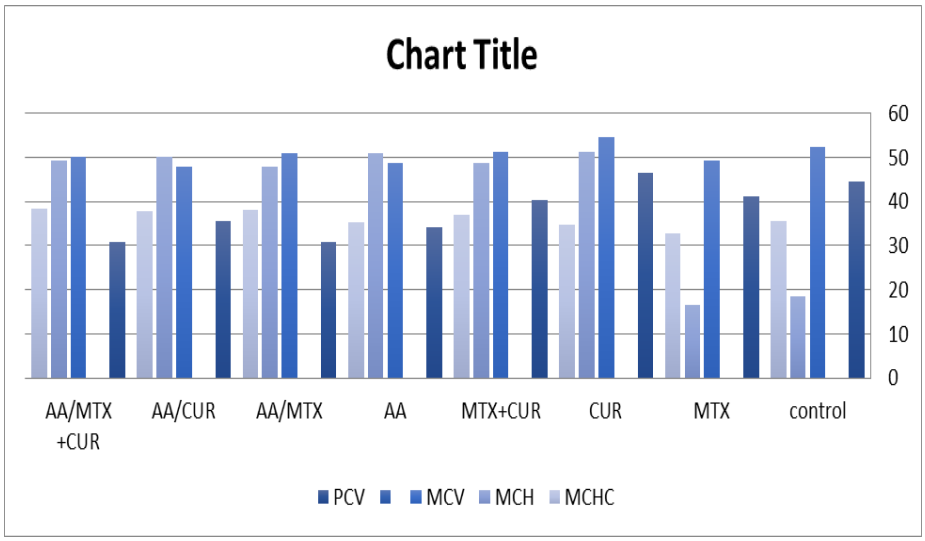

Figure 4: The mean values of Packed Cell Volume (PCV), mean corpuscular volume (MCV), corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) of control and experimental groups.

4.5. Antinuclear antibodies (ANA) level

The recoded values of serum ANA level in AA group showed a very highly significant increase *(p<0.05)* that recorded 4.16 ng/ml as compared with control group which recorded 1.11 ng/ml. On the other hand, the treatment with methotrexat (AA/MTX) or curcumin (AA/CUR) caused a significant decrease *(p<0.05)* in serum ANA level as compared to AA group. Remarkably, the treatment with both methotrexat and curcumin (AA/MTX+CUR group) significantly reduced *(p<0.05)* the ANA level as compared to AA group (fig. 5).

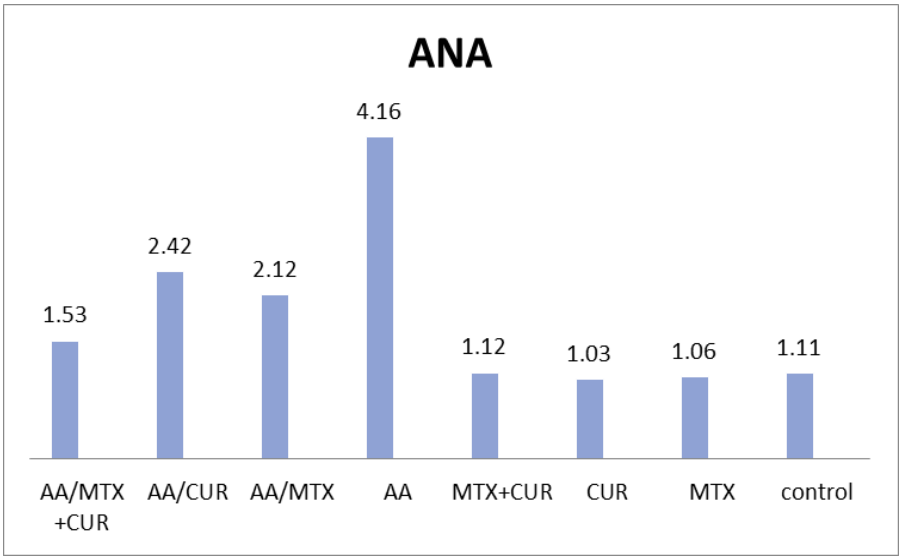

Figure 5: The mean values of ANA levels of Control and Experimental groups.

4.6. Histological study

Microscopically, livers from control group revealed normal histologic structure, and the hepatocytes arranged in normal lobular architecture with central veins surrounded by radiating hepatic cords. The portal triads showed normal histological structure containing branches of the hepatic artery, hepatic portal vein and bile duct fig. (6. a,c, and d). Liver sections of arthritis rats were illustrated in figs. (6.e). on the other hand liver tissue from MTX group showing few scattered fatty change in affected hepatocytes fig. (6. b).

Regarding arthritis group, several histopathological changes were detected in the affected hepatic parenchyma. Severe hepatic fibrosis was detected in several examined sections. Replacement of hepatic lobules with abundant fibroplasia was noticed as well. Intense number of mononuclear inflammatory cells infiltration were observed in the hepatic parenchyma accompanied by widespread hepatocellular necrosis. Regarding (ARM) group, multifocal areas of mononuclear inflammatory cells infiltration were commonly noticed in the hepatic parenchyma accompanied by less necrosis and karyorrhectic debris (Fig. 6.f).Group (ARC) showed apparently normal hepatic lobules in several examined sections. Few sections showed fewer number of inflammatory cells accumulation with focal area of oval cell hyperplasia (fig. 6.g.). Treated arthritis rats with both methotrexate accompanied with curcumin showed ameliorated structure of some cells of hepatocyte that contain nearly normal nuclei, while other cells were abnormal with either pyknotic or karyolitic nuclei. Slightly vaculated space was mostly still evident. The hepatic labels were made up of hepatic cords radiating from the central veins with normal hepatic sinusoids. (Fig. 6.h.).

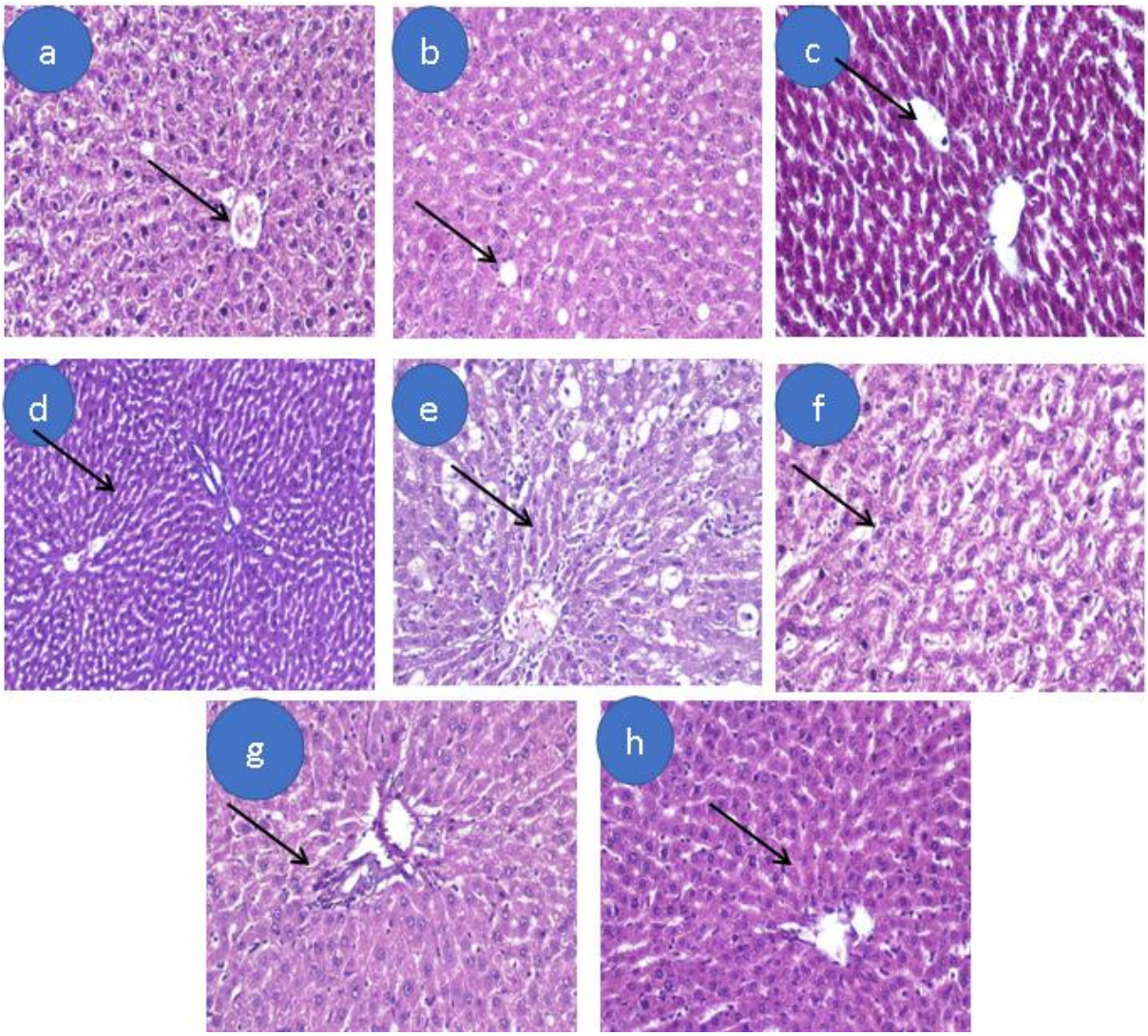

Fig. 6: Photomicrographs of histolgical staining of H&E in liver sections (x400): (a) photomicrograph of liver tissue from control group showing normal histological structure in which central vein surrounded by radiating cords of hepatocytes; (b) A photomicrograph liver tissue from MTX group showing few scattered fatty change in affected hepatocytes (arrows); (c) A photomicrograph of liver tissue of CCgroup, showing normal hepatic tissue; (d) Photomicrograph of liver tissue of CMCgroup, showing normal hepatic parenchyma; (e) A photomicrograph of liver tissue from AAgroup showing excessive vacuolated hepatocytes with variable number of inflammatory cells infiltration; (f) A photomicrograph of liver tissue from AA/MTX group showing sinusoidal dilation; (g) A photomicrograph of liver tissue from AA/CUR group showing apparently normal portal are; (h) A photomicrograph of liver tissue from AA/MTX+CUR group showing apparently normal hepatocytes surrounding central vein.

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that leads to articular cartilage and bone destruction.[20] The etiology of RA is unknown; however, epidemiological studies show that both genetic and environmental factors are responsible for the initiation of RA and the related pathological events. Continuous activity of immune cells in RA leads to persistent inflammatory synovitis in the peripheral joints; furthermore, hyperplasic synovial cells stimulate the release of more inflammatory cytokines such as interleukin (IL) -1, IL-6, IL-17 and tumour necrosis factor-alpha (TNF-α) with more articular damages.[21]

The present study revealed a significant decrease in body weight of the AA group as compared to the control group that may be due to paw inflammation causes in decrease food intake and anorexia.

A similar observation was reported by[22] who used 0.1 ml of CFA into the right hind paw intradermally to induce arthritis and observed a decrease in body weight in rats. They attributed the results to the inflammation resulting from arthritis might have affected the body weight in CFA induced animals than control animals. Also,[23] found a loss in rats' body weight. They attributed this reduction injurious effect of CFA which caused inflammation in paw produced necrotic lesions.

In addition,[24] and[25] attributed the decrease in body weight to inflammatory mediators which are released following CFA induction include cytokines, prostaglandins, lysosomal and hydrolytic enzymes.

On the other hand, the current work recorded a very highly significant increase in the body weight gain in the treatment of animals in both with Curcumin in the AA/CUR group and with methotrexate and Curcumin (AA/MTX +CUR) compared to the beginning of the experiment. A similar result was reported by[26] who observed that curcumin treatment to AA group improves the body weight that attributed to curcumin reducing the inflammatory markers, induction of the pro-apoptotic expression and inhibition of the anti-apoptotic expression and inhibition of osteoclasts RA cells.

Purpura *et al.*, 2018[27] found the same results that curcumin lead to increase in body weight duo to its ability to suppress inflammation. The present result was confirmed by[22] who observed improvement of body weight and attribute these results to strong anti-oxidative and anti-inflammatory activities of curcumin.

In the current work, there is no change in absolute and relative liver weights of AA group compared to control group. These suggestions may accordance with.[26-28] After 6 weeks, AA group has a significant increasing effect in absolute and relative spleen weights when compared to control group and this decline improved AA/MTX, AA/CUR and AA/MTX+CUR group.

Similar result was reported by Giles *et al*., 2015 and Hassan *et al.*, 2019[29-30] who observed increasing absolute and relative liver weights in arthritis group and attributed this result to oedema and inflammation of spleen after induced by CFA.

Also, Alghadir *et al.*, 2020[26] found decrease in absolute and relative spleen weights after treated with curcumin because that the Curcumin is an antioxidant, which means it can efficiently reduce the level of reactive oxygen species (ROS), weaken redox signaling, and reduce inflammation. In addition to having direct antioxidant properties, curcumin also blocks the activity of ROS-generating enzymes like lipoxygenase (LOX), cyclooxygenase (COX), xanthine dehydrogenase, and nitric oxide synthase (iNOS) Despite reducing ROS levels.

Moreover, Our results revealed that A high WBC count of 24.75 was considered a positive sign of induction of arthritis in AA group in comparison to normal WBC count of 9.2 in control group ($P<0.001$). Also, AA/MTX treated group showed a significant increase ($P<0.001$) in WBC count when compared to control group. Similary, Alghadir et al., 2020 found elevated level of WBCs in the induced arthritis that may due to the stimulation of the immune system against the invading antigens of arthritis. this might have led the gradual development of excessive leucocytes in arthritic rats. The restoration of leucocytes was maximum in iontophoretic.

Also, Asmawi *et al.,* 1993 attributed the change in the WBC count and its restoration can be explained in the way that induction of arthritis in the experimental rats was identified as a foreign body, leading to a cascade of reaction resulting in production of more WBCs to counteract the assault. This is might justify the gradual development of excessive leucocytes in the peripheral blood of the arthritic rats.

On the other hand, WBC count significantly decreased ($P<0.001$) in treated groups (AA/MTX, AA/CUR and AA/ MTX + CUR when compared to AA group. According to[30]

and[31] showed better efficacy of curcumin treatment using the iontophoretic drug delivery method which increased bioavailability and suppresses the migration of leukocytes into the inflamed area much better than the other conventional methods of herbal treatments. It was also found that oral curcumin along with plain local application was more effective in decreasing the WBC count as compared to oral curcumin alone. It could be due to the thin layer of the rat's skin that topical penetration was much greater causing curcumin to have reached the inflamed regions more effectively.

Our present finding was the agreement with[32] found that a decrease in WBC count in the group which treated with curcumin related to liposomal and micellar curcumin effectively.

Interestingly, In the current work, showed that both AA and AA/MTX were significantly decreased ($P<0.001$) in heamoglobin content and in RBCs count in compared to control group. Our present finding was the agreement with those of earlier studies found that significant reduction in the hemoglobin (Hb) and red blood corpuscles (RBC) levels across the study. The maximum reduction in RBC count was noticed on the 45th day when it decreased from a control value.

They attributed significant reduction in the RBC count and hemoglobin (Hb) concentration in the arthritis-induced rats d to two reasons: the first may be due to the premature destruction of red blood cells, and the second cause may be due to abnormal storage of iron in the reticuloendothelial system and synovial tissue, which might have caused low bone marrow iron availability due to decreased iron release by the mononuclear phagocyte system activation which had probably have resulted in ineffective erythropoiesis causing anemia.[33]

Our results observed that, The control group showed normal platelets count throughout the whole experimental period. Notably, both MTX and AA groups were decrease in platelets count relating to control group.While impovment in AA/CUR and AA/ MTX + CUR groups when compared to AA group.

According to (Wang *et al.*, 2020)[34] who attributed the restoration of platelet count to the curcumin could mask the hematological abnormalities induced by methotrexate in RA possibly by suppressing the release of interleukins and leukotriens. This implies that a reduction in the dose of methotrexate and incorporation of curcumin protects against the risk of hematological toxicity.

Corpuscular values such as MCV, MCH, and MCHC are good indicators of erythrocytes characteristics and can be obtained by computation. An elevation in MCV is a good predictor of hematologic toxicity. In the present study, reductions in PCV percentage were speculated in AA, AA/MTX, AA/CUR and AA/MTX+CUR groups recorded 34.20, 30.78, 35.58 and 30.92 percentage, respectively.

In this study, the MTX group showed decreased in both MCH and MCHC in comparison to control group. On the other hand, AA/MTX+CUR group revealed significantly decreased ($P<0.001$) in MCHC that recording 38.25 percentage in compared to 35.40 in AA group.

Contradictory to our result[35] reported that MCH and MCHC values were also improved with MTX and curcumin. Which can be related to Curcumin stabilizes the lysosomal membrane causing enhanced blood cell survival.

The current study recoded values of serum ANA level in the AA group showed a very highly significant increase as compared with the control group. This fact is supported by the results of several authors working on RA.[36]

A similar result was reported by Makar *et al.*, 2020[37] who recorded that increase in level of ANA. This finding is similar to the observations of Refaat *et al.*, 2012[38] who proved that injection of CFA led to significant elevation of serum ANA compared to control group. Also, these results are in agreement with the study of Patel and Pundarikakshudu who 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.

On the other hand, the treatment with methotrexat (AA/MTX) or curcumin (AA/CUR) caused a significant decrease serum ANA level as compared to AA group. Remarkably, the treatment with both methotrexat and curcumin (AA/MTX+CUR group) significantly reduced the ANA level as compared to AA group.

Also, this can be attributed to methotrexate and curcumin have established anti-inflammatory, antiproliferative, immunosuppressive effects on activated T lymphocytes, increasing the rate of apoptosis of T cells, increasing endogenous adenosine release, altering the expression of cellular adhesion molecules, influencing production of cytokines, humoral responses and bone formation, Similar result was reported by Shahin *et al.*, (2010).[39]

The present study, Regarding arthritis group, several histopathological changes were detected in the affected hepatic parenchyma. Severe hepatic fibrosis was detected in several examined sections. Replacement of hepatic lobules with abundant fibroplasia was noticed as well. Intense number of mononuclear inflammatory cells infiltration were observed in the hepatic parenchyma accompanied by widespread hepatocellular necrosis.

Similar observations were reported by Ibrahim *et al.*, 2012[40] who noted several histopathological changes in liver tissues in AA group.

Regarding (ARM) group, multifocal areas of mononuclear inflammatory cells infiltration were commonly noticed in the hepatic parenchyma accompanied by less necrosis and karyorrhectic debris.

The polyglutamated form of methotrexate will have a longer retention time inhepatic cells, thereby enhancing the chances of hepatotoxicity. Hence these areas are more susceptible to damage. Depletion of flats by MTX polyglutamate is a major contributing factor for hepatotoxicity.[40]

Our study, results showed that the arthritis rats with curcumin only or both methotrexate accompanied with curcumin showed ameliorated structure of some cells of hepatocyte that contain nearly normal nuclei while other cells were abnormal with either pyknotic or karyolitic nuclei. Slightly vaculated space was mostly still evident. The hepatic lobules were made up of hepatic cords radiating from central veins with normal hepatic sinusoids.

Furthermore, Zarandi *et al.*, (2020)[41] mentioned that rats were treated with curcumin or MTX alone or combination of both after RA induction. Then, histological examinatioin of liver sections were undertaken. Liver sections from normal control rats showed intact hepatic architecture, healthy hepatocytes, portal tracts containing bile ducts, portal veins and hepatic arteries attributed this result to the favourable effects of curcumin in reducing the inflammatory markers, induction of the pro-apoptotic expression and inhibition of the anti-apoptotic expression.[42]

5. CONCLUSION

Finally, it can be concluded that curcumin has a significant role in restoring the structural and functional efficacy of liver that may cause by methotrexate in adjuvant-induced arthritis rat model.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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