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The Effect of Ethyl Pyruvate on High Mobility Group Box I and Oxidative Stress in Induced Rheumatoid Arthritis in Rats

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Abstract

Background: Rheumatoid Arthritis (RA) is autoimmune disease characterized by chronic inflammation and by the destruction of synovial joints, leading to joint deformity and disability. The cause of RA is still unknown, but several factors have been documented. These include environmental factors, genetic factors, microbial pathogens, altered levels of inflammatory mediators, and defects in immune regulation, as autoimmune disease. The response of autoimmunity can be identified by the production of autoantibodies, such as Rheumatoid Factor (RF) or Anti Citrullinated Protein Antibodies (ACPAs), in serum. Recently, antioxidant supplementation has been the major focus of attention across the world among the health professionals to explore it as a strategy to protect against the injurious effects of oxidative stress.

Aim of Study: This study aimed to investigate the beneficial effects of Ethyl Pyruvate (EP) in a rat model of Complete Freund's Adjuvant (CFA) induced RA.

Material and Methods: The current study was carried out in Medical Biochemistry Department, Faculty of Medicine, Tanta University, in accordance to the guidance of Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt (Approval code 31802/10/17) during 2018.

The study was conducted on 60 male albino rats divided into four groups; Group I (control group), Group II (Rheumatoid arthritis group) and Group III (treated rheumatoid arthritis group) and Group IV (EP-control group) that are received the same dose of EP in treated rheumatoid arthritis group. All groups were subjected to estimation of level of anticyclic citrullinated peptide (AntiCCP) for diagnosis of RA, inflammatory marker as High Mobility Group Box1 (HMGB1) and Interleukin 1 Beta (IL1B) and also oxidative stress marker as Nitric Oxide (NO) and total antioxidant capacity respectively trying to detect effect of inflammation and oxidative stress in RA.

Results: This study showed that EP significantly decrease Anticcp, HMGB 1 and IL1B and NO levels, also significantly in TAC.

Conclusion: On basis of these results it could be concluded that EP exhibits anti-inflammatory and antioxidant effects in experimentally induced RA in rats.

Key Words: *Rheumatoid arthritis – Complete Freund's adjuvant – High mobility group box 1.*

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Introduction

RHEUMATOID Arthritis (RA) is a long-term inflammatory autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Most commonly, the wrist and hands are involved, with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body [1]. The pathogenesis of RA is a multistep process that starts with the development of autoimmunity, continues with local inflammation and finally induces bone destruction [2,3]. Rheumatoid arthritis is considered as an autoimmune disease since the production of the Rheumatoid Factor (RF) which is an autoantibody directed against determinants on the Fc fragment of immunoglobulin IgG but the most relevant autoantibodies appear to be Anti-citrullinated Protein Antibodies (ACPA). It are autoantibodies that are directed against proteins that are citrullinated [4]. High-Mobility Group Box chromosomal protein 1 (HMGB 1) was an important molecule in the pathogenesis of arthritis [5]. It is highly conserved non-histone chromosomal protein, Intra nuclear HMGB 1 binds DNA and regulates transcription. In addition, HMGB 1 may be extracellularly translocated, thereby acting as an inflammatory mediator of tissue invasion and tissue repair [6]. HMGB 1 may either be actively secreted from a wide number of cell types following stimulation with inflammatory mediators, including TNF, IL-1β, IFN-γ and multiple Toll-Like Receptor (TLR) ligands, or be passively released from dying nucleated cells [7]. The extracellular effects of HMGB1

are mediated via multiple receptors including the receptor for advanced glycated end-products (RAGE), some members of the TLR family and other are unidentified pathways [8]. Interleukin-1β are prototypic proinflammatory cytokines that exert pleiotrophic effects on a variety of cells and play key roles in acute and chronic inflammatory and autoimmune disorders. There are two IL-1 receptors: IL-1 type 1 receptor (IL-1RI) and IL-1 type 2 receptor (IL-1 RII). IL-1 α and IL-1 0 signal through IL-1RI. Binding to IL-1RII does not lead to cell signaling. Upon binding of IL-1 to IL-1RI, a second receptor termed IL1 receptor accessory protein (IL-1RAcP) gets recruited at the cell membrane to form a high affinity binding receptor complex leading to intracellular signaling [9]. On the other hand, previous reports suggested the role of oxidative stress in inflammation and destruction in the joints of arthritic animals and RA patients. Nitric Oxide (NO) is implicated in inflammation, angiogenesis and tissue destruction. The enzyme inducible Nitric Oxide Synthase (NOS) is responsible for the localized over-production of NO in the synovial joints affected by RA [10]. However, patients (RA) have increased oxidative stress, decreased antioxidant levels, and impaired antioxidant capacity. Ethyl Pyruvate (EP) is a stable and simple lipophilic ester derived from the endogenous metabolite pyruvate. It acts as a potent anti-inflammatory, anti-oxidant and free scavenger, which can rapidly and known to scavenge hydrogen peroxide [11]. The present study was conducted to investigate the modulatory effects of EP on some oxido -inflammatory (HMGB1and IL1B) as inflammatory parameter, (NO and TAC) as parameter of oxidative stress in a rat model of CFA induced rheumatoid arthritis.

Material and Methods

Chemicals: Complete Freund's Adjuvant (CFA) (SIGMA-ALDRICH Co., Egypt) CAS NO (8020-83-5). Ethyl pyruvate (>95 purity) (Alfa-Aesar Co., Egypt) CAS NO (617-35-6). Other chemicals and solvents used unless otherwise described were purchased from Sigma (Sigma, St Louis, USA). All chemicals and solvents were of high analytic grade.

Study design and animal grouping:

The current study was carried out in Medical Biochemistry Department, Faculty of Medicine, Tanta University, in accordance to the guidance of Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt (approval code 31802/10/17). This study comprised 60 albino male rats of approximately 180-200g body weight obtained from experimental animal colony of Tanta University. During 2018, rats housed in wire mesh cages, were fed standard rat chew and allowed free access to water. They were kept under constant environmental conditions (25ºC and lighting regimen of 12-h dark/12-h light cycle).

The rats were equally divided into the following four groups:

• *Group I:* Control group.

• *Group II:* Rheumatoid arthritis induced group.

Rheumatoid arthritis was induced by a single subcutanous injection (0.1ml) of Complete Freund's Adjuvant (CFA) (SIGMA-ALDRICH Co., Egypt). into the base of the tail [12,13].

• *Group III:* Treated Rheumatoid arthritis group.

After RA induction these rats were given daily intraperotenial injection of ethyl pyruvate 40mg/kg for 3 weeks [14].

• *Group IV:* (Ethyl pyruvate-control).

This group was given daily Ip injection of ethyl pyruvate 40mg/kg for 3 weeks.

Inflammation in each paw was graded for all rats on days 7, 14 and 21 after induction of RA according to the extent of erythema and edema of the periarticular tissues, using a scale of 0-4. The scores for each paw were then added to get the total arthritis score (maximum possible score 16 per animal), and designated as the arthritic index [15].

0 = No signs of arthritis

1 = Swelling and/or redness of the paw or one digit after 7 days.

2 = Two joints of the paw involved after 14 days.

3 = More than two joints involved after 18 days.

4 = Severe arthritis of the entire paw and digits after 21 days.

Blood and tissue sampling:

At the end point of the experiment (5 weeks), all rats were sacrificed by decapitation after anesthesia and blood samples were taken into dry sterile centrifuge tubes allowed to clot at room temperature for 30 minutes, and then centrifuged for 10 minutes at 3354 XG. Sera were separated and stored in aliquots at –70ºC till used. Samples were thawed at room temperature at the time of assay measurement.

All groups were subjected to the measurements of the following:

A- *Biochemical investigations:*

- Anti-Cyclic Citrullinated Protein antibodies (Anti CCP) by Elisa technique.
- High mobility group box I level by ElISA technique.
- Interleukin 1b level by ElISA technique.
- Colorimetric detection of Nitric oxide level.
- Colorimetric detection of total antioxidant capacity level.

B- *Histopathological examination of the knee joints of all groups:*

From each group, samples of knee joint were taken from 5 randomly selected rats, preserved in 10% formalin for 24 hours. Trimming was done on formalin fixed samples and washed in tape water for 12 hours. Serial alcohols (methyl, ethyl and absolute) were used for dehydration of tissue samples. Then tissue samples were cleared in xylene and embedded in paraffin. Paraffin blocks were sectioned at 3 [redacted] thickness by slide microtome. The obtained tissue sections were collected on glass slides and stained by haematoxylin and eosin (H & E) for histopathological examination by light microscope.

Statistical analysis was conducted as mean and standard deviation using Statistical Package for Social Sciences (SPSS), Version 16.0 for Windows (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was used for multiple comparisons to evaluate the statistical significance between experimental groups followed by post hoc test. The correlation study was calculated using Pearson's correlation. p-value <0.05 was considered significant.

Results

Table (2) summarized the comparative statistics of studied biomarkers between all groups. There was statistically significant increase in serum level of Anticcp, HMGB1, I L1B and NO as well as there was statistically significant decrease in serum level of TAC in Group II when compared to control and treated groups. Treated RA by EP showed significant decrease of the mentioned biomarkers (Anticcp, HMGB1, IL1B and NO), also showed significant increase TAC reflect its proactive role.

Table (3) showed correlation matrix between all studied parameters in RA Group II and treated RA group. There were statistically significant positive correlation between Anticcp, HMGB1, IL1B and NO in both Groups (II, III), as well as TAC showed negative correlation between other parameters.

Histopathological examination results:

The knee joint specimens of control Groups (Group I and IV) showed normal synovial lining, normal collagen fibers and normal bone as shown in Figs. (1,2). Rheumatoid Arthritis (Group II) showed chronic inflammatory infiltration of synovial tissue by macrophages, plasma cells and lymphocytes with fibrinoid necrosis and bony erosion as shown in Fig. (3). Treated rheumatoid arthritis groups (Group III) showed lesser chronic inflammatory infiltration of synovial tissue compared to rheumatoid arthritis.

Table (1): Shows comparison of arthritic index and paw thickness among the studied groups using ANOVA and Tukey's test.

	(Group I) Negative control (n=15)	(Group II) Rheumatoid arthritis (n=15)	(Group III) Treated Rheumatoid arthritis (n=15)	(Group IV) Positive control (n=15)	ANOVA	
					F-test	p-value
Paw thickness:						
7th day	2.11±0.17^a	4.58±0.30^b	4.52±0.16^b	2.16±0.12^a	736.717	0.001*
14th day	2.21±0.15^a	4.69±0.38^b	3.64±0.12^c	2.18±0.12^a	554.892	0.001*
21 th day	2.25±0.14^a	4.68±0.15^b	3.58±0.10^c	2.22±0.11^a	1594.519	0.001*
Arthritic index:						
7th day	0±0^a	8.90±0.46^b	8.07±0.47^c	0±0^a	3309.282	0.001*
14th day	0±0^a	13.03±0.43^b	6.06±0.54^c	0±0^a	4848.618	0.001*
21 th day	0±0^a	14.11±0.50^b	5.06±0.28^c	0±0^a	8207.314	0.001*

n : Number of each group. *: Significance ($p \leq 0.05$).
Data are mean ± standard deviation of a group of 15 rats.
Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test. SPSS computer program.
a.b.c. : Significant difference between groups at * $p<0.05$.
^{a} : Significance from Group I. c: Significance from Group III.
^{b} : Significance from Group II. a: Significance from Group IV

Table (2): Comparative statistics of studied biomarkers between all groups.

	(Group I) control (n=15)	(Group II) Rheumatoid arthritis (n=15)	(Group III) Treated Rheumatoid arthritis (n=15)	(Group IV) EP-control (n=15)	ANOVA	
					F-test	*p*-value
Total antioxidant capacity (mmol/l)	2.28±0.20a	1.24±0.17b	1.63±0.29c	2.18±0.15d	50.267	0.001 *
NO (µM/L)	134.88±13.42a	565.32±107.34b	242.54±40.39c	133.29±14.69d	184.382	0.001 *
HMGB 1 (ng/ml)	2.24±0.11a	8.97±1.14b	4.08±0.61c	2.32±0.16d	349.733	0.001 *
Interlukin1beta (pg/ml)	22.05±5.22a	65.66±5.92b	29.57±4.86c	21.15±4.53d	249.430	0.001 *
AntiCCP (ng/ml)	6.47±1.63a	596.53±36.86b	310.13±28.62c	5.49±1.39d	249.430	0.001 *

Abbreviations:
NO : Nitric Oxide.
HMGB1: High Mobility Group Box1.
IL1B : Inteleukin 1 Beta.
Anticcp: Anti.Cyclic Citrullinated Protein Antibodies.
TAC : Total Antioxidant Capacity.
Data are mean ± standard deviation of a group of 15 rats.
Statistical analysis is carried out using one-way ANOVA with Tukey's post hoc test. SPSS computer program.
a.b.c.: Significant difference between groups at $*p<0.05$.
a : Significance from Group I.
b : Significance from Group II.
c: Significance from Group III.
a: Significance from Group IV.

Table (3): Correlation matrix of all the studied parameters in RA group.

Group II Rheumatoid arthritis	Total antioxidant capacity (mmol/l)		NO (µM/L)		HMGB 1 (ng/ml)		Interleukin 1 beta (pg/ml)	
	r.	*p*.	*r*.	*p*.	*r*.	*p*.		
NO (µM/L)	–0.768	0.001*						
HMGB 1 (ng/ml)	–0.892	0.001*	0.590	0.021 *				
Interleukin 1 beta (pg/ml)	–0.825	0.001*	0.518	0.048*	0.821	0.001*		
AntiCCP (ng/ml)	–0.823	0.001*	0.608	0.016*	0.865	0.001*	0.691	0.004*

Table (4): Correlation matrix of all the studied parameters in treated RA group.

(Group III) Treated Rheumatoid arthritis group	Total antioxidant capacity (mmol/l)		NO (µM/L)		HMGB 1 (ng/ml)		Interleukin 1 beta (pg/ml)	
	r.	*p*.	*r*.	*p*.	*r*.	*p*.		
NO (µM/L)	–0.910	0.001 *						
HMGB 1 (ng/ml)	–0.957	0.001 *	0.960	0.001 *				
Interleukin 1 beta (pg/ml)	–0.615	0.015*	0.719	0.003*	0.695	0.004*		
AntiCCP (ng/ml)	–0.879	0.001 *	0.928	0.001 *	0.906	0.001*	0.535	0.040*

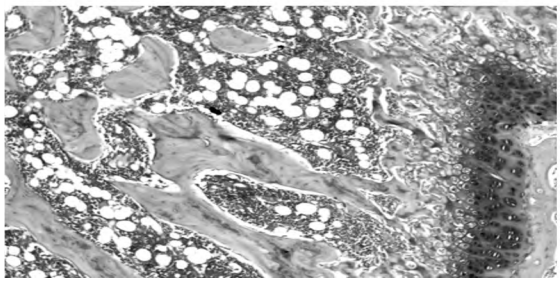

Fig. (1): Control Group I: Showed cartilaginous and bony fragments with bone marrow [H & E X200].

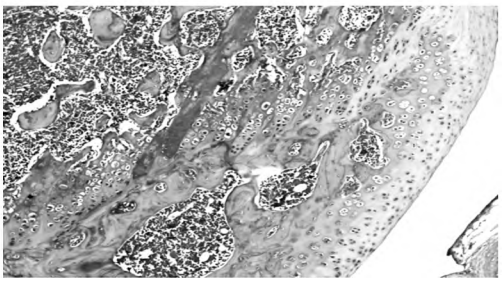

Fig. (2): EP-control Group (IV) showed cartilaginous and bony fragments with bone marrow [H & E X200].

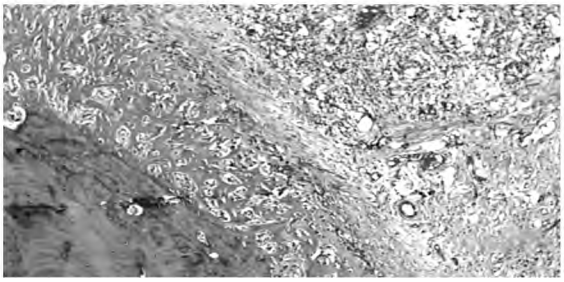

Fig. (3): RA Group II: Showed dense perivascular inflammatory infiltrate to subchondral bone [H & E X200].

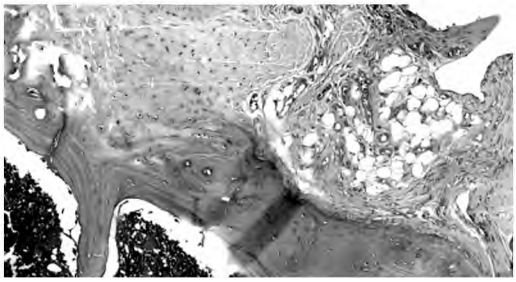

Fig. (4): Treated RA (group III) showed decrease in the number of inflammatory cells and fibrosis [H & E X200].

Discussion

Rheumatoid Arthritis (RA) is chronic progressive inflammatory autoimmune disorder that primarily affects the peripheral joints. It affects synovial lining of joints, causing a painful swelling that can eventually result in bone erosion and joint deformity [1]. The pathogenesis of RA is a multistep process that starts with the development of autoimmunity, continues with local inflammation and finally induces bone destruction [3,16]. Also the oxidative stress may play a role in developing of RA [17]. The present study was designed to induce RA in rat by CFA and determined serum levels of AntiCCP, HMGB1 and Inteleukin1 beta in RA as inflammatory markers. Moreover the oxidative stress markers were studied. A possibility of new line of treatment: Ethyl pyruvate for RA was investigated. Among the most widely used models of RA are the rat Adjuvant-Induced Arthritis model (AIA). It serves as an excellent model for human RA [18]. As Complete Freund's Adjuvant (CFA) is easy to be handled, produce rapid induction and excellent antibody responses that why we used it for induction of RA in experimental animals [19]. Injection of CFA causes an immunological changes and damaging in knee tissue in form of accumulate immune cells such as activated T-and B cells, monocytes and macrophages from activated fibroblasts, and hence releasing cytokines and chemokines [20]. Also RANKL and RANK signaling pathways trigger osteoclast over activation, which degrades bone tissue [21]. In our model, RA was induced in albino rats by a single subcutaneous injection of CFA into the base of the tail [12]. Rheumatoid arthritis in rat appeared in the form of swelling, redness and limitation of movement in knee joint. Furthermore presence of RA was confirmed by detection of histopathology changes in tissue of knee joint that showed dense perivascular inflammatory infiltration of synovial tissue to subchondral bone by macrophages, plasma cells and lymphocytes. The results of present study are in basic agreement with results of El-Shabrawy et al., [22] and Abildtrup et al., [23] who reported that RA could be induced in 21 days after injection of CFA. They showed that there was formation of granulation tissue at the edges of the synovial lining, pannus with extensive angiogenesis causing tissue damage in the joint of knee. Arthritic index is a clinical assessment of joint swelling in inflammatory diseases. The results of present study showed that there were a significant increase in arthritic indices and paw thickness of knee of rat up to 21 st day from induction of RA in Group II due to damage in joints and bones of the rats paw when compared with other groups. These results are confirmed and supported by work of Lei Yi et al., [24] and Praveen and Janarthan [25]. However treatment of RA rats with ethyl pyruvate decreased paw swelling, thickness and inflammation of joints indicating that EP might be an effective treatment for rheumatoid arthritis supported by work of Jung et al., [26]. It has been demonstrated that ethyl pyruvate component has a high pleiotropic activity due to its complex chemical properties. Ethyl pyruvate has ability to control various signaling pathways and capability of interacting with numerous molecular targets involved in inflammatory processes. These characters of EP make it able to suppress of inflammation and relived the pain of joints. In addition, the EP treatment could significantly alleviate the variation of arthritic scores [27].The present study demonstrated a significant increase in serum level of AntiCCP in RA rats when compared with other groups. Although the presence of anticcp in patient with RA has been investigated therapy and there is vast amount in research work that aspect has been published, there is only one or two research paper deal with presence of AntiCCP in rat with RA induced by CFA. The presence of anti-Cyclic-Citrullinated Protein antibodies (Anti-CCP) was determined in acute and chronic Pristane-Induced Rheumatoid Arthritis (PIA) rat model and it was found in that model [28].The most of other animal models of RA do not showed Anti-CCP in their sera. However RA rats treated with ethyl pyruvate demonstrated significantly lower serum level of AntiCCP. The exact mechanism for suppression of serum level of AntiCCP by EP is not known. EP may suppress the production of AntiCCP because it induces anti-inflammatory effects through NF- κβ inhibition and lead to inhibition of the inflammatory processes in the synovium of knee. Cytokines play a role in the pathogenesis of RA, among these cytokines: HMGB 1 , IL1B. HMGB 1 is a ubiquitous nuclear protein that can be released by damaged cell or by activated macrophages and monocytes [29]. HMGB1 interacts with multiple Pattern Recognition Receptors (PRRs), the receptor for advanced glycation end-products (RAGE) and Toll-Like Receptors (TLRs), TLR4 or TLR9 and activates intracellular signal of Mitogen-Activated Protein Kinases (MAPKs) and nuclear factor kappa B (NF- κb) [30]. In the present work, we found significantly higher serum levels of HMGB 1 in rat model versus control. These results are coincided with work of Wang et al., [31]. The increase of serum level of HMGB1 in the model group may be explained in the following basis. In CFA induced RA in rat, there is pathological changes in form of synovial hyperplasia, inflammatory infiltration that stimulate releas-

ing of phosphatidylinositol binding to specific receptor stimulate Ca release from endoplasmic reticulum which bound with calmudulin: Ca-calmodulin dependent protein kinase resulting in phosphorylation lead to release of HMGB 1 into the extracellular space **[32]**. However rats treated with ethyl pyruvate (Group III) showed significantly decrease serum level of HMGB 1. These results are supported by work of Seyhan et al., **[33]** who reported that EP reduce serum level of HMGB 1 in RA that induced in rat by inhibition of NF-κb DNA binding that mediate its effect. Also it is consistent with work of Li et al., **[34]** who studied effects of ethyl pyruvate on synovium of knee in collagen-induced arthritis rat. The present study showed that there were a significant increase in serum level of IL 1B in RA rats (Group II) when compared with other groups. These results are supported by work of Eastgate et al., **[35]** and Yang et al., **[36]** who reported that a lot of cytokines have been found to participate in the regulation of this complicated immune network and most important one is Interleukin-1-beta, also as result of increase serum level of HMGB 1 that bind with it specific receptor stimulate signaling pathway lead to stimulate NF KB that stimulate release of IL 1 B. However rats treated with ethyl pyruvate (Group III) showed significantly decrease in serum level of IL1B. These results are supported by work of Yin et al., **[37]** who stated that EP inhibit NF-κB DNA binding which stops the expression of pro-inflammatory cytokines as TNF-a and IL-1β. Nitric oxide is a pleiotropic free radical messenger molecule. NO has an important role in chronic inflammatory conditions as rheumatoid arthritis and neurodegenerative disorders **[10]**. The present study showed that there were a significant increase in serum NO level in RA rats (Group II) when compared with other groups. These results are supported by the work of Uttara et al., **[38]**. Biochemical mechanism of increase of NO in RA rat might be due to action or releasing pro inflammatory marker such as IL1B which stimulate iNOS. Furthermore NO enhances the inflammatory response by sustaining the nuclear localization of NFκB **[39]**. However rats treated with ethyl pyruvate (Group III) showed significantly decrease serum level of NO. These results are supported by work of by Zhang et al., **[40]** who reported that EP inhibits nitrite/nitrate (NO) release as a proinflammatory marker through inhibition of NO synthetase. Total antioxidant capacity considers the cumulative action of as all the antioxidants present in plasma and body fluids **[41]**. The present study showed that there were a significant decrease in serum level of TAC in RA rats (Group II) when compared with other groups. These results are supported by the work of Al-Rubaei et al., **[42]** who reported that oxidative stress produces free radicles which are involved in the pathogenesis of RA. However rats treated with ethyl pyruvate (Group III) showed significantly higher serum level of TAC. These results are supported by the work of by Rossmann et al., **[43]** who reported that EP promotes the antioxidant systems in the body and it has inhibitory effect on lipid peroxidation.

Our correlation study between immunological parameters such as Anticcp, inflammatory parameters such as HMGB 1and IL 1 B and oxidative stress parameters as TAC and NO indicated that there was a significant positive correlation between serum Anticcp level and serum level of HMGB1, IL1B and NO level in both RA group and treated RA groups as they have the same pathway between them. Furthermore there was a significant negative correlation between serum level of TAC and other parameters that involved in our study as TAC exhausted. In conclusion, this study, on the basis of histopathological data study supported the biochemical findings in the present study as there were significant positive correlation between histopathological and the biochemical markers in both RA group and treated RA groups, also shows that EP reduces inflammatory injury in an experimental RA model and improve histopathological changes. As result of the antioxidant and antinflammatory effects of EP supported by Algieri et al., **[44]**.

Conclusion:

On the basis of these results, the current study highlights evidences for the promising protective effects of EP in CFA induced RA model which resembles the histopathological appearance of the disease in human. These favorable actions were linked with its modulatory effect in maintaining adequate intracellular redox environment as evidenced by reducing some oxido-inflammatory with subsequent improvement of histopahological abnormalities of knee, moreover it is likely to be safe as EP is a common additive in beverages and confectionary products. These findings presented here also imply that EP has therapeutic potential in the prevention of RA and represent a promising therapeutic strategy.

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تآثير الإيثيل بيروفيت على بروتين صندوق المجموعة عالية التنقل آ وعلى الإجهاد التآكسدى فى إلتهاب المفاصل الروماتويدى المفتعل فى الجرذان

إلتهاب المفاصل الروماتويدى هو مرض من آمراض المناعة الذاتية وآعراضه ومضاعفاته الآساسية هو إلتهاب والآم فى المفاصل وتلف العظام. تزال المسببات المرضية لإلتهاب المفاصل الروماتويدى غير متضحة تماما بينما تم توثيق عدة عوامل منها البيئية والوراثية، والجينية. قد حازت المواد المضادة للآكسدة فى الآونة الآخيرة على آهمية خاصة وآصبحت محورا رئيسيا للإهتمام فى جميع آنحاء العالم لذا فالهدف من هذه الدراسة هو تقييم تآثير الإيثيل بيروفيت على إلتهاب المفاصل الروماتويدى المفتعل بمادة مساعد فرينويد بالكامل فى الجرذان وقد آجريت الدراسة على عدد ٦٠ من ذكور الجرذان البيضاء والتى قسمت إلى آربع مجموعات: المجموعة الآولى (مجموعة الضابطة)، المجموعة الثانية (مجموعة إلتهاب الروماتويد المفصلى تشمل ١٥ جرذا تم حقنها بجرعة واحدة ٠.١ ميللتر من مادة مساعد فرينويد الكامل تحت الجلد)، المجموعة الثالثة (مجموعة العلاج تشمل ١٥ جرذا إفتعل بها إلتهاب الروماتويد المفصلى وآعطيت محلول إيثيل بيروفيت المذاب بجرعة ٤٠ ملجم لكل كجم كل يوم لمدة ٣ آسابيع) والمجموعة الرابعة (مجموعة الإيثيل بيروفيت تشمل ١٥ جرذا آعطيت محلول إيثيل بيروفيت فقط بجرعة ٤٠ ملجم لكل كجم كل يوم لمدة ٣ آسابيع) وقد خضعت كل المجموعات إلى تقدير مستوى كلا من الآجسام المضادة لسترولانتيد بروتين، بروتين صندوق المجموعة عالية التنقل ١، الإنترليوكين ١ بيتا، آكسيد النيتريك ومستوى مضدات الآكسدة الكلية كمحاولة للكشف عن الدور الذى يلعبه كلا من الإلتهابات، الإضطرابات فى جهاز المناعة والإجهاد التاكسدى فى حدوث المرض. وقد آظهرت هذه الدراسة إرتفاعا بشكل ملحوظ فى مستوى كلا من الآجسام المضادة لسترولانتيد بروتين، بروتين صندوق المجموعة عالية التنقل ١ والإنترليوكين ١ بيتا ومستوى آكسيد النيتريك وإلى جانب ظهور إنخفاضا فى مستوى مضادات الآكسدة الكلية فى المجموعة الثانية بينما قد ظهر إنخفاضا بشكل ملحوظ فى مستوى كلا من الآجسام المضادة لسترولانتيد بروتين، بروتين صندوق المجموعة عالية التنقل، الإنترليوكين ١ بيتا ومستوى إكسيد النيتريك وإلى جانب ظهور إرتفاعا فى مستوى مضادات الآكسدة الكلية فى المجموعة الثالثة التى تم معالجتها بالإيثيل بيروفيت على آساس هذه النتائج يمكن آن نلخص إلى آن الإيثيل بيروفيت يتمتع بتآثير مضاد للإلتهابات ومضاد للآكسدة وإنه قد يمثل إستراتيجية علاجية واعدة.