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The Enhanced Beneficial Effect of Extra Virgin Olive Oil Over Evening Primrose Oil on Oxidative Stress, and Liver Function in Male Arthritic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MAK and BMM. suggested the design of the study. All authors have conducted research, provided research materials, and wrote the article. All authors critically reviewed, and approved the final draft and are responsible for the content and similarity index of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Rheumatoid arthritis (RA) is characterized by the onset of oxidative stress. This study aimed to evaluate the enhancing of extra virgin olive (EVOO) and Evening primrose oil (EPO) on oxidative stress and liver enzymes in male Wistar rats and compare between them.
Place and Duration: Faculty of Science biochemistry department, Between July 2018 and August 2018.
Methodology: A Subcutaneous injection of 200 µl of Freund's complete adjuvant into a footpad of the right hind leg of Wistar male rats at two consecutive days induced RA. Rats received EVOO and EPO daily by oral gavage needle with gauge 18 at doses of 5 mg/kg b.wt./day. for 10 and 21 days. No loss was recorded in the experimental rats.
Results: A significant depletion in serum Reduced glutathione content (GSH), glutathione

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peroxidase (GPX), and glutathione s transferase activities (GST) in arthritic rats compared to normal rats after 10 and 21 days of induction which improved significantly after 10 and 21 days of EPO and EVOO treatments. EPO and EVOO treatments for 21 days increased the GSH and GPX compared to 10 days treatments while no difference in GST activity. EVOO treatment improved GSH and GPX after 10 and 21 days than EPO treatment. The elevated uric acid levels in arthritic rats were markedly ameliorated as a result of EVOO and EPO treatment administration. Increased lipid peroxidation products (MDA), rheumatoid factor, and liver enzyme (Alanine transaminase ALT and Aspartate transaminase AST) were recorded in arthritic rats and they significantly progressed after EPO and EVOO treatments for 10 and 21 days but EVOO had the best effect at 21 days.
Conclusion: EVOO and EPO showed significant antioxidant efficacies and improved affected liver enzymes due to rheumatoid arthritis onset. When comparing olive oil has more antioxidant properties than evening primrose oil, so we recommend more studies on olive oil combination with anti-arthritic medications to improve their efficacies with less toxicity.

Keywords: Antioxidants; rheumatoid arthritis; oxidative stress; extra virgin olive oil; evening primrose oil; liver function.

1. INTRODUCTION

Rheumatoid arthritis (RA) causes swelling and pain in joints which lead finally to chronic functional impairment due to changes in autoimmunity function. It is characterized by synovial inflammation due to filtration of T lymphocytes, macrophages, B lymphocytes, PUFAs metabolites, and cytokines through the synovium [1]. Several reports from Middle East countries showed that RA injures about 0.14% to 0.55%. Both the environmental factors and genetic factors are implicated in the progress of clinical indication of RA [2]. A fivefold increase in proinflammatory cytokines and mitochondrial ROS production in whole blood and monocytes with decreased detoxifying enzymes and antioxidants like reduced glutathione, catalase, and heme oxygenase was recorded in RA [3,4]. Using FCA induces inflammation of joints, synovial tissues, hyperplasia, and finally bone and cartilage destruction which comparable to RA features [5]. Despite the advances in the diagnosis of RA, existing drug therapies have limited effectiveness and should be used cautiously due to their frequently adverse and toxic effects. New biologic agents are not easily accessible and too expensive so they do not give advantage to most patients, so more effective, safer, and economically justified alternative treatments for RA have always been a target for research. Using ω-3 polyunsaturated fatty acids like EVOO could not only decrease the onset but also the sternness of RA [6]. Evening primrose oil (EPO) is a common complementary medication, with different bioactive compounds. It is used as an antidiabetic drug and to treat inflammatory diseases such as rheumatoid arthritis, and atopic dermatitis. Several studies confirmed the radical scavenging and antioxidant effects of EPO [7,8].

2. MATERIALS AND METHODS

2.1 Experimental Animals

Male Wistar rats weighing from110 to 130 g were housed in plastic cages with good aeration at normal dark/light cycle of 12 h, humidity (55 ± 5%), and temperature (25 ± 5°C) and were provided with water and a standard balanced diet via ad libitum. We bought them from the National Research Centre, Egypt, and rats were then isolated under observation for 2 weeks in separate isolation units. Animals with weights over 140 g, less than 120 g, or who suffered from any illness were excluded from the experiment. Animals were distributed after their adaptation time into separate cages, each with 6 rats, and marked with treatment type and time. Animals were divided into eight groups of 6 rats and classified:

At 10 days Post inoculation

The first group (G1): normal rats were regarded as a control group and weren't suffering from any illness affecting measured parameters. The second group (G2): rheumatoid arthritis untreated rats. The third group (G3): EPO-treated rats. The fourth group (G4): EVOO-treated rats.

At 21 dpi

The fifth group (G5): normal rats were regarded as a control group and weren't suffering from any illness affecting measured parameters.

The sixth group (G6): RA untreated rats. The seventh group (G7): EPO-treated rats. The eighth group (G8): EVOO-treated rats.

2.2Creation of Rheumatoid Arthritis

Freund's complete adjuvant was purchased from Sigma Company, Cairo, Egypt; it mainly contains a suspension of *Mycobacterium tuberculosis* (heat-killed) in mineral oil. Approximately 0.2 ml of FCA was infused into rats' right hind footpad at two consecutive days as previously described by [9].

2.3 Treatment Protocol

2.3.1 Evening primrose oil (EPO)

5 mg/kg. wt./day EPO was given daily at 9:00 am by oral gavage needle (gauge 18, length 2–3 inch, and ball diameter 2.25mm) as previously described [10]. EPO was purchased from EVA pharm co. (Cairo, Egypt) as a gelatin capsule containing 1000 mg. EPO has an LD^{50} of 3.12×10^{4} µg/kg also Short-Term and Subchronic Exposure Male rats fed a diet supplemented with 11% EPO for 6 weeks showed no difference in body weights when compared to control [11].

2.3.2 Extra virgin olive oil (EVOO)

An EVOO bottle was purchased from a traditional market in Beni-Suef, Egypt. It was of Illiada product made in Italy. About five mg/kg. wt./day was given orally daily at 9:00 am as previously described by [12]. Rodríguez-Lara et al.,2019 stated that in rats the oral Lethal Dose fifty (LD^{50}) is >2,000 mg/kg and demonstrated no toxic effect of a dose of 300 mg/kg/d of a VOO extract rich in hydroxytyrosol, in an acute single dose, subacute 14-days supplementation with a maximum dose (2000 mg/kg/d), and after the sub-chronic supplementation during 90 days with 100 mg/kg/d, 300 mg/kg/d and 1000 mg/kg/d, according to the OECD-408 guidelines [13].

2.4 Serum Preparation

10 days after FCA injection (G1 to G4) under anesthetization by inhalation using light diethyl ether (5%) blood samples collected from the middle canthus of the eye in a vacutainer blood collection tubes then plasma separated into three aliquots and stored in -100 C till examination and this repeated for the remaining groups G4 to G8 at 21 days after induction.

2.5 Detection of Serum RF, MDA, and Antioxidants

Serum MDA, GSH, GPX (EC 1.11.1.9), and GST (EC 2.5.1.18) level was determined following [14,15,16, and 17] instructions with little modifications. RF level was determined using rat rheumatoid factor kit CUSABIO Company, USA, By ELISA Technique according to [18]. ALT and AST activities were assessed upon [18] recommendations using Kits purchased from Randox Laboratories Ltd. - Crumlin Company, Antrim, United Kingdom.

2.6 Statistical Analysis

We compared our groups' results with an unpaired t-test one-way analysis of variance (ANOVA) followed by a two-way analysis of Variance (ANOVA). Values of $P\leq 0.05$ were regarded as significant. Data were expressed in tables and figures as mean ±SEM, this was done using Graph Pad Instat software (version 8, ISS-Rome, Italy).

3. STUDY RESULTS

3.1 Serum Oxidative Stress Biomarkers

3.1.1 Serum lipid peroxidation

Measured as MDA products were significantly increased in untreated rats at 10 and 21 days compared to normal rats but the highest level at 10 days, EVOO and EPO treatment reduced it at 10 and 21 days with superior effect at 21 days. EVOO treated groups showed a better effect compared to the EPO groups at 21 days. (Table 1, Fig. 1).

3.1.2 Serum glutathione (GSH)

Serum GSH was significantly reduced in RA rats at 10 and 21 days compared to normal rats. Treatments by EVOO and EPO improved GSH levels in RA- treated rats at 10 and 21 days compared to untreated rats. EPO treatment's best effect was at 21 days compared to 10 days. EVOO treatment made an improvement in GSH levels than EPO at 10 days (Table 1, Fig. 2).

3.1.3Serum glutathione peroxidase (GPX) activity

Serum GPX was significantly reduced in RA rats at 10 and 21 days compared to normal rats. Treatments by EVOO and EPO heighten GPX

Table 1. effect of RA, EVOO, and EPO on oxidative stress markers

G test	10 dpi groups				21 dpi groups			
	Control	**Arth.**	**Arth+EPO**	**Arth+EVOO**	**control**	**Arth.**	**Arth+EPO**	**Arth+EVOO**
MDA	49.8±1.47	157.4±5.3 a	127.1±2.6a, b	127.9±3.14a, b	46.67±2.1	177.7±7.1#, R	93.0±3.0#, O, R	74.83±1.7#, O, ×, R
GSH	16.3±0.28	8.53±0.86 a	11.82±0.86 a, b	13.45±0.6a, b, c	15.6±0.3	8.95±0.2 #	13.35±0.36#, O, R	14.42±0.3#, O, ×
GPX	18±0.37	11.02±0.4a	13.47±0.11 a,b	15.05±0.18a, b, c	17.52±0.4	11.48±0.46 #	14.93±0.36 #, O R	16.18±0.38#, O, ×, R
GST	8..7±0.39	3.0±0.16a	4.92±0.32 a,b	5.14±0.25a, b	8.22±0.2	3.33±0.16 #	5.15±0.34#, O	5.36±0.26#, O

Values of P <0.001 were regarded as significant. Values followed by letters are significantly different (P≤ 0.05); [a] significantly different from control 10 dpi G. [#] significantly different from control 21 dpi G; [b] significantly different from arthritis 10 dpi G. [O] significantly different from arthritis 21dpi G; [C] significantly different from EPO 10 dpi G. [×] significantly different from EPO 21 dpi G; R significantly different from its corresponding 10 dpi G

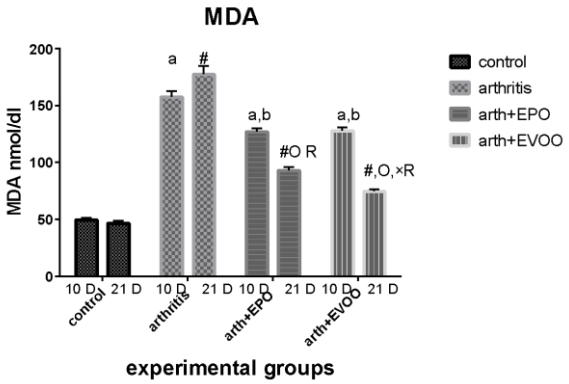

Fig. 1. shows Lipid peroxidation levels in arthritis, EPO and EVOO treated rats

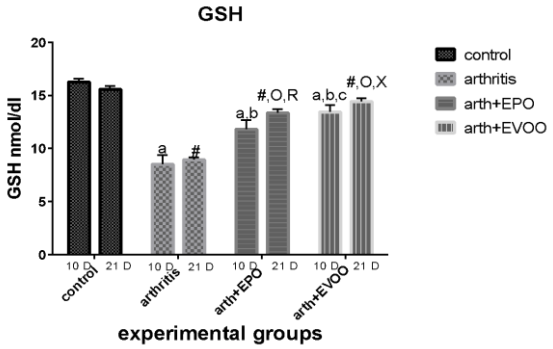

Fig. 2. shows Reduced glutathione levels in arthritis, EPO and EVOO treated rats

activity in RA- treated rats at 10 and 21 days compared to untreated rats. The most appeared effect of EPO and EVOO treatment was at 21 days compared to 10 days. EVOO treatment made an improvement in GSH levels than EPO at 10 and 21 days. (Table 1, Fig. 3).

3.1.4 Serum glutathione transferase (GST)

it was visible that GST activity was diminished in arthritis, EPO, and EVOO judge against Normal groups. GST activities increased in EPO and EVOO groups than in arthritis. There weren't any significant differences in GST activity between the used treatments. (Table 1, Fig. 4).

3.2 Serum Rheumatoid Factor, Uric Acid, and LIVER Enzymes Levels

3.2.1 Serum Rheumatoid factor: Serum RF was significantly increased in untreated rats at 10 and 21 days compared to normal rats but the highest level at 10 days, EVOO and EPO treatment decreased it at 10 and 21 days. EVOO treated groups showed a better effect compared to the EPO group at 21 days. (Table 2 and Fig. 5).

3.2.2 Serum uric acid levels

The uric acid level was significantly increased in arthritis, EPO, and EVOO than in the control groups. Uric acid showed no significant change in EPO and EVOO treated groups compared to the arthritis groups, while the level in EVOO treated group was significantly increased compared to EPO at 10 days (Table 2 and Fig. 6).

3.2.3 Serum liver enzymes ALT (GPT) activity

A significant augmentation in Glutamate pyruvate transaminase (GPT) activity was detected in EPO treated, EVOO treated, and untreated arthritic rats compared to normal rats. EVOO showed non-significant change after 10 days treatment when compared to untreated groups while after 21 days a significant GPT activity decrease achieved while compared to its activity in EPO treated group they were increased. EPO treatment for 10 and 21 days reduced the activity compared to untreated rats' groups and there was a significant reduction in the activity after EPO 21 days treatment compared to 10 days treatment.

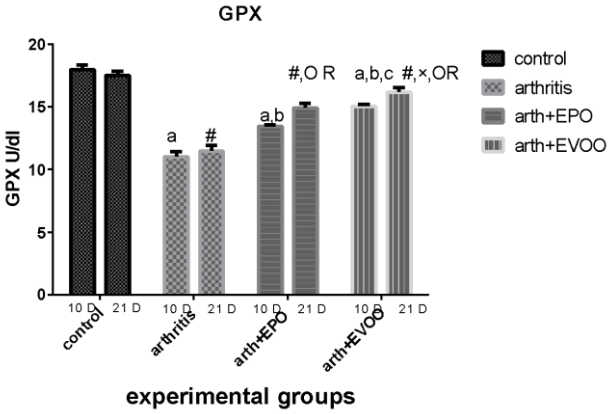

Fig. 3. shows Serum glutathione peroxidase activities in arthritis, EPO and EVOO treated rats

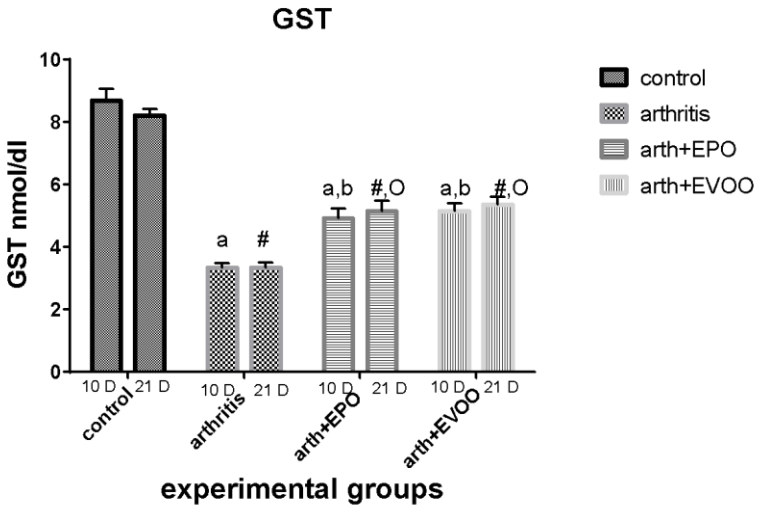

Fig. 4. shows glutathione S transferase activities in arthritis, EPO and EVOO treated rats

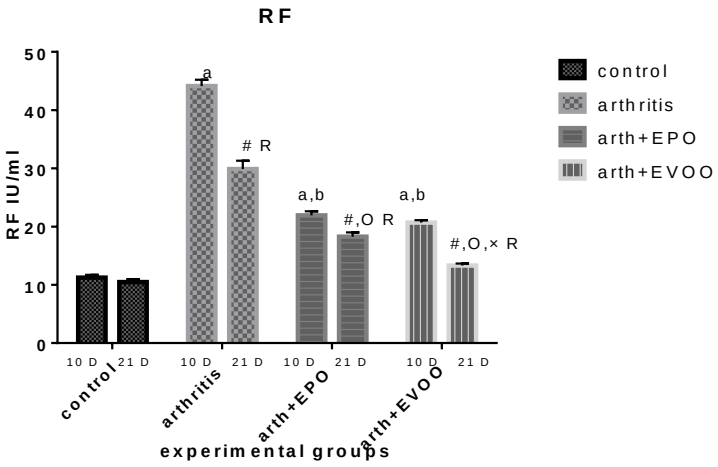

Fig. 5. shows Rheumatoid factor levels in arthritis, EPO and EVOO treated rats

Table 2. effect of RA, EVOO, and EPO on arthritis biomarkers and liver enzymes

G test	10 dpi groups				21 dpi groups			
	Control	**Arth.**	**Arth+EPO**	**Arth+EVOO**	**control**	**Arth.**	**Arth+EPO**	**Arth+EVOO**
RF	11.2±0.5	44.1±1.1a	21.9±0.73a, b	20.7±0.41a, b	10.48±0.5	29.9±1.46 #, R	18.27±0.7 #, O, R	13.32±0.34# O × R
Uric acid	3.89±0.2	6.23±0.3a	5.33±0.13a	6.56±0.48a, c	3.84±0.3	5.5±0.7#	5.53±0.45 #	5.99±0.79#
ALT	26.37±1.8	48.03±3.8a	42.18±1.8a, b	49.38±2.37 a, c	25.7±1.4	42.4±2.67 #, R	35.97±1.5 #, O, R	37.65±1 #, O, R
AST	160.0±5.6	257.8±7.2a	228.2±1.85a, b	196.5±11.5a, b, c	160.0±5.7	250.3±9.6 #	201.0±5.1 #, O, R	188.7±10.5#, O

Values of P <0.001 were regarded as significant. Values followed by letters are significantly different (P≤ 0.05). [a] significantly different from control 10 dpi G [#] significantly different from control 21 dpi G; [b] significantly different from arthritis 10 dpi G [O] significantly different from arthritis 21dpi G; [C] significantly different from EPO 10 dpi G [×] significantly different from EPO 21 dpi G; R significantly different from its corresponding 10 dpi G

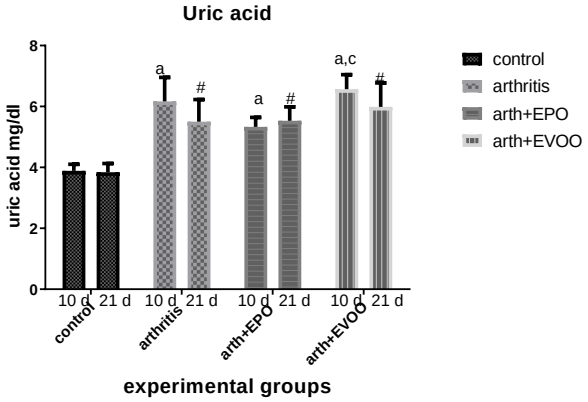

Fig. 6. shows Serum Uric acid levels in arthritis, EPO and EVOO treated rats

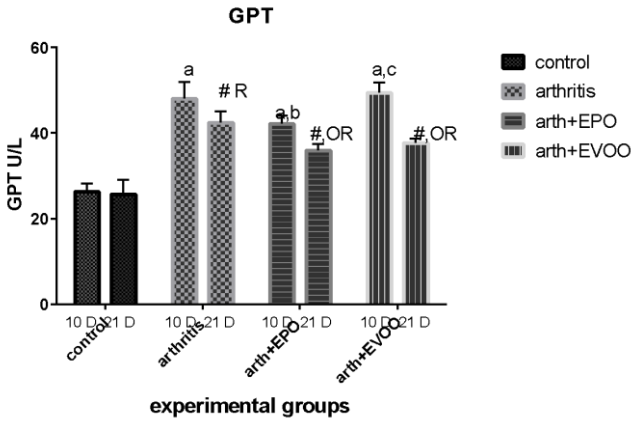

Fig. 7. shows GPT activity in arthritis, EPO and EVOO treated rats

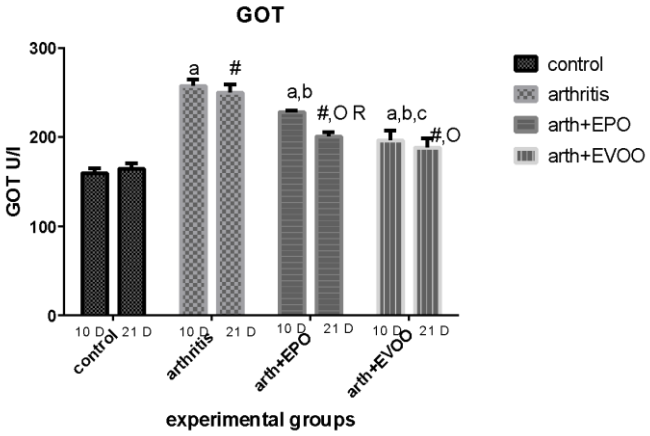

Fig. 8. shows GOT activity in arthritis, EPO, and EVOO groups

3.2.4 Serum liver enzymes AST (GOT) activity

A significant augmentation in Glutamate oxaloacetate transaminase activity (GOT) activity was detected in EPO treated, EVOO treated, and untreated arthritic rats compared to normal rats. EVOO treatment decreased the GOT activity at 10 and 21 days compared to untreated (10 and 21 dpi) and 10 days EPO treated groups. EPO treatment for 10 and 21 days reduced the activity

compared to untreated rats' groups. EPO 21 days treatment caused a significantly reduced activity compared to 10 days EPO treatment.

4. DISCUSSION

Freund's Complete adjuvant-induced Rheumatoid arthritis mirrors several rheumatoid arthritis features like Pannus formation, T and B cells infiltration, augmented proinflammatory chemokines, and amplified oxidative stress. RA is diagnosed by assessing autoantibodies formation including rheumatoid factor and anticitrullinated proteins which are secreted from stimulated B cells via activated CD4+ T cells and by treating the inflammation, the titer of these proteins improves.

Our results proved that Extra virgin olive oil and evening primrose oil can decrease RF levels in arthritic rats and this may attribute to high polyunsaturated fatty acids contents which increases the anti-inflammatory eicosanoids, decreased nitric oxide (NO) and ROS production, down-regulated iNOS, COX-2, reduced MAPK (JNK, p38) phosphorylation, and prevented the nuclear NF-κB translocation [19].

Our results showed that RF amplified after CFA injection at 10 and 21 but the level began to decline at 21 days without treatment; these findings were the same as other results [20,21], also an augmentation in MDA at 10 and 21 days of induction; this was in the accordance with Akyol et al., founded a remarkable elevation in MDA levels in patients with RA compared to controls [22], this was also observed by [23]; discussed that increased levels of S-nitrosothiols are present in RA plasma and knee-joint synovial fluid, which correlated with measures of inflammation in RA [24].

Several studies discussed COX-2 present in small amounts in healthy tissues and it is amplified in stimulated macrophages and immune cells in inflammation sites. COX-2 also activates prostaglandins 2 PGE2 which is an inflammatory mediator.

EVOO polyphenols and DGLA contained in EPO could diversify the inflammation, decreasing ROS and RNS production. inducible NO synthetase activity. The deleterious expression of COX-2 and iNOS gene was inhibited by EVOO HT, thus decreasing nitrate synthesis. It is well known that EPO leads to the accumulation of DGLA in tissue phospholipids. DGLA is a competitive inhibitor with arachidonic acids for the COX pathways, which in turn will reduce inflammation [25]. GLA increases the fluidity and integrity of cell membranes. Collectively, this reflects the suppression of membrane damage.

Our data showed that the serum antioxidant contents (GSH content, GPX, and GST activities) in the arthritic rats were greatly depleted at 10- and 21-days' post-inoculation these were confirmed by others [26,27]**.** Hydroxyl radical and other ROS species attack cell membranes causing alterations in the protein structure, antigenicity, and conformation, also destroys antioxidants within the synovial joints and spleen producing amplified malondialdehyde MDA and decreased GSH, GSHPx, GR, and GST activities [28]. In RA, the antioxidant pathway is controlled by the transcription nuclear factor (erythroid-derived 2)-like 2(Nrf2) [29].

EVOO polyphenols like HT, oleuropein, and tyrosol decreases oxidative stress [30] by increasing glutathione reductase, glutathione-S-transferase, ɤ-glutamyl cysteine synthetase, and intracellular GSH levels. EVOO polyphenols increase serum antioxidants through direct pathway including activation of Akt, ERK, and Nrf2 pathways [31], and indirect assists including inhibition of phospholipase A2, lipoxygenase, and cyclooxygenase enzymes, thus reducing the production of chemotactic agents [32].

Our results elucidated that RF [33,34], and MDA products [35] levels were significantly reduced after EVOO treatment for 10 and 21 days while serum antioxidants levels were significantly increased. EVOO N-3 PUFA intake increases the gene expression of the anti-inflammatory cytokines such as IL-10, [33,36] and intrinsic redox enzymes (GPX1 and GPX4) which reduces the adhesion molecule expression and regulates the nuclear factor-kB (NF-kB) oxidative stress-activation pathway [36].

Our results showed that EPO treatment for 10 and 21 days after RA induction could improve the augmented RF and lipid peroxidation. EPO treatment increased serum antioxidants levels in 10 and 21 days; this was the same as others [25,37,38]. EPO administration not only attenuated lipid peroxidation as evident by the significant decrease in MDA level, but also increased the antioxidant enzymes pool, authors revealed that EPO suppresses the formation of ROS by its Phenolic compounds which inhibit lipid peroxidation, chelate metal ions, and stop

the chain reactions [39]. EPO does not only have a powerful antioxidant effect but also revealed intense radical-scavenging activity [40]. EPO is rich in omega-6 essential fatty acids (EFAs) including α-linoleic acid and γ-linolenic acid GLA, which have a direct influence on immune cells action [41] as well as its indirect effect on the eicosanoid production [39].

Our results showed that ALT and AST were significantly enhanced in arthritis and EVOO treatment improved their activities, these were the same as [42] results they illustrated that the liver histological structure of arthritic rats showed mild degenerative changes and they attributed these changes to increased TNF and NF-kB levels.

EVOO decreased ALT, AST activities and restored them towards normal values via preservation of functional integrity of hepatic cell membrane due to olive oil's ability to scavenge free radicals [43]. El Bohi et al., stated that administration of EVOO alone or with 5-HMF reduced hepatotoxicity of 5-HMF and implemented an antioxidant defense system, liver function biomarkers, and histoarchitecture of the liver. EVOO also inhibited the genotoxic and apoptotic effects of 5-HMF [43]**.**

Our results showed that EPO decreased liver enzymes This was also established by Rezapour-Firouzi et al; stated that EPO has improved effects on the activity of liver enzymes in Multiple Sclerosis patients [44]. GLA could reduce various lipogenic enzyme activities, increased the activity and mRNA levels of various fatty acid oxidation enzymes [45]. We found that EVOO enhanced AST activity more than EPO.

5. CONCLUSION

We could conclude that Rheumatoid arthritis inflammation and oxidative stress lead to liver enzyme leakage which proves that RA onset harms the hepatocytes. EVOO and EPO showed significant antioxidant efficacies and improved affected liver enzymes in arthritic rats. When comparing olive oil has more antioxidant properties than evening primrose oil, so we recommend more studies on olive oil combination with Disease-modifying antirheumatic medications to improve their efficacies, decrease their known hepatotoxicity, and enhance arthritic patient Discomforts.

DISCLAIMER

There is no conflict of interest between the authors. This research didn't receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors, rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The ethics approval number for our study is BSU-IACUC 021-0149. All animal dealings followed Institutional Animal Care and Use Committee of Beni-Suef university guidelines.

IACUC oversees the university's animal care and use program ensuring the appropriate care and use of animals used for research, testing, and teaching.

The IACUC is also responsible for reviewing and approving requests to use vertebrate and aquatic animals, and ensuring compliance with the Guide for the Care and Use of Laboratory Animals 8th Edition 2011 (the Guide).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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