IMPACT OF NATURAL OILS SUPPLEMENTS ON DISEASE ACTIVITY AND ANTIOXIDANT STATE OF EGYPTIAN PATIENTS WITH RHEUMATOID ARTHRITIS

SAHAR Y. AL-OKBI*
NAGWA M. AMMAR**
KH.A. SOROOR***
DOHA A. MOHAMMED*

SUMMARY: The present research was established to study the effect of oral supplementation of different natural oils on the anti-oxidant state, plasma trace elements, prostaglandin E_2, activities of transaminases, uric acid and creatinine and erythrocyte sedimentation rate (ESR) in Egyptian patients with rheumatoid arthritis (RA). The natural oils used in this study were fish oil, evening primrose and Nigella sativa, in conjunction with vitamin E and were supplemented for two months to RA patients. Additional group of patients was given synthetic anti-inflammatory drugs.

Results showed that supplementation of any of the above referred natural oils, significant reduction of the activities of erythrocyte superoxide dismutase (SOD), ESR, plasma copper, prostaglandin E_2, and creatinine with significant increase of plasma zinc were noticed in RA patients. Administration of either fish oil or primrose oil produced significant increase of plasma vitamin C and decrease of plasma uric acid. The serum of RA patients that were given primrose showed significant increase of plasma vitamin E. Fish oil was the only supplement that produced reduction in plasma activities of transaminases. It was noticed that administration of synthetic anti-inflammatory drugs produced significant increase of plasma vitamin C and significant decrease of plasma copper and prostaglandin E_2.

The present research included studying the antioxidant state and the different biochemical parameters when the previously mentioned natural oils were administered simultaneously during consumption of either low or high caloric diet in RA patients.

Concerning the antioxidant state, especially the elevating effect of plasma vitamin C and the reducing effect of the activities of erythrocyte SOD, the best natural oil in this respect was fish oil followed by primrose then Nigella, the synthetic drug had the best least effect. Primrose was superior in elevating plasma vitamin E.

Plasma prostaglandin E_2 was efficiently reduced by fish oil supplementation followed by synthetic drugs then primrose oil which was more efficient than Nigella oil.

Key Words: Rheumatoid arthritis, antioxidant state, natural oils, disease activity.

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease characterized by joint swelling, synovial inflammation and cartilage destruction (1). Essential fatty acids have a unique role as precursor molecules of chemical regulators of inflammatory cell function (2). These regulators are the prostaglandins and the leukotrienes, compounds synthesized and released by almost every tissue in the body and participating in many biological functions, including the inflammatory and immune processes (3).

*From Department of Food Sciences and Nutrition,
**From Department of Pharmacology, National Research Centre, Dokki, Cairo, Egypt.
***From Department of Rheumatology and Rehabilitation, Medical Military Academy, Cairo, Egypt.

Altering the essential fatty acids content of the diet or administering different essential fatty acids as supplements may modify the production of the various prostaglandins and leukotrienes. Supplementation of fish oil rich in eicosapentaenoic acid (20:5 ω-3) and docosahexaenoic acid (22:6 ω-3) might have successful results in the treatment of rheumatoid arthritis. The mechanisms which might account for these anti-inflammatory effects, including conversion of these polyunsaturated fatty acids to biologically less active eicosanoids (prostaglandin E_3, thromboxane B_3 and leukotriene B_5) (4, 5).

The ingestion of a diet rich in evening primrose (*Oenothera biennis* L. family Oenotheraceae) oil rich in δ-linolenic acid (18:3 ω-6) elevates serum dihommo- δ-linolenic acid (20:3 ω-6) concentrations, resulting in an increase in the 1-series prostaglandins, prostaglandin E_1 (6). Prostaglandin E_1 has a negative feedback role in chronic inflammation, initially aiding the development of the cardinal signs of inflammation but later suppressing inflammation. So evening primrose oil might be useful in a disease characterized by inflammation such as rheumatoid arthritis.

The whole powdered seeds of *Nigella sativa* L. Family Ranunculaceae as well as the successive petroleum and alcoholic extracts revealed a remarkable anti-inflammatory activity in experimental rats (7). Fixed oil of *Nigella sativa* and derived thymoquinone has been shown to inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation (8). Only scanty studies of the anti-inflammatory action of *Nigella sativa* were conducted on human and even the majority of these studies were done on asthma (9), no previous studies have been in rheumatoid arthritis, the present study might be the first in this concern.

The main goal of the present work was to study to what extent oral administration of natural oils (fish oil, evening primrose oil and *Nigella sativa* oil) together with vitamin E might have therapeutic activity towards rheumatoid arthritis patients in comparison to synthetic drugs. To fulfill this aim a follow up study of certain biochemical parameters, including antioxidants, trace elements and parameters reflecting disease activity, before and after the natural oils' administration was carried out in rheumatoid arthritis patients.

MATERIALS AND METHODS

Materials

Three natural oils in form of capsules were used in our study.

1. Animal origin

Fish oil (Salmon oil): Commercial name: EPA, obtained from Minapharm-Egypt from Pharmagel-Switzerland.

2. Botanical Origin

Evening primrose seeds oil (Oenothera Biennis L. family Oenotheraceae*):* Commercial name: Primaleve, obtained from Glaxo Wellcome Egypt,

Nigella sativa oil (Nigella sativa L. family Ranunculaceae*):* Commercial name: Nigellar, obtained from Kahira Pharmaceuticals and Chemical Industries Company Cairo-Egypt,

In addition, Vitamin E, obtained from Pharco Pharmaceuticals, Alexandria-Egypt were used.

Subjects

The study included 112 rheumatoid arthritis male patients as outpatients aged 30-70 years attending the Rheumatology Clinic, Kobry El-Koba Military Hospital.

Methods

Determination of fatty acids and unsaponifiable fractions of the different natural oils:

Three grams of each of the three natural fixed oils (fish oil, evening primrose oil and *Nigella sativa* oil) under investigation, were saponified by refluxing with 10% alcoholic potassium hydroxide. After dilution with distilled water, the unsaponifiable fraction was extracted with ether. Both aqueous (saponifiable) and non-aqueous portions (unsaponifiable) were separated in separating funnel. The ether was evaporated, and the extract was weighed and kept for further investigation of unsaponifiable matter. The aqueous mother liquor, in each case, was acidified with 10% hydrochloric acid and the liberated fatty acids were extracted with ether. Ether was evaporated and the residue was weighed and kept for studying the total fatty acids (10, 11). The unsaponifiable fraction of the three natural oils under investigation was analyzed by Gas Liquid Chromatography (GLC). Identification of hydrocarbons and sterols contents of the unsaponifiable fraction was carried out by comparison of their retention times and co-injection with the available authentic reference compounds [(cholesterol, campesterol, β-sitosterol, β-amyrine (E. Merck, Darmstadt, Germany)]. Quantitation was based on peak area integration. GLC conditions were stationary phase: Chromosorb W-HP, detector temperature 290°C, injector temperature 28°C, carrier gas N_2, flow-rate 30 ml/min, air flow-rate 300 ml/min, H_2 flow-rate 30 ml/min, detector FID, chart speed 0.5 cm/min, oven program: Initial temperature 70°C, final temperature 270°C, programmed 4°C/min. For 50 min maintained for 35 min at 270°C, total

Table 1: GLC analysis of unsaponifiable matter of the different natural fixed oils (as percentage of total hydrocarbon).

Hydrocarbons	Fish oil	Primrose oil	*Nigella sativa* oil
Dodecane C12	-	0.528	-
Tetradecane C14	0.467	1.020	0.111
Eicosane C20	0.926	0.752	25.071
Docosane C22	-	35.675	-
C25	1.764	9.135	0.804
C26	13.612	2.401	1.133
C28	0.320	2.111	0.298
C29	0.137	2.741	0.193
β-amyrine	-	7.884	-
Total (Identified)	17.226	62.247	27.61

time 85 min. Identification of the fatty acid methyl ester was carried out by GLC through direct comparison of retention times of each of the separated compounds with those of certain available authentic samples of the fatty acid methyl esters analyzed under the same conditions. Quantitation was based on peak area integration. GLC conditions were stationary phase: 10% diethylene glycosuccinate (DEGS) packed column, oven temperature 170°C, detector temperature 300°C, injector temperature 250°C, carrier gas N_2, flow-rate 30 ml/min, air flow-rate 350 ml/min; H_2 flow-rate 350 ml/min, detector FID, chart speed 2 cm/min.

Design of the clinical study

Patients with active rheumatoid arthritis were divided into four groups, each group comprised of twenty-eight patients. Three groups (after stopping medication with synthetic drugs) were given daily oral dose of 2 g of either fish oil, evening primrose oil or *Nigella sativa* oil separately in forms of capsules (in two divided doses), in addition, they were given 100 mg vitamin E/day orally as an antioxidant. The fourth group of patients was given synthetic anti-inflammatory drugs (combination of steroidal, non-steroidal and immunosuppressive) used in the treatment of rheumatoid arthritis, this group served as reference group. The experiment continued for two months.

Biochemical analysis of blood of rheumatoid arthritis patients were carried out at the start and end of the clinical study. Duration of morning stiffness and time of walking were also followed.

Biochemical analysis of blood

Blood samples were obtained from subjects after an overnight fast. Each blood sample was divided into three parts one mixed with trisodium citrate for determination of erythrocyte sedimentation rate (12), the second part mixed with heparin for separation of plasma and determination of copper (13), zinc (14), vitamin C (15), vitamin E (16), aspartate transaminase (17), alanine transaminase (17), uric acid (18), creatinine (19), and prostaglandin E_2 (20), and the third part without addition of anticoagulant for separation of sera for the determination of C-reactive protein (21) and rheumatoid factor (22). Plasma and serum were kept at -20°C until analyzed. For erythrocyte superoxide dismutase determination (23), 0.9% NaCl was added to the red cell layer of the centrifugate, the red cells were centrifuged and washed three times and stored in a plastic tube at -20°C until analyzed.

The biochemical parameters of patients were compared before and after natural oils or synthetic drug supplementation using statistical analysis of Student's t-test (2-tailed).

Biochemical parameters after natural oils or synthetic drug supplementation were analyzed by one-way ANOVA when significant difference were found, a Tukey test was performed at a probability of p=0.05 (24).

RESULTS AND DISCUSSION

The results of investigation of unsaponifiable matter (Table 1) showed that the percentage of hydrocarbons (as percentage of total hydrocarbons) identified in unsaponifiable matter was 17.226, 62.247 and 27.61 in fish oil, primrose oil and in *Nigella sativa* oil respectively. Hexasane the main hydrocarbon identified in fish oil C26 was (13.612%), while the major hydrocarbone in primrose oil is Docosane (C22) (35.675%), but the main hydrocarbon in *Nigella sativa* oil is eicosane (C20) (25.071%). β-amyrine, a triterpenoidal matter, is identified only in primrose oil (7.884%). GLC analysis of the

fatty acids (Table 2) showed the presence of palmitic, oleic and linoleic acids in the three oils under investigation, while myristic and linolenic acids were only present in *Nigella sativa* oil and fish oil. δ-linolenic acid was present only in primrose oil. Linoleic acid may be considered as the major fatty acid present in primrose oil and *Nigella sativa* oil, while eicosapentaenoic acid was the major fatty acid present in fish oil.

Concerning primrose oil, the percentage of saturated fatty acids was 10.843%, palmitic acid was the major one (9.367%), while the percentage of unsaturated fatty acid was 87.978%, linoleic acid was the major one (62.941%). This oil contains δ-linolenic acid as 12.607%.

In the present study the percentage of saturated fatty acids in *Nigella sativa* oil was 9.658% and they were represented only by palmitic acid, while the unsaturated fatty acids represent 85.141%, and linoleic acid was the major one (46.036%). The percentage of ω-3 fatty acid was 18.188% as linolenic acid.

In the current study the percentage of identified saturated fatty acids of fish oil was (14.403%) and palmitic acid was the major one (9.602%), while the percentage of identified unsaturated fatty acids was (36.155%), and eicosapentaenoic acid was the major one (11.534%). The percentage of identified ω-3 fatty acids were 23.353% in fish oil which were represented by linolenic, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic (3.212, 11.534, 3.143 and 5.464% respectively).

Biochemical changes in rheumatoid arthritis patients after 2-month-treatment with synthetic drugs or natural oils

In a previous study (25) when the biochemical parameters of rheumatoid arthritis patients were compared with control healthy subjects, there was significant increase of ESR, serum CRP, RF, plasma PGE_2, Cu, activity of AST, ALT, creatinine, uric acid and activity of erythrocyte SOD with significant reduction of plasma zinc, vitamin C and vitamin E. The biochemical parameters of rheumatoid arthritis patients before and after 2 months supplementation of the natural oils or synthetic drugs are present in Table 3. There was a significant reduction in erythrocyte sedimentation rate in fish oil, primrose oil, and *Nigella sativa* oil groups ($p<0.001$, <0.005 and <0.05 respectively), while a non-significant reduction was shown in synthetic drugs group. It was reported that erythrocyte sedimentation rate is the best parameter (better than C-reactive protein and rheumatoid factor) to be followed for evaluating the efficacy of new anti-inflammatory agents in rheumatoid arthritis (26). This means that natural oils used in our study might have a therapeutic effect towards rheumatoid arthritis.

Table 2: Fatty acids of the different natural fixed oils (as % of total fatty acids).

Fatty acids	Fish oil	Primrose oil	*Nigella sativa* oil
Myristic acid (C14:0)	3.682	-	Traces
Palmitic acid (C16:0)	9.602	9.367	9.658
Stearic acid (C18:0)	1.119	1.476	-
Oleic acid (C18:1 ω-9)	2.666	12.430	20.917
Linoleic acid (C18:2 ω-9)	10.136	62.941	46.036
δ-Linolenic acid (C18:3 ω-6)	-	12.607	-
Linolenic acid (C18:3 ω-3)	3.212	-	18.188
EPA (C20:5 ω-3)	11.534	-	-
DPA (C22:5 ω-3)	3.143	-	-
DHA (C22:6 ω-3)	5.464	-	-
Total identified saturated fatty acids	14.403	10.843	9.658
Total identified unsaturated fatty acids	36.155	87.978	85.141
Total ω-6 fatty acid	10.136	75.548	46.036
Total ω-3 fatty acid	23.353	-	18.188

EPA: Eicosapentaenoic acid DPA: Docosapentaenoic acid DHA: Docashexaenoic acid

Serum C-reactive protein showed a non-significant reduction in all groups. Serum rheumatoid factor was non-significantly reduced in synthetic drugs group and did not change from baseline in natural oils groups. Synthetic drugs used in the present study included immune suppressive drugs and corticosteroidal that has been reported to inhibit the immune function (27). In the present study natural oils were administered after stopping synthetic drugs, so natural oils did not produce any deterioration of these parameters (C-reactive protein, rheumatoid factor) but they keep the level as it is which reflect the beneficial effects of these oils. ω-3 and ω-6 polyunsaturated fatty acids (which are present in high percentage in the natural oils under study) can modulate immune and inflammatory cell functions by eicosanoid-mediated effects (28). Long chain fatty acids such as palmitic, stearic, docosahexaenoic acid, eicosapentaenoic acid and δ-linolenic ..... etc. (primrose oil, fish oil and *Nigella sativa* oil are rich in long chain fatty acids) have been reported to have anti-denaturant activity, protect against protein denaturation, which might have beneficial effects in rheumatoid arthritis and other rheumatic conditions (29). These fatty acids have the capability of stabilizing endogenous protein such as serum albumin and perhaps other proteins (through binding the proteins). This stabilization could prevent changes of antigenicity due to protein denaturation and glycosylation, which may trigger pathological autoimmune responses (30) suggesting that this action may be involved in the mode of action of fish oil, *Nigella sativa* oil and primrose oil in rheumatoid arthritis and other chronic inflammatory diseases. It has been reported that fish oil anti-denaturant activity was higher than non-steroidal anti-inflammatory drugs like phenylbutazone and indomethacin (29).

Plasma level of prostaglandin E_2 was significantly reduced in all patients groups; fish oil group ($p<0.001$), primrose oil, *Nigella sativa* oil ($p<0.05$) and synthetic drugs ($p<0.005$), which means that the natural oils were comparable with synthetic drugs in inhibiting the inflammatory mediator prostaglandin E_2.

Arachidonic acid, the mother substance of the pro-inflammatory eicosanoid, is released from membrane phospholipids through phospholipase A_2 in the course of inflammatory activation and is metabolized by cyclooxygenase and lipoxygenase to prostaglandins and leukotrienes respectively.

Synthetic drugs such as steroidal anti-inflammatory drugs inhibit prostaglandin E_2 by stimulating lipocortin (proteins) which inhibit phospholipase A_2 activity preventing the release of arachidonic acid (31). However, non-steroidal anti-inflammatory drugs block prostaglandin and thromboxane formation by inhibiting cyclooxygenase activity. Indomethacin, in addition inhibits phospholipase by increasing intracellular cyclic adenosine monophosphate (31). Steroidal anti-inflammatory drugs are able to inhibit both cellular and fluid egress from the vascular space to inflammatory sites and inhibit the function of cell involved in the inflammation process (27).

Dietary supplementation of long chains ω-3 fatty acids like eicosapentaenoic acid and docosahexaenoic acid competitively inhibits the oxygenation of arachidonic acid by cyclooxygenase. In addition eicosapentaenoic acid is able to act as a substrate for both cyclooxygenase and 5-lipoxygenase leading to inhibition of the inflammatory mediator prostaglandin E_2 and leukotriene B_4. Ingestion of fish oils decrease membrane arachidonic acid and concomitantly decrease the capacity to synthesize eicosanoids from arachidonic; eicosapentaenoic acid gives rise to the 3-series prostaglandins and thromboxans and the 5-series leukotrienes (28,32,33).

Evening primrose oil contain large amount of δ-linolenic acid (18:3, ω-6) which can be converted by an elongase enzyme to dihommo -δ- linolenic acid (20:3, ω-6). Dihommo-δ-linolenic acid competes with arachidonate for oxidative enzymes, thereby reducing production of cyclooxygenase products derived from arachidonate. Dihommo-δ-linolenic acid is oxidized by cyclooxygenase to prostaglandin E_1, a monoenoic prostaglandins that has altered biologic activities from the dienoic prostaglandin E_2. Prostaglandin E_1 has known anti-inflammatory and immunoregulating properties (34-36). Dihommo-δ-linolenic acid itself cannot be converted to leukotrienes but can from a 15-hydroxyl derivative that blocks the transformation of arachidonic acid to leukotrienes. So dihommo-δ-linolenic acid acts as a competitive inhibitor of 2-series prostaglandins and 4-series leukotrienes and thus suppresses inflammation (3,37-42).

It has been reported that the crude fixed oil of *Nigella sativa* seed which is rich in thymoquinone and

Table 3: Different biochemical parameters of RA patients before and after 2- month treatment with synthetic drugs or different natural oils.

Parameters	Synthetic drugs		Fish oil		Primrose oil		*Nigella sativa* oil		Control healthy
	Before	After	Before	After	Before	After	Before	After	
	Means±SD		Means ±SD		Means±SD		Means±SD		
ESR (mm/h)	63.3±4.7	52.7±4.6	52.7±3.7	34.3±3.7*****	58.7±3.7	42.7±2.8****	52.8±5.6	37.7±4.7*	9.8±0.431
Change (%)		-21		-35		-27		-29	
Serum									
CRP (mg/l)	50.1±5.6	46.5±6.1	43.5±5.9	40.7±6.1	45±5.7	40.7±5.5	46.3±5.6	44.1±5.9	0
Change (%)		-7		-6		-10		-5	
RF (IU/ml)	111.4±25.5	108.6±25.7	51.4±16.821	51.4±16.821	114.3±22.2	114.3±22.2	104.3±22.6	104.3±22.6	0
Change (%)		-3		0		0		0	
Plasma									
PGE_2 (pg/ml)	1112.5±30.9	962.5±25.5****	1097.5±21.3	937.5±24.5*****	1011.3 ±22.9	921.3±26.5*	1057.5±18.6	991.3±19.3*	184.3±10.4
Change (%)		-14		-15		-9		-6	
Cu (ug/dl)	141.1±2.8	116.7±2.6*****	130.6±2.7	106.8±2.9*****	128.6±2.9	116.7±2.6****	133.4±2.8	119.8±2.4*****	97.2±2.837
Change (%)		-17		-19		-9		-10	
Zn (ug/dl)	51.3±1.7	55.1±1.7	58.3±2.2	75.2±2.2*****	64.9±1.5	73.2±1.2*****	61.1±2.6	68.9±2.4*	87.03±1.83
Change (%)		7		29		13		13	
Vit. E (mg/100 ml)	0.557±0.02	0.582±0.01	0.549±0.091	0.596±0.016	0.526±0.013	0.597±0.01*****	0.565±0.02	0.623±0.03	0.663±0.013
Change (%)		5		9		13		10	
Vit. C (mg/100 ml)	0.623±0.019	0.678±0.015*	0.614±0.02	0.721±0.02*****	0.539±0.018	0.612±0.019***	0.676±0.029	0.77±0.023	0.819±0.025
Change (%)		9		17		14		14	
Uric acid (mg/100 ml)	5.9±0.292	5.1±0.226*	5.9±0.275	5.2±0.195**	6.3±0.275	5±0.203*****	5.8±0.372	5.3±0.23	4.1±0.166
Change (%)		-16		12		-21		-9	
Creatinine (mg/100 ml)	0.914±0.032	0.941±0.032	1.02±0.026	0.892±0.03****	1.05±0.033	0.883±0.02*****	1.01±0.038	0.874±0.21****	0.872±0.038
Change (%)		-3		13		-16		-14	
AST (IU/ml)	25.1±1.68	25.5±1.69	23.8±1.45	19.1±1.03**	25.9±1.20	23.3±1.09	26.3±1.53	22.6±1.41	16.6±0.913
Change (%)		-2		-20		-10		-14	
ALT (IU/ml)	28.9±1.59	29±1.50	29.8±1.18	24.8±1.003****	28.6±1.25	25.8±0.99	28.8±1.48	24.9±1.58	18.9±0.865
Change (%)		-1		-17		-10		-14	
Erythrocyte									
SOD (U/g Hb)	2710.9±68.8	2532.1±62.8	2779.9±89.3	2502.8±81.3*	2657.8±72.9	2431.2±56.8**	2681.4±54.1	2461.1±61.2***	2256±39.65
Change (%)		-7		-10		-9		-8	

Values statistically significant when after compared with before:
*p<0.05, **p<0.025, ***p<0.01, ****p<0.005, *****p<0.001

ω-3 fatty acids inhibited the cyclooxygenase and 5-lipoxygenase pathways of arachidonate metabolism resulting in eicosanoid inhibition (8). β-sitosterol which has been reported to present in *Nigella sativa* oil (43) has been shown previously to possess anti-inflammatory activity (44). ANOVA and Tukey tests (Table 4) showed non-significant difference between the effects of the different modes of treatment on erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor and prostaglandin E_2 i.e. no treatment was superior on the other.

Concerning the antioxidant state, erythrocyte superoxide dismutase activities was reduced significantly in *Nigella sativa* oil, primrose oil and fish oil groups ($p<0.01$, <0.025, <0.05 respectively), while a non-significant reduction was noticed in synthetic drugs group. Reduction of erythrocyte superoxide dismutase activities may result in decreased reactive oxygen species generation although erythrocyte superoxide dismutase is considered as antioxidant (45). When the increased superoxide dismutase activity in rheumatoid arthritis patients was not compensated by increase in glutathione peroxidase or catalase (46) there would be increased due to accumulation of superoxide ion. So the superoxide dismutase reducing effect of the natural oil in the present study has a beneficial effect in reducing the oxidation stress of patients. Plasma copper showed significant reduction in all patients' groups after treatment with fish oil, *Nigella sativa* oil, synthetic drugs groups ($p<0.001$) and primrose oil group ($p<0.005$). There were significant increases in plasma zinc in fish oil, primrose oil and *Nigella sativa* oil groups ($p<0.001$, <0.001, <0.05 respectively), while that of synthetic drugs group increased non-significantly. Copper and zinc are not antioxidant until they are incorporated into the antioxidant enzyme (superoxide dismutase). Plasma vitamin E was increased non-significantly in fish oil, *Nigella sativa* oil and synthetic drugs groups, while significantly increased in primrose oil group ($p<0.001$). Significant increases in plasma vitamin C were observed in fish oil, primrose oil and synthetic drugs groups ($p<0.001$, <0.01, <0.05 respectively). *Nigella sativa* group showed a non-significant increase in plasma vitamin C. Plasma uric acid was reduced sig-

Table 4: Changes of different biochemical parameters (mean±SE) (These values were obtained when data before treatment were subtracted from those after treatment) of rheumatoid arthritis patients after administration of the different natural oils or synthetic drugs.

Parameters	Synthetic drugs	Fish oil	Primrose oil	*Nigella sativa* oil	ANOVA p values
ESR (mm/h)	-13±1.809	-17.4±1.809	-16±1.661	-15±1.551	NS
Serum					
CRP (mg/l)	-3.6±1.357	-3.6±1.616	-4.3±2.045	-2.1±1.078	NS
RF (IU/ml)	-2.86±1.982	0	0	0	NS
Plasma					
PGE_2 (pg/ml)	-150±17.42	-160±15	-90±10.856	-66.3±6.798	NS
Cu (μg/dl)	-8.79±0.649	-23.9±2.33	-11.9±1.179	-13.6±1.266	0.000068**
Zn (μg/dl)	3.83±0.647	16.9±1.76	8.32±1.406	7.8±0.736	0.000006**
Vit. E (mg/dl)	0.025±0.007	0.078±0.016	0.074±0.024	0.059±0.018	0.049
Vit. C (mg/dl)	0.055±0.012	0.109±0.015	0.071±0.011	0.074±0.019	NS
Uric acid (mg/100ml)	-0.823±0.119	-0.75±0.121	-1.36±0.182	-0.445±0.337	NS
Creatinine (mg/100ml)	0.27±0.022	-0.132±0.024	-0.164±0.025	-0.141±0.031	0.00003**
AST (U/l)	0.357±0.799	-5±0.901	-2.61±0.464	-3.61±0.698	0.003*
ALT (U/l)	0.143±0.822	-4.96±0.948	-2.79±0.770	-3.86±0.473	0.001*
Erythrocyte					
SOD (U/gHb)	-178.6±17.57	-281.9±38.23	-226.6±26.86	-220.3±18.54	NS

Values statistically significant: * $p<0.05$, ** $p<0.01$, NS: Non-significant

nificantly in fish oil, primrose oil and synthetic drugs groups ($p<0.025$, <0.001, <0.05 respectively). Non-significant reductions were noticed in *Nigella sativa* oil group. Uric acid has an antioxidant activity in normal level (47), however nothing was reported about this activity when elevated over normal so the reduction towards normal level may have a beneficial effect as antioxidant. When applying the ANOVA and Tukey test on the plasma antioxidant levels, no significant differences were shown between the different treatments.

In the present study 100 mg vitamin E was given to each patient as daily oral dose concomitantly with the natural oils to optimize the beneficial anti-inflammatory effect of polyunsaturated fatty acids and minimize lipid peroxidation and free radical generation. It was reported that ingestion of ω-3 polyunsaturated fatty acids without adequate antioxidant protection could result in increased free radical formation and lipid peroxidation (48, 49), and decreased antioxidant (especially vitamin E) leading to reduction in T cell-mediated function, natural killer cell activity, and macrophage cytotoxicity. These risks associated with the intake of ω-3 polyunsaturated fatty acids may be minimized without compromising its beneficial effects by the intake of appropriate levels of antioxidants such as vitamin E. Moreover, some authors suggested that the beneficial effects of fish oil could be enhanced by the addition of 500 IU of vitamin E in the diet (50).

The study of DeLa-Cruz *et al.* (51) showed that dietary supplementation with evening primrose oil decreased tissue oxidative stress, through reduction of lipid peroxidation where glutathione was increased in all tissues and percentage of oxidized glutathione decreased since the oil increased the activities of glutathione reductase.

The inhibition of oxidative stress by the oil of *Nigella sativa* is expected from its content of thymoquinone and polyphenol and extra tocopherol that were reported to be associated with *Nigella sativa* oil (43).

Improving the state of rheumatoid arthritis due to anti-eicosanoid activity and protein anti-denaturant effect of natural oils may lead to consequent reduction of reactive oxygen species generation and simultaneous increase of antioxidant state. In addition administration of vitamin E concomitantly with the natural oil elevate plasma vitamin E level and consequently vitamin C level that will not be consumed in regeneration of vitamin E involved in breaking the free radical chain. Moreover during improvement of the disease, vitamin C involved in collagen resynthesis will diminish leading to increasing its plasma level.

Trace elements (copper and zinc) showed restoration towards normal level after natural oils supplementation due to improved disease state since the cause affecting their changes (as reported previously) (25) were diminished. ANOVA and Tukey test showed fish oil to be superior in reducing plasma copper and elevating plasma zinc ($p=0.000068$ and 0.000006 respectively).

A significant decrease in plasma creatinine was noticed in fish oil, primrose oil and *Nigella sativa* oil groups ($p<0.005$, <0.001, <0.005 respectively), while synthetic drugs group showed a non-significant increase in plasma creatinine. ANOVA and Tukey test showed primrose oil to have superiority in reducing creatinine plasma level compared with other natural oils (p 0.00003). Plasma activities of aspartate transaminase and alanine transaminase decreased significantly in fish oil group ($p<0.025$, <0.005 respectively). Non-significant decreases were noticed in primrose oil and *Nigella sativa* oil groups. Synthetic drugs group showed a non-significant increase in plasma aspartate transaminase and alanine transaminase. The study of El-Dakhakhny *et al.* (52) showed that daily administration of *Nigella sativa* oil did not adversely affect the serum transaminase (ALT and AST), alkaline phosphatase, serum bilirubin or prothrombin activity. In the present study ANOVA and Tukey test showed that fish oil was the best in reducing plasma aspartate transaminase and alanine transaminase activities compared with the other natural oils (p, 0.003 and 0.001, respectively).

These results are of beneficial effect since in a previous study (25) we have noticed that the rheumatoid arthritis patients (baseline) have elevated serum level of creatinine, aspartate transaminase, alanine transaminase which might be due to either the disease itself or the synthetic drug used in the treatment. It was reported that methyl prednisolone therapy in rheumatoid arthritis produced elevation in alanine amino transferase (53). Previous studies have shown significant elevation of serum activities of transaminases and creatinine level associated with methotrexate therapy in patients with chronic inflammatory diseases (54-56).

It is worthy to mention that in the present study the duration of morning stiffness of joints has been diminished after natural oils' administration, which was 19.5 ± 1.529 min at the start of study and became 14.5 ± 1.295 min after natural oil administration. Morning stiffness occurred due to synovial congestion, effusions and thickening of joint capsules, its duration is considered an index of activity of the disease (57).

Rheumatoid arthritis patients (when foot is affected) suffered difficulty in walking and standing. The maximum time of walking in the present study was noticed to be 36.6 ± 1.386 min at the start and became 46.8 ± 0.899 after oils' treatment. Dietary supplementation with fish oil was shown previously to improve clinical parameters of disease activity including the number of tender joint and morning stiffness (58-61). It has been also reported that supplementation of human diet with dihommo-δ-linolenic acid attenuates clinical symptoms of chronic inflammatory disorders including rheumatoid arthritis (62).

In the current study gastrointestinal tract disturbances of rheumatoid arthritis patients (as side effects of synthetic drugs), have been improved after stopping the synthetic drugs and starting the administration of natural oils.

CONCLUSION

Fish oil, primrose oil and *Nigella sativa* oil have anti-inflammatory activity and can be used with successful results in rheumatoid arthritis patients. Vitamin E (as antioxidant) might essentially have provided in adjunct with the natural oils to optimize the beneficial anti-inflammatory effects of the polyunsaturated fatty acids. Liver and kidney functions were improved during natural oil treatments.

On recommendation, fish oil, primrose oil and *Nigella sativa* oil (with vitamin E) are recommended to be given in rheumatoid arthritis patients. These natural oils might be given alone or during treatment with synthetic drugs to permit reduction of dose level of the later, so as to minimize their side effects. We recommend prospective studies on the use of these natural oils either alone or in conjunction of different anti-inflammatory synthetic drugs (which may have synergistic effects with each other) for longer periods of time in rheumatoid arthritis patients.

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Correspondence:
Sahar Y. Al-Okbi
Department of
Food Sciences and Nutrition,
National Research Centre,
Dokki, Cairo,
EGYPT.
e-mail:S_Y_alokbi@hotmail.com