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Investigation of the immunomodulatory, mitigating and cell reinforcement exercises of night primrose oil in adjuvant-actuated joint inflammation in rodents

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Abstract

The present study was carried out to evaluate the anti-arthritic nature of evening primrose oil (EPO) rich in -linolenic acid (GLA), on adjuvant -induced arthritic rats. Results were compared to those of diclofenac sodium, a reference standard non-steroidal anti-inflammatory drug. Arthritis was induced by subcutaneous injection of complete Freund's adjuvant (CFA) in the right hind paw of male Wistar rats. EPO (5 g/kg b.w.) was orally administered from day 0 till day 28, while diclofenac sodium (5 mg/kg b.w.) was orally given from day 10 to day 28 after adjuvant injection. In the arthritic group, the results revealed significant decrease in body weight together with increased spleen weight, increase in serum immunoglobulin G (IgG), immunoglobulin M (IgM) and tumor necrosis factor- (TNF-) levels whereas serum interleukin-4 (IL-4) level was decreased. Serum N-acetyl- -D-glucosaminidase (NAG) and -glucuronidase enzyme activities were also elevated. Splenic antioxidant status was suppressed as manifested by increased malondialdehyde (MDA) level, decreased glutathione (GSH) content along with decreased enzymatic activity of glutathione peroxidase (GSHPx), glutathione reductase (GR) and glutathione-S-transferase (GST). Moreover, the splenic activity of the pathophysiological enzymes, -glutamyltransferase (-GT) and lactate dehydrogenase (LDH) was enhanced. Oral administration of EPO exerted a significant elevation in serum IgG and IgM levels. In addition, normalization of body weight, serum IL -4 and TNF- levels together with serum NAG, -glucuronidase and splenic LDH enzyme activities were observed. It could be suggested that administration of plant oils rich in GLA may provide a way to modulate the inflammatory response through alteration of certain inflammatory indices.

Keywords: Adjuvant-induced arthritis, evening primrose oil, immunoglobulins, IL- 4, TNF- , rat.

INTRODUCTION

Adjuvant-induced arthritis (AIA) is an erosive autoimmune polyarthritis involving both humoral and cell mediated immune responses that resemble human rheumatoid arthritis (RA) (van Eden et al., 1998). It can be induced in rats by a single intra-dermal injection of complete Freund's adjuvant (CFA) (Pearson and Wood, 1959). Adjuvant-induced arthritis has been extensively studied as a model for T cell-mediated immune disease. Although, the initiating phase of AIA is mediated by T cells, and can even be induced by a single T cell clone, there is little doubt that, at the later stages of the disease, T cells are of little importance for the maintenance of inflammation. In this chronic phase the local synovial infiltrate in AIA is dominated by monocytes, macrophages and fibroblasts (van Eden et al., 1998).

It has been suggested that immune-stimulatory DNA sequences (ISS) may be a critical factor contributing to the chronicity of inflammation in chronic autoimmune arthritis. ISS or CpG motifs are palindromic sequences of unmethylated CpG dinucleotides that are present in bacterial DNA, but not in mammalian DNA. ISS form molecular patterns that are readily recognized by the innate immune system, resulting in a first-line defense against bacterial infections. They can stimulate the expression of co-stimulatory molecules and the production of cytokines such as interleukin-12 (IL- 12), tumour necrosis factor- (TNF-) and interferons by macro-phages, dendritic cells, B cells and natural killer cells (Krieg, 2000) and are capable of skewing an immune response towards a strong and prolonged T helper 1 (T_h1) type of immunity (Chu et al.,1997).

In rheumatoid joint, the majority of T cells products are derived from cells exhibiting the T helper 1 phenotype, including gamma interferon (IFN). T helper subset 2

(T_h2)- derived cytokines, including IL-4 and IL- 13, suppress T_h1 function as well as pro-inflammatory cytokine production. The rheumatoid joint is essentially devoid of T_h2 lymphokines, and T_h1/T_h2 imbalance has been suggested as a mechanism for disease perpetuation in RA (Miossec and van den Berg, 1997). In animal models of arthritis, increased endogenous T_h2 cytokine production was found to be correlated with disease resolution (Mauri et al., 1996) and administration of exogenous IL-4 or IL-10 was reported to ameliorate disease progression (Joosten et al., 1997). B cells proliferate and differentiate into immunoglobulin secreting plasma cells under the influence of a combination of cytokines derived from T cells (Finkelman et al., 1991).

Non-steroidal anti-inflammatory drugs (NSAID), steroidal agents and immunosuppressants are usually used for the treatment of rheumatoid arthritis. However, these drugs are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances. Therefore, efforts need to be made to seek therapeutic agents that can be used for long-term administration. A number of vegetable oils have been claimed to provide benefit in rheumatoid arthritis. Cleland et al. (1988) found improvement in the symptoms of RA in patients taking olive oil for 14 weeks. Improvement was also seen in RA patients consuming evening primrose oil (EPO), which is rich in -linolenic acid (GLA) (Brzeski et al., 1991). Morning stiffness in patients with RA has been reported to be significantly ameliorated after taking blackcurrant seed oil, which also contains GLA. Moreover, monocytes cultured from those patients exhibited a lower secretion of the inflammatory cytokines such as IL-1 , IL-6 and TNF- when compared with control subjects (Watson et al., 1993).

The present study was undertaken to investigate the immunomodulatory, anti-inflammatory and antioxidant activities of evening primrose oil in adjuvant-induced arthritis in rats.

MATERIAL AND METHODS

Chemicals

Complete Freund's adjuvant was purchased from Sigma –Aldrich chemical company (St. Louis, USA). CFA consists of 0.1% heat killed Mycobacterium tuberculosis suspended in mineral oil. Evening primrose oil and diclofenac sodium were provided by NOW FOODS (USA) and Novartis Pharma, respectively. All other chemicals were of analytical pure grade.

Experimental design

Adult male Wistar rats weighing 180 - 200 g were used in the experiment. The animals were allowed free access to water and food and kept under suitable laboratory conditions throughout the experimental period. After two weeks acclimatization period, rats were divided into four groups. One group served as a normal group. In the other three groups, arthritis was induced by subcutaneously injecting 0.25 ml of CFA in the palmar surface of the right hind paw (Piliero et al., 1966). The first group was kept without treatment for 28 days and served as control arthritic rats. The second group was given orally evening primrose oil (5 g/kg b.w.) (Matsuo et al., 1996), from day 0 till the end of the experiment. The third group was orally given diclofenac sodium (5 mg/kg b.w.), a reference standard non-steroidal anti-inflammatory drug, from the day 10 till the end of the experiment.

After the experimental period, the animals were weighed then sacrificed by cervical decapitation. Blood was collected and serum was separated and used for the estimation of serum immunoglobulins (IgG and IgM) levels using kits of Dade Behring Inc., using Behring nephelometer 100 (New York, USA). Serum levels of TNF- using rat TNF- ELISA kit (Euroclone, Viafigino, Italy) and IL-4 using EASIA kit (BioSource, Europe S.A.) were also deter-mined. In addition, serum activity of N-acetyl- -D-glucosaminidase (NAG) (Beaufay et al., 1974) and - glucuronidase (Fishman et al., 1947) activities were assessed.

The spleen of each rat was excised and rinsed in ice-cold saline, blotted dry and weighed. Two portions of the spleen were homogenized in 1.15% KCl and 5% sulfosalicylic acid, centrifuged at 1000 x g and the resulting supernatants were used for the assessment of splenic malondialdehyde (MDA) (Uchiyama and Mihara,1978) and glutathione (GSH) (Beutler et al., 1963) levels, respectively. Another portion was homogenized in Tris -EDTA buffer pH 7.6 and ultracentrifuged at 105,000 x g at 4 °C for 30 min. The resulting cytosolic fraction was used for the estimation of glutathione peroxidase (GSHPx) (Paglia and Valentine, 1967), glutathione reductase (GR) (Long and Carson, 1961), and glutathione-S-transferase (GST) (Gawai and Pawar, 1984) activities. Lactate dehydrogenase (LDH)(Buhl and Jackson,1978) and - glutamyltransferase (-GT) (Szasz, 1969) activities were also estimated in the same fraction using commercially available kits of Stanbio, San Antonio, USA, and QCA, Amposta, Spain, respectively. The protein content was measured by the method of Lowry et al. (1951).

Statistical analysis

Values are given as mean ± S.E.M. The level of statistical significance was taken at p 0.05 using one-way analysis of variance (ANOVA) followed by Tukey-Kramer's multiple comparison test to judge the difference between various groups.

RESULTS

Concerning CFA inoculation, a significant decrease in body weight and a significant increase in spleen weight were observed in arthritic rats which reached to 88 and 137% of their levels in normal rats, respectively (Table 1). Significant increase in serum immunoglobulins (IgG and IgM), TNF- levels , NAG and - glucuronidase activities were observed in arthritic rats that reached to 169, 162, 210, 150 and 245% as compared to their values in normal rats, respectively. On the other hand, serum IL-4 level was significantly decreased in arthritic rats that reached to 75% of its normal level (Table 2). A significant increase in MDA level with significant decrease in GSH level and GSHPx, GR and GST activities were observed in spleen of arthritic rats which reached 224, 56, 64, 56 and 53% of their normal values, respectively. Splenic - GT and LDH activities were shown to be significantly increased in arthritic rats amounting to 273 and 227% of their normal activities, respectively (Table 3).

Table 1. Body weight and spleen weight in arthritic rats treated orally with either evening primrose oil or diclofenac sodium.

	Spleen weight (g)	Body weight (g)
Normal rats	0.7±0.046	187.5±2.5
Arthritic rats	0.96±0.049[a]	165±2.31[a]
Arthritic rats treated with evening primrose oil	0.92±0.064 [a]	180.62±1.75[b]
Arthritic rats treated with diclofenac sodium	0.95±0.05 [a]	179.37±2.9[b]

Values expressed as mean ± S.E.M., n = 8 rats per group. [a]Significant difference from normal group at p<0.05; [b]significant difference from arthritic group at p<0.05.

Table 2. Serum levels of immunoglobulins (IgG and IgM), TNF- and IL-4 as well as serum activities of NAG and - glucuronidase in arthritic rats treated orally with either evening primrose oil or diclofenac sodium.

	Normal	Arthritic rats	Arthritic rats treated with evening primrose oil	Arthritic rats treated with diclofenac sodium
IgG (mg/ml)	2.55±0.25	4.3±0.2[a]	5.2±0.25[a,b]	4.2±0.15[a]
IgM (mg/ml)	0.26±0.02	0.42±0.03[a]	0.57±0.02[a,b]	0.4 ±0.03[a]
TNF- (pg/ml)	31.19±2.9	65.36±3.5[a]	35.42±3.09[b]	39.06±2.98[b]
IL-4 (pg/ml)	262.75±14.26	197.5±10.81[a]	272.37±8.13[b]	217±12.72 [a]
NAG (nmoles/min/ml)	35.7±3.17	53.71±4.24[a]	32.55±2.76[b]	30.41±2.67[b]
-Glucuronidase (U/ml)	1.23±0.12	3.01±0.32[a]	1.62±0.25[b]	1.67±0.2[b]

Values expressed as mean ± S.E.M., n = 8 rats per group. [a]Significant difference from normal group at p<0.05; [b]significant difference from arthritic group at p<0.05.

Regarding the effect of EPO treatment, restoration of body weight with no significant change in spleen weight were observed in EPO treated rats (Table 1). Normalization of serum TNF- and IL-4 levels along with NAG and - glucuronidase activities were observed in EPO treated rats. This effect was accompanied by significant elevation of immunogloubulins (IgG and IgM) as compared to arthritic rats which reached 121 and 136%, respectively (Table 2). EPO did not affect spleen antioxidant status, as compared to arthritic rats where MDA, GSH levels along with GSHPx, GR and GST activities were not significantly changed. Normalization of splenic LDH was detected while -GT activity was not altered in EPO treated rats as compared to its activity in arthritic rats (Table 3).

Data shown in Tables 1, 2 and 3 clarified that diclofenac sodium restored the body weight with no significant effect on spleen weight as compared to arthritic rats. Normalization of serum TNF- level, NAG and - glucuronidase activities were observed with no significant change in serum immunoglobulins (IgG and IgM) and IL-4 levels. Normalization of splenic LDH activity was observed with no significant change in the other splenic measured parameters.

DISCUSSION

Results of the present study showed a significant decrease in body weight along with a significant increase in spleen weight, IgG and IgM levels in the serum of arthritic rats when compared with normal group. Adjuvant-induced arthritis is an immune response to an antigen present on the capsule of the mycobacterium. Following adjuvant arthritis induction with CFA, rats not only develop arthritis but also systemic features of inflam-mation, such as uveitis, inflammation of the gastro-intestinal tract and body weight loss that starts 24 – 48 h before the clinical onset of arthritis (Prakken et al., 2003). The spleen is an important lymphoid organ involved in immune responses against all types of antigen that appear in the circulation and it provides a readily available source of cells known to be involved in adjuvant arthritis. Increased cellularity in the spleen of adjuvant injected rats engendered interest as to the potential for concomitant classical antibody formation where the increased antibody titre in arthritic animals further sup-ports the hyperimmune status by humoral immunity (Jerne et al., 1963).

Oxygen free radicals such as superoxide and hydrogen peroxide are produced by polymorphonuclear leukocytes when they ingest bacteria or immune complexes. In rheumatoid arthritis, it has been suggested that hydroxyl radical or a similar oxidizing species, contributes to membrane damage, alteration in the protein structure, conformation and antigenicity, production of autoantibodies and destruction of antioxidants within the synovial

Table 3. Splenic levels of MDA and GSH as well as activities of GSHPx, GR, GST, -GT and LDH in arthritic rats treated orally with either evening primrose oil or diclofenac sodium.

	Normal rats	Arthritic rats	Arthritic rats treated with evening primrose oil	Arthritic rats treated with diclofenac sodium
MDA (nmoles/mg protein)	2.6±0.31	5.83±0.51[a]	4.6±0.29[a]	4.38±0.50[a]
GSH mg/g tissue)	143.46±7.03	80.71±4.72[a]	104.23±8.45[a]	98.5±5.5[a]
GSHPx (U/mg protein)	105.75±6.19	67.53±5.1[a]	79.63±5.08[a]	72.38±6.53[a]
GR (U/mg protein)	43.06±3.85	23.97±2.13[a]	27.8±3.02[a]	24.82±1.82[a]
GST U/mg protein)	22.83±2.02	12.08±1.46[a]	16.33±1.54[a]	14.48±1.50[a]
-GT (U/mg protein)	2.08±0.22	5.68±0.47[a]	4.53±0.4[a]	4.95±0.46[a]
LDH (U/mg protein)	1.82±0.15	4.14±0.26[a]	2.61±0.26[b]	2.41±0.29[b]

Values expressed as mean ± S.E.M., n = 8 rats per group. [a]Significant difference from normal group at $p<0.05$; [b]significant difference from arthritic group at $p<0.05$.

joints (Gutteridge, 1986). Many cellular defense mechanisms are presented against the toxic effect of these radicals in inflammation including serum sulfhydryl groups, ceruloplasmin, and blood glutathione (Fahim et al., 1995). In the current study, the depressed splenic antioxidant status manifested by the significant increase in MDA and decrease in GSH levels along with reduction in GSHPx, GR and GST activities is found to be in harmony with a recent study of Rasool and Varalakshmi (2007). The authors referred such result to the weak free radical defense system against oxidative stress that might explain the pathogenic state associated with arthritis.

The present study showed a significant increase in splenic activity of lactate dehydrogenase, which is one of the cytoplasmic cellular enzymes that serve as indicator suggestive of disturbances of the cellular integrity induced by pathological conditions. In addition, it has been reported that increased splenic LDH may be an index of natural killer activity of spleen cells (Jain and Stevenson, 1991). -GT is an enzyme with a conside-rable role in inflammation and its enhanced activity has been described during AIA (Singh et al., 1986) as well as in RA (Karp et al., 1999). Bauerova et al. (2006) reported that -GT activity in tissues such as spleen and joints could provide a simple and inexpensive marker for AIA development at systemic as well as local level. -GT has been implicated in cell repair and growth, where it catalyses the resynthesis of GSH involved in antioxidant processes (Dominici et al., 1999). In the present study, its elevated activity in the spleen of arthritic rats was perhaps due to the persistent need for the enzyme to cope with continuing oxidative stress.

One of the characteristic features of AIA in rats is the correlation between the development of inflammatory process and the release of lysosomal enzymes into the extra-cellular compartment (Weissman,1972). Lysosomes are membrane enclosed cytoplasmic organelles, containing many hydrolytic enzymes such as NAG and -glucuronidase. Lysosomal enzymes are responsible for intracellular breakdown of complex macromolecules and degradation of endothelial membrane glycoconjugates. The significant elevation in serum NAG and -glucuronidase activities observed in our study in arthritic rats is found to be in harmony with the results of Narendhirakannan et al. (2007). The altered enzyme activities in arthritis can be regarded as an index of lysosomal enzyme activation occurring in response to metabolic need of degrading various constituents of cells such as mucopolysaccharides and glycoproteins accumulated in tissues due to arthritis associated with vasculopathies (Naparstek et al., 1984).

Adjuvant-induced arthritis involves a single injection of CFA into an area of potent lymphatic drainage in susceptible rats that results in arthritis-like symptoms for many weeks in distal joints. The infiltration of leukocytes into the synovial fluid and tissues is a hallmark of chronic joint inflammation. Initiation of a “flare” or reactivation of the inflammatory reaction in arthritic disease has been attributed to activation responses of polymorphonuclear neutrophils (PMNs) recruited into the joint space by local production of inflammatory cytokines such as TNF- and IL-1 in response to an antigen driven immune complex deposition (Greenwald, 1991). Neutrophils can then contribute to joint damage by the production of reactive oxygen metabolites and the production of cytokines that further amplify the inflammatory response by their effects on lymphocytes and macrophages (Nurcombe et al., 1991). Results of the current study of increased TNF-and decreased IL-4 in the serum of arthritic rats is found to be in accordance with the previous studies of Cai et al. (2006) and Bush et al. (2001), respectively.

IL-4 is a pleiotropic cytokine that plays a number of important roles including the regulation of inflammation. IL-4 acts on lipopolysaccharide (LPS)-stimulated monocytes *in vitro* to down-regulate the production of the inflammatory cytokines TNF- , IL-1 , IL- 6 and IL-8 (Brown and Hural, 1997). Complementary to its anti-inflammatory properties on monocytes, IL-4 inhibits RA synoviocyte proliferation and production of prostaglandin

E_2 (PGE_2) (Dechanet et al., 1995). IL-4 enhances monocyte apoptosis, thereby decreasing monocyte accumulation (Mangan et al., 1992), and acts as an autocrine growth factor promoting the differentiation of naive T cells to Th_2 cells (Brown and Hural, 1997). The immunoregulatory Th_2 cytokines such as IL-4 has been reported to be deficient in RA (Feldmann et al., 1996) and in AIA (Ayer et al., 2000). Furthermore, Boyle et al. (1999) demonstrated that intra-articular IL-4 gene therapy caused an increased level of IL-4 in the injected paw along with the serum with consequent reduction in paw swelling and decreased radiographic evidence of bone destruction.

Epidemiologic and experimental data indicate that changes in the source of lipid consumed in the diet may modify the fatty acid composition of many cell types, including those involved in the development of many inflammatory and immunologic diseases (Zurier, 1993; James et al., 2000). The dietary essential fatty acid linoleic acid (LA) is metabolized within the body, giving rise to -linolenic acid (GLA, 18:3, n-6) and dihomo- -linolenic acid (DGLA, 20:3, n-6). The use of evening primrose oil as a source of GLA has been investigated, and several clinical applications on arthritis and immunologic diseases have been described (Zurier et al., 1996; Rosenstein et al., 2003). The rationale of its therapeutic use is based on the high amount (9%) of -linolenic acid, which is metabolized into dihomo- -linolenic acid (Kunkel et al., 1982). DGLA itself cannot be converted to leukotrienes (LTs) but can form a 15-hydroxyl derivative that blocks the transformation of arachidonic acid to leukotrienes. Thus, increased DGLA intake may allow DGLA to act as competitive inhibitor of the 2-series PGs and the 4- series LTs and thus suppress inflammation (Voorhees, 1983).

In the current study, intake of EPO in arthritic rats caused normalization of body weight as compared with the control arthritic group. Kunkel et al. (1982) reported that, in rats fed on borage oil (rich in GLA), the accumulation of DGLA was marked in both liver phospholipids and spleen total lipids .DGLA is the natural precursor of the 1-series prostaglandins and it has been reported that oral administration of EPO elevated the tissue PGE_1 level that possess anti-inflammatory activity by suppressing LTB_4 synthesis. Kaku et al. (2001) demonstrated that LTB_4 production by peritoneal exudate cells was significantly suppressed in rats fed diet enriched with GLA. Moreover, immunoglobulin productivity in mesentric lymph node (MLN) lymphocytes was promoted by dietary GLA suggesting a strengthening of gut immune system and amelioration of allergic reactions. Such effect might help in the explanation of body weight normalization that has been observed in the present study.

The current study demonstrated a significant increase in serum IgG and IgM levels in arthritic rats fed EPO whereas no amelioration of the splenic antioxidant status when compared with control arthritic rats. Immunoglobulin production is regulated by T lymphocytes, and T cells are classified into CD4 and CD8 positive T cells having helper and suppressive functions, respectively where CD stands for "cluster of differentiation", a historical term that was coined to define cell-surface molecules that are recognized by a given set of monoclonal antibodies (Sprent, 1993). Matsuo et al. (1996) showed that the relative proportion of $CD4^+$ T cells in the spleen lymphocytes significantly increased in rats fed EPO, but the $CD8^+$ T cell population remained unchanged where the ratio of $CD4^+/CD8^+$ T cells was therefore increased. They also demonstrated that dietary EPO increased serum total Igs in an early phase after immunizing rats with intraperitoneal ovalbumin. Hung et al. (1997) demonstrated that polyunsaturated fatty acids (PUFAs) enhanced IgE production by the rat spleen and MLN lymphocytes. Since the elevation of IgE level was inhibited in the presence of lipophilic antioxidative -tocopherol (Hung et al., 1997), but not in the presence of hydrophilic ascorbic acid (Yamada et al., 1996), the oxidation of PUFAs under lipophilic circumstances such as the cell membrane seems to be essential for enhanced IgE production suggesting that lipid peroxidation is partly responsible for the enhancement of IgE level induced by PUFAs (Hung et al., 1997).

$CD4^+$ T_h lymphocytes can be divided into two functionally distinct T_h cell types, based on their cytokine secretion patterns. T_h1 cells produce IL- 1 and IFN- and are involved in cell-mediated immune responses and tissue injury in many organ-specific autoimmune diseases, whereas T_h2 cells secrete IL-4, IL-5, IL-10 and IL-13, and are responsible for T cell-dependent antibody responses and allergic reactions (Mosmann and Sad ,1996). There is cross–regulation between these cellular subsets with IFN- inhibiting T_h2 cells and IL -4 / IL-10 inhibiting T_h1 cells (Morel and Oriss, 1998). In the present study, normalization of serum TNF- and IL-4 levels was observed as a result of administration of EPO to arthritic rats. Dietary GLA has been reported to suppress spleen lymphocytes proliferation (Peterson et al., 1999) and influence cytokine actions such as sup-pression of IL-2 and TNF- production in human blood lymphocytes (Purasiri et al., 1997). Kavanagh et al. (2004) demonstrated that the LPS-induced decrease in hippocampal concentration of anti-inflammatory cytokines IL-10 and IL-4 are blocked in rats treated with GLA .It is possible that GLA anti-inflammatory action is linked to its ability to up regulate expression of peroxisome proliferator-activated receptors gamma (PPAR), which are ligand-dependent transcription factors (Panigrahy et al., 2005). Natural PPAR ligands were discovered, which include PUFA and essential fatty acids, such as linoleic and linolenic acids (Kliewer et al., 1997) . Anti-inflammatory effects of PPAR ligands have been reported in animal models of arthritis (Cuzzocrea et al., 2003) and inflammatory bowel disease (Sanchez-Hidalgo et al., 2005). PPAR activation can produce anti-

inflammatory effect via downregulating the expression of transcription factors including nuclear factor kappa B(NF-B) which govern the expression of pro-inflammatory cytokines such as TNF- and IL- 1 (Alarcon de la Lastra et al., 2004). In addition, Vale et al. (2003) demonstrated that IL-4 significantly inhibited the release of TNF- by mice peritoneal macrophages obtained after intraperitoneal injection of zymosan.

Regarding the effect of dietary EPO on cellular enzymes, the data presented indicated a normalization in serum activities NAG and -glucuronidase along with splenic LDH activity. It has been shown that dietary EPO has the ability to elevate PGE1. The biological effects of PGE1 in various cells are mediated by activation of adenylate cyclases with subsequent elevation of intracellular cyclic adenosine monophosphate (cAMP) levels (Owen, 1986). The increase in intracellular cAMP was reported to reduce the release of lysosomal enzymes (Belch and Hill, 2000). Moreover, western blot analysis showed that NAG synthesis was repressed by increasing the levels of intracellular cAMP (Silva et al., 2004).

In fact, oral supplementation of EPO showed homologous effects on the biochemical parameters studied as compared to the administration of diclofenac sodium with few qualitative and quantitative distinctions.

As reported from previous studies, there is no doubt that dietary manipulation of fatty acids levels cause different immune responses including immunoglobulin and inflammatory mediators production. With the increasing evidence that GLA play a pivotal role in disease process underlying arthritis and other inflammatory disorders, dietary control of fatty acid intake would be expected to modify the disease progression and provide a useful adjunctive strategy in the treatment of these disorders. However, exact mechanisms causing these responses warrant further investigations.

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