The potential anti-inflammatory effect of Omega-3 polyunsaturated fatty acids on experimentally induced ulcerative colitis in rats

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1. INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic frequent inflammatory disorders recognized by the development of intestinal inflammation produced from the transmural infiltration of neutrophils, macrophages, lymphocytes and mast cells, eventually giving rise to mucosal disruption and ulceration (Masoodi et al., 2013). IBD represents an increasing public health interest due to increasing incidence worldwide, according to recent reports, there is an increase in the global incidence of Ulcerative Colitis (UC). Currently, its propagation in Europe and North America is 24.3 and 19.2 per 100,000 individuals, respectively, and 6.3 per 100,000 people in Asia and Middle East. Most patients improve UC between the ages of 15 and 30 years, although individuals aged 50–70 years consider as another prospect risk group (Molodecky et al., 2012). Ulcerative colitis is IBD in which the inflammation is in superficial layers of the intestinal mucosa, typically ascending in a continuous manner from the rectum and can expand to the whole colon (pancolitis). Clinical features of this disease are abdominal pain or cramping, diarrhea, fatigue, fever, weight loss and anorexia, loss of appetite and nutrients deficiency (Tatiya-aphiradee et al., 2017). Many components of the mucosal immune system are involved in the pathogenesis of IBD and contain intestinal epithelial cells, innate lymphoid cells, cells of the innate (macrophages/monocytes, neutrophils, and dendritic cells) and adaptive (T- and B-cells) immune system, and their secreted mediators (cytokines and chemokines) (Wallace et al., 2014).

Acetic acid-induced colitis is usually utilized and easily an inducible model that is much like human IBD in terms of pathogenesis and inflammatory mediator profile (Hartmann et al., 2012).

MPO, a heme-containing peroxidase in myeloid cells (PMNs and monocytes), has a vital role in the expansion of the inflammatory response. A considerable growing in the
activity of MPO in the colon tissues was recorded in acetic acid induced ulcerative colitis in rats which may reached to six-fold higher than normal (Rezayat et al., 2018). L-Malondialdehyde (L-MDA), the end product of lipid peroxidation, is a good marker of free radical–mediated injury and oxidative stress (Ozturk et al., 2015). The concentration of serum MDA is elevated in colitis induced in rats by acetic acid. The lower concentration of antioxidants in the colitis group with additional increase in MDA and hydroperoxide levels which are indicator for the tissue injury.

Catalase is a key enzyme in the metabolism of hydrogen peroxide (H₂O₂) and reactive nitrogen species, and its expression and localization is significantly change in this diseases (Glorieux and Calderon, 2017). Because of the importance of excess and uncontrollable Reactive Oxygen Species (ROS) in the pathogenesis of ulcerative colitis, the value to determine free radical scavenger such as reduced Glutathione. The endogenous antioxidant GSH was reduced significantly in the colonic tissues of the acetic acid-induced ulcerative colitis in rats (Karakoyun et al., 2018).

Colon Caspase-3 levels were higher in the colitis than in the control group rats (Akcan et al., 2008). Caspases (cysteine aspartic acid proteases) are a family of cysteine peptases that have vital roles in apoptosis, development and inflammation (Marton et al., 2009). Caspase-3 has an important function in this cascade, via activation of cytoplasmic DNase, which in turn migrates to the nucleus and fragments the DNA. So, DNA fragmentation especially when it is inter-nucleosomal is one of the gold standards for detection of apoptosis, therefore, Colon caspase-3 levels were higher in the colitis than in the control group rats. The B-cell lymphoma-2 (Bcl-2) family of proteins are key regulators of programmed cell death (Chipuk et al., 2010). They minimized mitochondrial membrane permeability and inhibit the release of cytochrome C into the cytoplasm (Orenius et al., 2007). Studies indicate that an increment in Bcl-2 level in acetic acid induced ulcerative colitis in rats when measured immunohistochemically (Aksit et al., 2016).

Cytokines are small signal molecules, released by immune cells, facilitate communication between cells. Cytokines increase or down regulate different cell types in an autocrine or paracrine way. Cytokines can be functionally divided into pro-inflammatory, such as IL-1β, tumor necrosis factor (TNF)-α and IL-6, and anti-inflammatory, like IL-10 and transforming growth factor (TGF)-β (Sanchez-Munoz et al., 2008). IL-1β secreted from activated macrophages activates formation of other cytokines, arachidonic acid metabolites, and proteases by intestinal macrophages, neutrophils, smooth muscle cells, fibroblast, and epithelial cells. A key role of IL-1β is its ability to stimulate IL-2 and IL-2 receptor on T lymphocytes. IL-1β appears to be a stimulator of diarrhea, the major symptom of intestinal inflammation. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most effective Omega-3 (n=3) fatty acids found in oily fish and fish oil supplements. These fatty acids are able to partly inhibit a number of aspects of inflammation including leukocyte chemotaxis, adhesion molecule expression and leukocyte–endothelial adhesive interactions, production of eicosanoids like prostaglandins and leukotrienes from the Omega-6 (n=6) fatty acid arachidonic acid, production of inflammatory cytokines, and T-helper 1 lymphocyte reactivity (Calder, 2015). Among the possible mechanisms underlying the anti-inflammatory effects of omega-3 PUFAs are the diversion of eicosanoid synthesis to non-inflammatory products, the production of anti-inflammatory lipid mediators (resolvins and protectins), the inhibition of nuclear factor-κB (NF-κB) activation, and the induction of peroxisome proliferator activated Receptor-γ (PPAR-γ) synthesis in dendritic cells (Yates et al., 2014). Both animal and clinical work suggest that omega 3-fatty acids are anti-inflammatory in their action and so have the potential to be of use in the treatment of inflammatory bowel disease (IBD) (Farrukh and Mayberry, 2014). Accordingly, the purpose of the present study was to investigate the effect of Omega-3 against acetic acid induced colitis in rats. Also, to determine whether Omega-3 when administered to ulcerative colitis induced-rats would attenuate the oxidative stress in colon tissue, beneficial for prevention and treatment of colitis complications.

2. MATERIAL AND METHODS

2.1. Experimental animals

Forty male albino rats, 12-16 weeks old and average body weight 220-250 g were used in the experimental assessment of this study. Rats were obtained from Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University. Animals were kept in individual metal cages, and fresh and clean drinking water was supplied ad-libitum. Rats were kept at constant environmental and nutritional conditions during the period of experiment. The animals were kept 14 days for acclimatization before the beginning of the experiment.

2.2. Chemicals and drugs:

The chemicals and drugs used in the present study were: Omega-3 (fish oil) fatty acids (Puritan’s Pride, INC, Holbrook, NY 11741, U.S.A). Omega-3 fatty acids present in a soft gelatin capsulated form with 1360 mg in each capsule. Ester-omega fish oil 1360 mg provides 950 mg of total fatty acids comprising of Eicosapentaenoic acid (EPA) 625 mg, Docosahexaenoic acid (DHA) 244 mg and other fatty acids. Omega-3 fatty acids were dissolved in propylene glycol and administered orally in a daily dose of 300 mg/kg body weight using stomach tube. Dose of omega-3 fatty acids was chosen to be within the therapeutic range levels reported in the pamphlet according to (Paget and Barnes, 1964).

Glacial acetic acid was manufactured by Merck Millipore, Darmstadt, Germany.

2.3. Induction of ulcerative colitis:

After 36 hours of fasting, the rats were anesthetized by intra-peritoneal injection of sodium thiopental (500 mg dissolved in 12.5 ml of normal saline) at the dose level of 0.2 ml/200gm body weight (40 mg/kg b.wt i.p.). To induce colitis, the rats were intra-colonially treated with 3 ml of (3% v/v) glacial acetic acid via a cannula that was placed 8cm proximal to the anus using a rectally inserted flexible polyethylene catheter. Rats were positioned head-down for 2-3 minutes to preclude immediate anal leakage of the instillate and thereafter returned to their cages with access to food and water ad libitum. Also, 0.5ml physiological saline was intra-colonially administered to the rats in the control group via a cannula according to (MacPherson and Pfeiffer, 1978).
2.4. Experimental design:
Rats were randomly divided into five equal groups, 8 rats each, placed in individual cages and classified as follow:

- **Group I**: (Control Normal group): Rats received no drugs, served as control non-treated for all experimental groups.
- **Group II**: (Early Ulcerative colitis-induced group): Rats received glucic acid (2ml/rat) at first day of experiment for 3 successive days, and the rats were sacrificed after 21 days from induction.
- **Group III**: (Early Ulcerative colitis+ Omega-3 protected group): Rats received Omega-3 (300 mg /kg b.wt) orally for 21 days, then ulcerative colitis was induced by 3% acetic acid (v/v) on day 21st for 3 successive days and on day 24th the animals were sacrificed and the samples were taken.
- **Group IV**: (Late Ulcerative colitis - induced group): Rats received glucic acid (2ml/rat) at first day of experiment for 3 successive days, and the rats were sacrificed after 21 days from ulcerative colitis-induction.
- **Group V**: (Late Ulcerative colitis + Omega-3 treated group): in this group the ulcerative colitis was induced in rats by 3% acetic acid (v/v) for 3 successive days, then rats received Omega-3 (300 mg /kg b.wt) orally for 21 days, and on day 24th the animals were sacrificed and the samples were taken.

2.5. Sampling:
Colonic tissue specimen was collected at the end of experiment on day 24th for all animal groups (control and experimental groups).

2.5.1. Colon tissue for biochemical analysis:
After 21 days of treatment with Omega-3 the rats of each group were sacrificed by cervical decapitation. The abdomen was opened and the colon specimen was quickly removed and opened using a scraper gently, cleaned by rinsing with ice-cold isotonic saline to get rid of any blood cells, clots and scraps of food, then it between 2 filter papers and quickly stored in a deep freezer at (-20 °C) for further biochemical analysis. Briefly, colon tissues were divided into appropriate portions, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 rpm for 15 min at 4 °C then the resultant supernatant was used for the determination of Hepatic peroxidase (MPO), L-Malondialdehyde (L-MDA), Catalase (CAT), reduced glutathione (GSH) and Myeloperoxidase (MPO) were determined according to the methods described by Mesbah et al. (2004), Xu et al. (1997), Patterson and Lazarow (1955), Shi et al. (2005), and Qiu et al. (2015), respectively.

2.6. Molecular analysis:
RNA was isolated from colon tissue of rats using RNeasy Mini Kit (Thermo Qiagen, #74104) according to the manufacturer’s protocol. Following determination of RNA concentration and purity by Quawell nanodrop Q5000 (USA), 5 mg of total RNA from each sample was reverse transcribed using Quant script reverse transcriptase. The produced cDNA was used as a template to determine the relative expression of Caspase-3, Bcl-2 and IL-1β using Step One Plus real time PCR system (Applied Biosystem, USA) and gene specific primers. The reference gene, β actin, was used to calculate fold change in target genes expression. The thermal cycling conditions, melting curves temperatures, and calculation of relative expression was done. For the treated groups, assessment of 2-ΔΔCt determined the fold change in gene expression relative to the control.

2.7. Statistical Analysis:
For biochemical analysis, the obtained data were analyzed and graphically represented using the statistical package for social science (SPSS, 13.0 software, 2009), for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences between groups. Duncan’s test was used for making a multiple comparison between groups for testing the inter-grouping homogeneity. However, for molecular analysis the difference between the groups was determined by One-way ANOVA using Graph Pad Prism 5 (Graph Pad Software, Inc., LaJolla, CA, USA). Comparison of means was carried out with Tukey’s Honestly Significant Difference (Tukey’s HSD) test. Data were presented as mean SEM (standard error of mean) and significance was declared at P < 0.05.

3. RESULTS
Effect of Omega-3 administration on colon tissue L-MDA, CAT, GSH and MPO of acetic acid-induced ulcerative colitis in male rats is presented in table (1). The obtained results showed significant increase in L-MDA concentration and MPO activity with marked decrease in CAT activity and GSH concentrations in colon of acetic acid -induced...
ulcerative colitis in rats as compared with control normal group. However, a significant depletion of colon tissue L-MDA, MPO activity and marked increase in CAT activity and GSH concentration were observed after omega-3 administration compared to colitis untreated rats. Effect of Omega-3 administration on the relative mRNA expression of IL-1β, Caspase-3 and Bcl-2 in colon of acetic acid -induced ulcerative colitis in rats is shown in table (2). The obtained qPCR results revealed a significant up-

regulation of IL-1β and Caspase-3 and a significant down-regulation of Bcl-2 gene expression levels in colon of acetic acid-induced ulcerative colitis in rats (G2 and G4) as compared to control normal group (G1). This expression was significantly down-regulated with significant up-regulation in Bcl-2 in colon tissues following administration of Omega-3 either before (G2) or after (G4) induction of UC, with lower expression in preventive group (G3).

Table 1 Effect of omega-3 administration on colon tissue MPO, L-MDA, CAT and GSH in acetic acid -induced ulcerative colitis in male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal groups</th>
<th>MPO (mU/mg tissue)</th>
<th>L-MDA (µmol/g tissue)</th>
<th>CAT (mU/min/mg tissue)</th>
<th>GSH (µg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Normal control</td>
<td>0.03 ± 0.005</td>
<td>2.86 ± 0.89</td>
<td>11.30 ± 5.11</td>
<td>12.38 ± 4.64</td>
<td></td>
</tr>
<tr>
<td>Group II: (early UC-induced group)</td>
<td>0.50 ± 0.030</td>
<td>8.25 ± 3.43</td>
<td>19.28 ± 1.04</td>
<td>1.00 ± 0.040</td>
<td></td>
</tr>
<tr>
<td>Group III: (early UC+ Omega 3 protected group)</td>
<td>0.18 ± 0.014</td>
<td>4.56 ± 1.22</td>
<td>6.24 ± 1.03</td>
<td>5.19 ± 0.27</td>
<td></td>
</tr>
<tr>
<td>Group IV: (late UC-induced group)</td>
<td>0.67 ± 0.037</td>
<td>9.36 ± 2.60</td>
<td>12.61 ± 0.94</td>
<td>0.45 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Group V: (late UC+ Omega 3-treated group)</td>
<td>0.27 ± 0.028</td>
<td>51.42 ± 1.47</td>
<td>31.87 ± 2.13</td>
<td>1.91 ± 0.11</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as (Mean ± S.E). S.E = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 2 Effect of omega-3 administration on the relative expression of caspase-3, Bcl-2 and IL-1β gene in colon tissue of acetic acid -induced ulcerative colitis in male rats.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Caspase-3</th>
<th>Bcl-2</th>
<th>IL-1β</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fold change mean</td>
<td>SEM</td>
<td>Fold change mean</td>
</tr>
<tr>
<td>Group I: Normal control</td>
<td>1.00 ± 0.09</td>
<td>0.09</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td>Group II: (early UC-induced group)</td>
<td>5.57 ± 0.47</td>
<td>0.20</td>
<td>0.01</td>
</tr>
<tr>
<td>Group III: (early UC+ Omega 3 protected group)</td>
<td>3.86 ± 0.19</td>
<td>0.59</td>
<td>0.04</td>
</tr>
<tr>
<td>Group IV: (late UC-induced group)</td>
<td>10.70 ± 0.52</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>Group V: (late UC+ Omega 3-treated group)</td>
<td>6.73 ± 0.38</td>
<td>0.39</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Means within the same column carrying different superscript letters are significantly different at (P≤0.05).

4. DISCUSSION

Inflammatory bowel diseases (IBD) is classified into two diseases are ulcerative colitis (UC) and Crohn’s disease (CD) are multi-factorial, relapsing disorders of the gastrointestinal tract. Although, the etiology of these diseases involves altered immune responses, epithelial dysfunction, environmental factors, and nutrition (Banerjee et al., 2019). Ulcerative colitis is characterized by intestinal mucosa inflammation with an extension from the rectum to
the colon in a uniform manner (Hosseinzadeh et al., 2017). The present study, the experimental induction of colitis with instillation of 3 ml of 3% acetic acid into the colon was successfully achieved and confirmed by macroscopic damage and histopathological findings such as congestion, haemorrhages, edema, and leukocytic infiltration. This result is in agreement with previous reports in animal models of colitis, which demonstrated similar macroscopic and microscopic pictures and tissue damage after the induction of colitis using acetic acid (El-Akabawy and El-Sherif, 2019). The use of acetic acid for the production of ulcerative colitis in rats is a standardized model, and it has been reported to have similar histological features to that of human. It is usually associated with deep ulceration and inflammation, enhanced vasopermeability as well as severe influx of neutrophils and macrophages to the site of injury (Kandhare et al., 2016).

Michalak et al. (2016) reported that polyunsaturated fatty acids (PUFAs) are bioactive lipids which regulate inflammation and immunity. They had increased importance in nutritional therapy and are recommended dietary supplements. Gastrointestinal diseases are one of the diseases that ameliorate through the administration of (PUSFA). Lipid mediators, such as prostanoids and leukotrienes play pivotal roles in the initiation of acute inflammation, where as resolvs and protectins, inflammation mediators derived from long chain poly unsaturated fatty acids omega-3 (LC-PUFA n-3) (Serhan et al., 2015), promote and stimulate the active resolution of inflammation, the control of neutrophil infiltration is most interest in this situation, which underlines the importance of resolvins and protectins omega-3 lipid mediators; resolution of acute inflammation is a central component of host defense and the return of tissue to homeostasis (Serhan et al., 2015).

In the current study of Myeloperoxidase MPO, a glycosylated hemo protein enzyme in the granules of neutrophils and monocytes. It is an important enzyme used in the defense of the organism, MPO also takes part in the defense of the organism. MPO also modulates inflammation by generating ROS (Bastaki et al., 2016). The activity of this enzyme is used as an indirect measurement for the severity of tissue damage (Klebanoff et al., 2013).

Increasing in activity of MPO is often observed in inflammatory gastrointestinal disorders. In present study, MPO was up regulated in the acetic acid-induced ulcerative colitis according to (Bastaki et al., 2016; Kumar et al., 2019), and significantly down-regulated after administration of Omega-3 both as a protection or treatment to acetic acid-induced ulcerative colitis in rats. Although it not reach the normal values as reported in (Soliman et al., 2010; Tyagi et al., 2012), decreasing of MPO activity after Omega-3 administration, may be due to effect of Eicosapentaenoic acid EPA-derived 18-hydroxyeicosapentaenoic acid (18-HEPE) and 15-hydroxyeicosapentaenoic acid (15-HEPE) each produced by hypoxic vascular endothelial cells and show some activity in reducing polymorph macrophage neutrophil (PMN) trans-endothelial migration and thus decreasing Myeloperoxidase activity as reported in (Marcon et al., 2013; Serhan et al., 2018). Hamabata et al. (2018) suggested that treatment with the n-3 fatty acid-derived lipid mediator, resolvin D1/D2, decreased neutrophil activity and resulted in the amelioration of the symptoms of Dextran Sulfate Sodium (DSS)-and Trinitrobenzene Sulfonic Acid (TNBS)-induced colitis in mice.

Lipid mediators derived from EPA and Docosahexaenoic acid (DHA) have an important role in stopping the inflammation cascade as explained by (Bannenberg and Serhan, 2010; Balas et al., 2014). Resolving E1, resolvin D1 and protectin D1 all inhibited trans-endothelial migration of neutrophils, so preventing the infiltration of neutrophils into the sites of inflammation; resolvin D1 inhibited interleukin-1β (IL-1β) production; and protectin D1 inhibited tumor necrosis factor α (TNF-α) and (IL-1β) production. Oxidative stress Biomarkers (Malonaldehyde MDA, Catalase CAT, reduced glutathione GSH) Oxidative stress plays vital role in the progression of the disease. Oxidative stress induces and triggers IBID through two pathways, including oxidative injury to intestinal mucosal cells and up regulation of inflammatory cytokines (Khodayar et al., 2018). Acetic acid causes severe oxidative stress in colonic tissue, which is manifested as an enhancement in lipid peroxidation that occurs via an increase in the L-MDA content; also this sever oxidative stress leads to the consumption of GSH and decreasing its content in colonic tissue as well as decreasing CAT activity in tissue, simply GSH and CAT trying to scavenge the free radicals from cells to ameliorating the happening oxidative stress that can be destruct the cells and to return Redox balance to our cells; this evidence is reported (Dogan et al., 2014; Tekeli et al., 2018; Soliman et al., 2019). In current study we found that decreasing in L-MDA and increasing in both CAT activity and GSH concentration were happened also due to the administration of Omega-3; this result is agreed with other previous studies (Tyagi et al., 2012) which improve the antioxidant activity of Omega-3.EPA was discovered as a precursor of resolvin E1 and resolvin E2; lipid mediators with potent resolution and anti-oxidant effects as reported in (Soliman et al., 2010; Sharma et al., 2019). Apoptosis is a programmed cell death process involved in the normal turnover of damaged cells (Shalkami et al., 2018). Apoptosis also considered as a component of a defense mechanism triggered during tissue damage and immune reactions caused by many pathological conditions (Ola et al., 2011). Caspase subfamilies are divided into initiator caspsases (initiate apoptosis) includes the Caspase-8 , -9, and -10 and the effectors caspases include Caspases -3,-6,-7 (Salmena et al., 2003).

Kountouras (2003) during the process of death by apoptosis, the cell shrinks, its mitochondria break down with the release of cytochrome c (Cyt c), it develops bubble-like blebs on its surface, the chromatin (DNA and protein) in its nucleus degrades and breaks into small, membrane-wrapped, fragments (apoptotic bodies). Consequently, the phospholipid phosphatidyl serine which normally hidden within the plasma membrane, is exposed on the cell surface, and is then bound by receptors on phagocytic cells (macrophages and dendritic cells) which then engulf the cell fragments. Lastly, the phagocytic cells secrete cytokines that inhibit inflammation. Caspase-3 has a central role in this cascade, and is known to activate cytoplasmic DNase, which subsequently migrates to the nucleus and fragments the DNA. Therefore, DNA fragmentation particularly when it is inter-nucleosomal is one of the gold standards for detection of apoptosis. Ackan et al., (2008) suggested that, Colon Caspase-3 levels were higher in the colitis than in the control group rats. B cell lymphoma (Bcl-2) proteins are the key.
regulators of the intrinsic apoptosis pathway. Each member of this family contains one or more BCL-2 homology (BH) domains, BH1–BH4. Apoptosis is triggered by pro-apoptotic, BH3-only proteins, the name of which stems from the fact that these proteins contain only a single BH domain, in this case, BH3. The key effectors of apoptosis commitment are BH3-only ‘activator’ proteins (Singh et al., 2019). Proteins of the Bcl-2 family are regulated by multiple mechanisms and their expression levels differ widely depending on tissue type, cell lineage, developmental stage, organismal age and any stress or damage that is experienced as described by (Sarosiek et al., 2017). Dysregulation of apoptosis recognized as a pivotal key in the pathogenesis of UC, this dysregulation involved two ways up-regulation of pro-apoptotic genes like Caspase-3 and down-regulation of anti-apoptotic genes like Bcl-2 this concept is agreed with our findings that acetic acid significantly induced epithelial apoptosis in colon; the administration of acetic acid resulted in an increased expression of caspase-3, which is specified as an apoptotic marker as reported in (Labib et al., 2016; Ghalwash et al., 2017; Shalkami et al., 2018; Saber et al., 2019; El-Akabawy and El-Sherif, 2019) and decreasing in expression of BCL-2 as reported in (Saber et al., 2019; Akisit et al., 2016). In the acetic acid group, Caspase-3 protein expression was up-regulated This can be explained by the results of (Labib et al., 2016) who reported that binding of TNF-α to its receptors activates receptor-dependent pathways of apoptosis through activation of caspases including Caspase-3. A number of studies suggest that N-3 PUFA s exert beneficial biological functions in carcinogenic processes. In vitro studies have reported that EPA and DHA induced apoptosis in the colon cancer cell lines HT-29, Caco-2, and DLD-1 through an increase in caspase-3 activity and inhibition of Bcl-2. Cytokines are associated with inflammatory diseases, with higher levels of TNF, IL-1β, interleukin-6 (IL-6), and interleukin-8 (IL-8) being a common feature of many inflammatory conditions (Calder et al., 2013). Various studies have shown that acetic acid-induced colitis resemble acute human intestinal inflammation by stimulating the production of inflammatory mediators this evidence is reported in (Boobalan et al., 2015; Hosseini et al., 2017). In this study the administration of Omega-3 significantly down-regulate pro-inflammatory cytokine IL-1β gene expression level, our findings is supported by other previous studies on human (Calder, 2015; Diab et al., 2018) and previous studies on animal (Triantafyllidis et al., 2015; Serhan et al., 2018). Calder (2017) suggested that EPA and DHA give rise to anti-inflammatory and inflammation resolving mediators called resolvins, protectins, and maresins. Mechanisms underlying the anti-inflammatory actions of EPA and DHA include altered cell membrane phospholipid fatty acid composition, disruption of lipid rafts, inhibition of activation of the pro-inflammatory transcription factor nuclear factor κB so reducing expression of inflammatory genes and activation of the anti-inflammatory transcription factor peroxisome proliferator-activated receptor γ. Marion-Letellier et al. (2015) reported in his review that ‘ The transcription factor NF-κB is sequestered in the cytoplasm linked to its inhibitory protein IκB. Upon stimulation, IκB is phosphorylated and then degraded into the proteasome. Free NF-κB translocates to the nucleus and activates the gene expression of pro-inflammatory targets. Numerous studies have observed that n-3 PUFA can inhibit NF-κB pathway in inflammatory conditions. Omega-3 PUFA themselves are also ligands that directly bind to nuclear receptors that impact on gene expression. Feeding fish oil to mice decreased the production of TNF, IL-1β, and IL-6 by endotoxin-stimulated macrophages and decreased circulating TNF. IL-1β and IL-6 concentrations in mice injected with endotoxin. Some animal studies as (Sierra et al., 2008) report that marine n–3 fatty acids increase the concentration of the anti-inflammatory cytokine interleukin 10 (IL-10). Several studies providing marine n–3 fatty acid supplements to colitis-induced animals have reported decreased production of TNF, IL-1β and IL-6 by endotoxin stimulated monocytes or mononuclear cells (Cho et al., 2011). It has been shown that in cell culture pretreatment with EPA and DHA decreases lipopolysaccharide-stimulated THP-1 macrophage TNFα, IL-1β and IL-6 production compared with control cells (Weldon et al., 2007) which evidence the protective effect of omega-3 against inflammation. Several studies providing marine n–3 fatty acid supplements to colitis-induced animals have reported decreased production of TNF, IL-1β and IL-6 by endotoxin stimulated monocytes or mononuclear cells (Marcon et al., 2013). The histological changes due to acetic acid were vivid. They included massive haemorrhage, complete absence of epithelial lining on the mucosal surface, severely damaged villi and crypts with subsequent loss of their architecture except in a few crypts, depletion of goblet cells, and severe infiltration of inflammatory cells. Acetic acid has been documented to induce localized inflammation and loss of mucosal integrity leading to epithelial injury. Omega-3 administration in groups 3 and 5 in this work resulted in significant increase in goblet cells containing acidic mucins, reflecting higher degree of mucins maturation. This may be attributed to stimulation of goblet cell via prostaglandin, which in turn activates synthesis and secretion of glycophenets. This altered mucin maturation could be due to accelerated eflux of immature mucins in response to acetic acid administration as reported by Soliman et al., 2010.

5. CONCLUSION

These findings suggested that oral treatment with Omega-3 shows a significant protective effect in acetic acid induced-UC animal models confirmed by antioxidant and anti-inflammatory activities. Also, the protective effect of Omega-3 on colonic tissues might be mediated by adjustment of inflammatory mediators and increasing antioxidants as well as attenuating oxidant/antioxidant imbalance. Moreover, Omega-3 administration may have anti-apoptotic effect and increasing proliferation of colon cells.

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6. REFERENCES


