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Short Communication

Effect of Different Types of Omega-3 Fish Oil on the Physiological, Biochemical and Immunological Parameters in Male Rabbits

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ABSTRACT

Omega-3 oil has been associated with several health benefits. It is challenging to get the required dose from one source. Therefore, the present study was aimed at evaluating the effects of three different types of omega-3 fish oil on the physiological, biochemical, and immunological parameters of domestic male rabbits. Twenty male rabbits were divided into four groups and used for the study. The grouping included animals that were administered: water (control) (Group 1); 400 mg/kg/day commercial fish oil (Group 2); 400 mg/kg/day pure fish oil (Group 3); 400 mg/kg/day complex omega-3, 6, 9 oil (Group 4). The experiment was terminated after three weeks and blood samples were collected for biochemical and immunological assays. The results indicated that omega-3 oil treatment affected lipid profiles, biochemical parameters and serum cytokine levels differently. There was no significant difference in the levels of cholesterol and VLDL-C among all the treatment groups in relation to the control. A high level of serotonin was observed in Group 2 animals (47.18±12.00 ng/mL). Also, high amounts of glutathione were recorded for Groups 2 (26.32±7.09 µmol/L) and 3 (26.04±7.22 µmol/L) animals in relation to the other experimental groups. There was no significant difference in the cytokine levels of IL-10 and TNF-α among all the treatment groups. Furthermore, the amount of IL-6 was low in both Groups 2 and 3 compared with the other groups. The findings from this study revealed that the commercial omega-3 fish oil has a positive effect on health parameters compared to other omega-3 sources.

Keywords: Antioxidants, Omega-3 fatty acids, Acetylcholinesterase, Interleukins, Serotonin, TNF-α.

Introduction

Attention to the roles of fatty acids in human health and diseases has been on an increase in recent decades. The most influential biochemicals in human physiology are alpha-linolenic acid (ALA), eicosapentaenoic acids (EPA), and docosahexaenoic acid (DHA). Both EPA and DHA are the predominant structural fatty acids in the brain and comprise 40% of the polyunsaturated fatty acid content. Almost 50% of the weight of neural membranes is composed of DHA. Many clinical and epidemiological studies have shown positive roles for omega-3 fatty acids in cardiovascular diseases.[1,2] Cholinesterases (CHEs) constitute a group of esterases that hydrolyze choline esters at a greater rate. CHEs are fundamentally important for the quick destruction of acetylcholine (ACH) within a millisecond after its discharge at cholinergic neural connections. In this way, it permits definitive control of muscle contraction. There are two types of cholinesterases in the vertebrates: acetylcholinesterase and butyrylcholinesterase. The former may be a key component of cholinergic brain neural connections and neuromuscular intersections.[3] Therefore, acetylcholinesterase is the essential enzyme participating on neurotransmission within the central, as well as peripheral nervous system. Its primary part is the end of the neuronal transmission through the cleavage of the neurotransmitter acetylcholine (ACh).[4] Moreover, serotonin, known as 5-hydroxytryptamine, is classified as a biogenic monoamine that may be found in plants and the human body. Within the human body, serotonin is synthesized in two free compartments that are isolated by the blood-brain barrier. The greater part of serotonin is synthesized in enterochromaffin cells of the gastrointestinal tract, discharged within the bloodstream, and transported in the blood platelets. Almost 5% of serotonin is synthesized within the brain inside serotonergic neurons. As a neurotransmitter, serotonin plays a vital role in the control of physiological activities like body temperature, rest, vomiting, sexuality, craving, behavior, and cognitive capabilities such as learning and memory. The breakdown of the serotonergic framework has been embroiled within the etiology of an assortment of psychiatric conditions (sadness, schizophrenia, liquor abuse), neurological conditions (headache, Alzheimer's disease, epilepsy), and clutters.[5] Glutathione (γ-glutamyl-cysteinyl glycine) is considered as a major intracellular antioxidant and plays a key role in reducing the effects of oxidative stress. It is a tripeptide (cysteine, glycine, and glutamic residues) and exists in the cells in two states: reduced (GSH) and oxidized (GSSG) forms. The proportion of GSH to GSSG determines the redox status of cells. Glutathione is additionally recognized as a thiol buffer, keeping up sulfhydryl groups of numerous proteins in their decreased state. It is delivered only within the cytosol and effectively pumped into the mitochondria, playing a significant function in protecting cellular macromolecules from endogenous and exogenous reactive oxygen species (ROS). Also, receptive nitrogen species (RNS) guards the body against oxidative stress as a strong antioxidant. Moreover, glutathione is included within the detoxification of both xenobiotic and endogenous compounds by

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which glutathione encourages the plasma layer transport of poisons. Besides, glutathione secures cells from oxidants through the recycling of vitamins C and E.[6,7]

Malondialdehyde (MDA) is a compound occurring from lipid peroxidation that may result in free radical-mediated damage of the cells. MDA is the foremost abundant chemical aldehyde, which results from lipid peroxidation leading to oxidative stress by which free radicals start a cascade. These series of events cause lipid peroxidation, DNA damage, cell passage, and neurological issues.[8] MDA has been broadly utilized as a helpful biomarker for lipid peroxidation of omega-3 fatty acids, because of its response to thiobarbituric acid (TBA). The TBA test is predicated upon the reactivity of TBA with MDA to generate an intense colored chromogen fluorescent colour.[9]

IL-6 is a pleiotropic pro-inflammatory cytokine that is included in different pathways such as the direction of intense stage reactions, cell development, and separation, as well as metabolic processes. Critically, IL-6 has risen as a powerful controller of safe reactions and aggravation.[10] Also, it influences an assortment of frameworks, counting on the neural and endocrine frameworks, bone metabolism, digestion system, and skeletal muscles.[11] IL-6 is synthesized by fibroblasts, monocytes, macrophages, T cells, and endothelial cells. Its amalgamation and emission are induced among provocative conditions, such as upon incitement of Toll-like receptor (TLR)-4 by lipopolysaccharide or upon incitement of cells by IL-1 or tumor necrosis factor (TNF)-a.[12]

IL-10 is a major immunosuppressive anti-inflammatory cytokine. It plays a very important immunoregulatory roles in host defense and immune homeostasis. Nearly all cells of the intrinsic and versatile arms of the resistant framework can generate IL-10. Such cells include the dendritic cells, macrophages, pole cells, NK cells, eosinophils, neutrophils, B cells, $CD8^+$ T cells, $CD4^+$, Th1, Th2, and Th17 cells, and administrative T cells. The major role of IL-10 is to restrain the degree of ensuring both the intrinsic and the versatile safe cells to preserve a homeostatic state. This part of IL-10 is critical in the prevention of infection-associated immunopathology, autoimmunity, and hypersensitivity. In addition to these processes, IL-10 directs the development and separation of B cells, NK cells, cytotoxic and partner T cells, granulocytes mast cells, dendritic cells, endothelial cells, and keratinocytes.[13]

Tumour necrosis factor (TNF) is a pluripotent pro-inflammatory cytokine which plays a central role in aggravation, expansion, and apoptosis. It is a fiery cytokine primarily produced by enacted macrophages and monocytes. TNF activates a cascade of other inflammatory cytokines and chemokines which has been considered as one of the key elements in inflammation.[14] Improved TNF generation can relieve cellular signaling that can cause cells to experience necrosis or apoptosis. Besides, TNF is well characterized as a pathogenic mediator in different infections, including Alzheimer's malady, Parkinson's illness, stroke, psoriasis, joint pain, septic stun, and pneumonic clutters.[15] The aim of the current research was to investigate the effects of three different types of omega-3 fish oil on the physiological, biochemical and immunological parameters of experimental domestic male rabbits.

Materials and Methods

Source and growth condition of experimental animals

Domesticated male rabbits, *Oryctolagus cuniculus*, subspecies domesticus, between the ages of 4 to 5 months old were purchased from the local market in Kirkuk city, Iraq and used for the study. The rabbits were raised in the animal house of the Faculty of Education-Al-Hawija, University of Kirkuk, Kirkuk, Iraq. These experimental animals were kept in cages of 100 x 80 x 50 cm, placed in a room measuring 4 × 5 m at a temperature of 30 - 35°C and humidity of 27 - 32% for 21 days before the commencement of the experiments. The cages were cleaned and sterilized twice a week. Sawdust was used to sprinkle the floor of the cages. The rabbits were fed with a special feed (25% corn, 10% animal protein, 10% dried milk, and 1% food salt), which was placed in special containers to prevent faecal contamination.

Treatment and experimental grouping

Twenty male rabbits, weighing between 0.9 – 1.3 kg were separated into four experimental groups: Group 1 (control) which was made up of animals that were only given water; Group 2, animals that were administered with commercial fish oil at a dose of 400 mg/kg/day; Group 3, animals that were given pure fish oil at a dose of 400 mg/kg/day; and Group 4, animals that were administered with complex omega 3, 6, 9 at a dose of 400 mg/kg/day. The gels were suspended in distilled water and administered orally the experimental rabbits daily for 3 weeks.

Blood sample collection and preparation

Blood samples were collected from the ear veins of the rabbits with glass test tubes that were rinsed with heparin anticoagulant (1:10 dilution) before collection. The blood samples were centrifuged at 3,000 rpm for 15 minutes to separate the blood plasma. Separated plasma was transferred to another dried and clean glass tubes for analysis.

Biochemical and immunological analyses of blood samples collected from experimental rabbits

The level of TNF in the blood plasma was determined by Cusabio Biotech, China.16 IL-6 was assayed using a Kit Assay Peprotech.[17] Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum levels of IL-6 and IL-10 using an ELISA kit (Diaclone Research, Besancon, France) following the manufacturer's instruction. Serum glutathione was estimated using a modified method by Sedlak and Lindsay.[18] Measurement of plasma peroxidation levels, malondialdehyde (MDA), a secondary product of lipid peroxidation was based on the colorimetric reaction of TBA.[19] Serotonin activity of blood plasma was determined by Assay Kit.[20] Estimation of lipid profile was conducted using Assay Kit (Biolabs, SA, France). CRP level was determined by Assay Kit.[21] Estimation of acetylcholine esterase activity in serum was done via spectrophotometer employing Ellman's method.[22]

Statistical analysis

All data were expressed as mean ± standard error (M ± SE) and statistically analysed using SPSS statistical software version 25 (IBM Corporation, USA). Duncan Multiple Range test was used to evaluate the statistical significance of differences among the group means.[23] Differences between the means of different parameters were considered significant at $p < 0.05$.

Results and Discussion

Effect of omega-3 oil on lipid profiles in blood serum of experimental male rabbits

The results obtained for the lipid profile of experimental animals (Table 1) revealed no change in cholesterol level in the treatment groups administered with different types of omega-3 oil compared to the control group. Group 2 animals showed a significant decrease in TG (36.50 ± 2.72 mg/dL), while Group 3 showed a significant increase (104.25 ± 18.27 mg/dL) when compared to the Group 1 animals (73.00 ± 14.00 mg/dL). HDL-C level was observed to be significantly high in Group 2 and Group 4 animals with values of 592.38 ± 14.31 and 572.18 ± 16.24 mg/dL, respectively compared to the control group (273.24 ± 11.10 mg/dL). There was no change observed in the HDL-C level of Group 3 animals compared to the control. In contrast, there was a significant decrease in the levels of LDL in rabbits that were fed with complex omega 3,6,9 (Group 4) and commercial omega (Group 2) with values of 129.44 ± 3.61 and 132.01 ± 3.55 mg/dL, respectively with the control Group 1 that had a value of 251.25 ± 4.07 mg/dL. Besides, there was no significant difference in the LDL level in animals administered with pure omega (Group 3) in comparison with the Control. VLDL level was significantly decreased in Group 2 animals with a value of 7.31 ± 0.53 mg/dL

compared to the Control group which had a VLDL value of 16.14 ± 0.92 mg/dL. There was no change in the level of VLDL observed in the other experimental groups with the Control.

The results obtained in this study are in agreement with the study of Movahedian *et al.*[24,25] Omega-3 fatty acids play an important role in quality control that is required for regulating lipid homeostasis. This occurs by the enhancement of the peroxisome proliferator actuated receptor (PPAR) which in turn controls quality expression of few key components of the greasy corrosive digestion system. Increased levels of the anti-inflammatory resources (delivered from omega-3 fatty acids), enactment of PPAR-α target qualities of the lipid digestion system, and up-regulation of a nuclear factor 2 (NRF2) mediate antioxidant chemicals, that may have an effect on cell integrity. Hence, the resultant increment in lipolytic movement changes the lipid profile parameters.[26] So, Omega-3 fatty acids has two predominant influences on the lipoprotein lipid profile which include the reduction in hepatic excretion of TG-rich VLDL and an increased rate of processing of VLDL to LDL particles. Reduced hepatic secretion of triglycerides (TG)-rich VLDL particles include a few components for case EPA and DHA of omega-3 fatty acids. The chemical constituents of hepatic TG biosynthesis include diacylglycerol, acyltransferase and phosphatidic acid, phosphatase/phosphohydrolase. EPA and DHA increase the level of hepatic β-oxidation of fatty acids, which may be auxiliary to the capacity to activate PPAR expression. There are some pieces of evidence that EPA and DHA utilization increases lipoprotein lipase-induced TG hydrolysis.[27] Furthermore, omega-3 fatty acids diminish VLDL, leading to a decrease in triacylglycerol generation through diminished action of sterol receptor element-binding protein-1c, which is the key switch in controlling lipogenesis. Besides, omega-3 fatty acids may induce β-oxidation at the same time in the mitochondria and peroxisomes through the activation of peroxisome PPAR-a, leading to a decrease in oily acid substrate for triglyceride amalgamation.[28]

Effect of omega-3 oil on biochemical parameters in blood serum of experimental rabbits

Table 2 depicts the results obtained for the estimation of cholinesterase activity, serotonin, glutathione, and malondialdehyde level in blood serum of experimental animals. There was a significant decrease in AchE activity in Group 2 and 3 animals with values of 0.020 ± 0.02 and 0.030 ± 0.04 µmol/min/mL, respectively compared to the control group, which had a value of 0.086 ± 0.04 µmol/min/mL. Meanwhile, there was no change in AchE activity in Group 4 animals (0.077 ± 0.04 µmol/min/mL) in relation to the control group. Serotonin level was observed to be high in the Group 2 animals (47.18 ± 12.00 ng/mL) compared to the control group (29.40 ± 5.51 ng/mL). Also, the Serotonin level in the other experimental groups remained unchanged with the control group. These observations are in support of the study carried out by Abd Allah,[29] who reported a similar effect in his study on omega-3 fatty acids. Glutathione level was significantly high in the Group 2 and 3 animals (26.32 ± 7.09 and 26.04 ± 7.22 µmol/L, respectively) in comparison with the control group (10.01 ± 2.12 µmol/L). There was no significant difference between Group 4 (11.43 ± 2.72 µmol/L) and the control group animals. These results are in agreement with other studies.[30,31] A decrease in the level of malondialdehyde was recorded in Group 2 and 3 animals with values of 0.15 ± 0.04 and 0.20 ± 0.05 µmol/L, respectively compared to the control group (0.82 ± 1.01 µmol/L). Meanwhile, there was no significant difference between Group 4 (0.82 ± 1.01 µmol/L) and the control group animals in the level of MDA. These observations correlate with other studies.[32,33]

The fundamental DHA of omega-3 fatty acids is specifically improved upon in the neuronal tissues, which are uncommon in neuronal cells and synaptic areas, oligodendrocytes, conjointly sub-cellular elements such as myelin and nerve terminating. DHA appeared to play a basic role in directing neural quality expression. Also, DHA acts as an endogenous ligand for retinoic acid receptors (RAR) and retinoid x receptors (RXR). RAR and RXR have been implicated to diminish with age and these receptors are associated with age-related memory shortfalls. DHA balances different cellular capacities with improved layer, ease of amyloid forerunner proteins, passage towards non-amyloidogenic preparation, that restrains α and β secretase in this manner, lessening amyloid-β release. The impact of DHA on the layer smoothness of DHA has a critical effect on neural layer work. DHA in specific, has been revealed to balance quality expression at the translation level. This is achieved through inducing peroxisome proliferator-activated receptor (PPAR), in addition to a few proteins related to glucose and lipid digestion system. Studies on rodents showed that treatment with angle oil led to overexpression of quality related to synaptic versatility, flag transduction, vitality digestion system, and administrative proteins,[34,35] and all the effects of omega-3 supplementation will reflect on AchE levels.

Serotonin and dopamine within the brain are likely included within the instrument of activity of omega-3 PUFAs. In humans, a positive relationship was found between plasma DHA level and serotonin metabolites in cerebrospinal liquid.[36] Also, omega-3 PUFAs are related to the working of neurotransmitters. This encourages the discharge of neurotransmitters, such as dopamine, serotonin, and nor-epinephrine by which omega-3 supplementation essentially progressed serotonin level and switched the stress-induced lessening in serotonin within the hippocampus, frontal cortex, and striatum. Also, it was found that reduction in DHA is related to dysfunctions of neuronal film and transmission of serotonin, norepinephrine, and dopamine. Besides, omega-3 PUFA controls flag transduction by upgrading G-protein-mediated flag transduction, membrane-bound chemicals (Na/K-dependent ATPase), and protein kinase C. Hence, the change in layer brought about by omega-3 PUFA administration influences distinctive neurotransmitter framework modifying the direction of dopaminergic and serotonergic neurotransmission.[37-39] Therefore, all these explain the elevation of serotonin level in the commercial omega group. Omega-3 PUFAs has been reported to act as powerful antioxidant operator in both ways, specifically by supplanting arachidonic acid as an eicosanoid substrate and hindering arachidonic acid digestion system. This is achieved by modifying the expression of incendiary qualities through impacts on translation.[40] Furthermore, omega-3 PUFAs initiate and enhance antioxidant proteins and reactive oxygen species (ROS).[41] These effects elevate glutathione levels as part of the antioxidant system directly by activating it and indirectly by reducing ROS leading to decrease glutathione consumption. Moreover, omega-3 fatty acids upregulate the quality expression of antioxidant proteins and down control qualities related to the generation of reactive oxygen species.[42] Therefore, omega-3 fatty acid will lower MDA through these mechanisms and by scavenging free radicals leading to inhibition of lipid peroxidation.

Effect of omega-3 oil on blood serum cytokine levels of experimental male rabbits

In the experimental animals, the levels of the C-reactive protein (CRP), IL-6, IL-10 and TNF-α were estimated and the outcomes were presented in Table 3. There was a significant decrease ($P \leq 0.05$) in the CRP in Group 2 rabbits (1.11 ± 0.90 mg/dL) with respect to the control group (273 ± 1.08 mg/dL). It was observed in the remaining experimental groups that there was no significant difference in the levels of the CRP compared to the control group. These results are in accordance with other studies.[43,44] Omega-3 fatty acid inhibits inflammatory processes within the body. These include suppression of transcription factors that control the production of circulating biomarkers such as CRP.[45] Additionally, omega-3 fatty acid reduces CRP indirectly by lowering the production of pro-inflammatory IL-6 which is well known to induce secretion of CRP from the liver.[46,47] IL-6 level was significantly low in Group 2 (1.09 ± 0.05 pg/mL) and 3 (1.74 ± 0.68 pg/mL) animals in comparison with the control group (4.67 ± 0.91 pg/mL). Group 4 animals did not show any significant difference with the control group. These results are supported by previous studies.[48-50] There was no change in TNF-α and IL-10 levels in all the experimental groups when compared with the control group, an observation similar to the study of Abdolahi *et al.*[51] Supplementation with omega-3 fatty acids prevents the pro-inflammatory generation of a cytokine such as IL-6 and TNF by influencing layer composition. This observation impacts flag transduction, moment flag-bearer atom, or NRF2, which play a key role in directing cytokine quality translation. In this way, anti-

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inflammatory impacts of omega-3 fatty acids are due to these effects and down-regulation of NF-κB activity by which this inhibition of cytokine production usually occurs at the transcriptional level.[46,47]
These results showed a significant increase in IL-10 level in pure omega-3 (6.63 ± 1.34 pg/mL) compared with the control group (5.87 ± 0.65 pg/mL). Meanwhile, the other group did not show any difference from the control group (Table 3). These results support other findings such as the study conducted by Hao *et al.*,[49] and Satoh–Asahara.[52] The elevation of IL-10 level after omega-3 fatty acid supplementation was due to direct activation of omega-3 fatty acids on macrophages and monocyte cells to produce anti-inflammatory IL-10 by which omega-3, especially EPA increased the expression of IL-10 through PPARg.[49,50]

Table 1: Lipid profiles in blood serum of experimental male rabbits

Group	Parameter				
	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Control	32.56 ± 5.02	73.00 ± 14.00	273.24 ± 11.10	251.25 ± 4.07	16.14 ± 0.92
Commercial omega	33.87 ± 5.35	36.50 ± 2.72	592.38 ± 14.31	132.01 ± 3.55	7.31 ± 0.53
Pure omega fish oil	42.09 ± 8.52	104.25 ± 18.27	395.10 ± 11.06	271.26 ± 4.05	15.66 ± 1.43
Complex mega 3,6,9	41.79 ± 8.69	59.00 ± 8.62	572.18 ± 16.24	129.44 ± 3.61	15.04 ± 1.84

Table 2: Concentrations of AchE, serotonin, glutathione and malondialdehyde in blood serum of experimental animals

Group	Parameter			
	Ach E (µmol/min/mL)	Serotonin (ng/mL)	Glutathione (µmol/L)	Malondialdehyde (µmol/L)
Control Group	0.086 ± 0.04	29.40 ± 5.51	10.01 ± 2.12	0.82 ± 1.01
Commercial omega-3 fish oil	0.020 ± 0.02	47.18 ± 12.00	26.32 ± 7.09	0.15 ± 0.04
Pure omega 3 fish oil	0.030 ± 0.04	26.02 ± 5.10	26.04 ± 7.22	0.20 ± 0.05
Complex omega 3,6,9	0.077 ± 0.04	28.40 ± 5.00	11.43 ± 2.72	0.80 ± 1.11

Table 3: Concentrations of CRP, IL-6, IL-10 and TNF-*α* in blood serum of experimental animals

Group	Parameter			
	CRP (mg/dL)	IL-6 (pg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)
Control	2.73 ± 1.08	4.67 ± 0.91	5.87 ± 0.65	36.53 ± 3.00
Commercial omega-3 oil	1.11 ± 0.90	1.09 ± 0.05	5.89 ± 0.89	34.27 ± 2.91
Pure omega-3 oil	2.77 ± 1.23	1.74 ± 0.68	6.63 ± 1.34	37.86 ± 3.38
Complex omega 3,6,9	2.84 ± 1.51	3.99 ± 0.12	6.00 ± 0.37	35.95 ± 3.75

CRP: C-reactive protein; IL-6: Interleukin 6; IL-10: Interleukin 10; TNF: Tumor necrosis factor

Conclusion

The results of the current study indicated a positive effect of the commercial omega-3 fish oil on the analyzed parameters compared to the other omega-3 groups. A significant decrease in lipid profile was observed, except for the HDL-C, which showed a significantly higher level than the control. Also, the commercial omega-3 fish oil increased the level of serotonin hormone with its positive effect, while reducing the levels of the cholinesterase enzyme and inflammatory factors. These characteristics give the commercial oil the positive effect of increasing mental and cognitive capabilities in humans and maintenance of a healthy life style.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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