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Effects of Omega 3 on brain performance and immunity of albino Rats

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Abstract: The purpose of this study was to examine the effects of omega-3 fatty acids on brain performance and immune function in albino rats. The study involved 40 male albino rats, each weighing between 200 and 230 grams, which were divided into two equal groups. The control group ingested placebo for 4 weeks, once daily, Experimental group ingested omega 3 300mg/Kg/day for 4 weeks, once daily. Blood was withdrawn for analysis of IL-1B, IL-6, TNFα, SOD, serotonin, GABA, GSH, MDA and nitrite. Results indicated that Omega 3 induced in MDA, IL-1B, IL-6, TNFα significant decreased levels while increased SOD, Serotonin, GABA, GSH, and nitrate, in conclusion, Omega 3 may induce beneficial effects on brain performance and immunity of albino rats

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Key Words: Albino Rats; Omega 3 fish oil; brain performance; immunity.

1. Introduction

Fish oils that are rich in ω-3 fatty acids, also referred to as n-3, come from various marine organisms, including tuna, halibut, salmon, mackerel, herring, cod liver, seal blubber, and whale blubber.

ω-3 PUFAs are classified into two groups: those with a long chain length (20 carbon atoms or more) and those with a short chain length (18 carbon atoms or fewer). This distinction is important because the body seems to have no direct use for the short-chain forms of ω-3, whereas it does require the long-chain forms. The body needs an external supply of ω-3 long-chain variants. At the base of the marine food chain, algae, plankton, and planktonic crustaceans produce the majority of long-chain ω-3 polyunsaturated fatty acids. These are then transferred to higher fish in the food chain (Mason, 2000).

Moreover, fish oils are rich in EPA and DHA (long chain PUFAs); EPA, also known as timnodonic acid, is the primary ω-3 polyunsaturated fatty acid found in the majority of seafood. DHA, the second most prevalent long-chain ω-3 polyunsaturated fatty acid, is found in higher concentrations in some fish, including tuna, but in smaller proportions than EPA in the majority of other fish. It plays a significant structural role in the development of the brain and nerves (Mason, 2000)

Omega 3 induces a series of effect in rats namely: it reduces oxidative stress as its increased antioxidant enzymes such SOD and decreased MDA; malondialdehyde.

also induced anti-inflammatory action by downregulated IL-1B gene expression and reduced MPO activity together with antiapoptotic and decreased Caspase-3 and increased Bcl2 expression and induced neuroprotection of cerebellum and brain tissue from oxidative damage (Agren et al., 1997)

According to Guyton and Hall (2006), serotonin is released by nuclei that originate in the brain stem's median raphe and extend to various parts of the brain and spinal cord, particularly the hypothalamus and the spinal cord's dorsal horns. Serotonin inhibits the pain pathways in the spinal cord, and it is believed that an inhibitor within the higher nervous system helps regulate mood and may even promote sleep.

According to Steinlein (2004), the serotonin system typically stimulates the limbic regions of the brain to enhance an individual's sense of well-being, resulting in satisfaction, happiness, a healthy appetite, proper sex drive, and psychomotor balance. He also mentioned that the serotonin system supplies a lot of nerve endings to the reward and pleasure centers in the hypothalamus and its surrounding regions.

According to Heshmat et al. (2004), individual differences exist in areas such as sex, shape, abilities, efficiency, and skill. These disparities in brain capacities and efficiency may lead individuals to achieve higher levels of success in leadership, wisdom, and championships.

Fish oil (Omega 3) is a polyunsaturated fatty acid affecting many dangers diseases including atherosclerosis and may induced beneficial effect in some cardiovascular diseases such as myocardial infarction (Dewailly et al 2002) Administration of cod liver oil induced important changes in fatty acid of plasma, and platelets (Lorenz et al 1983), Needleman

et al (1979) added that Fish oil may induce replacement of omega 6 by omega 3 in platelets phospholipids. Also, fish oils can treat Rheumatic arthritis, and inflammation and immunity and diabetes (Heller et al, 2002; Connor, 2000) reported the important action of Omega 3 in health and disease.

Free Radicals are atoms or molecules with at least one unpaired electron. Under normal conditions, an equilibrium exists between reactive oxygen species and antioxidants. The over production of ROS in cells induce an imbalance between pro-oxidants and antioxidants (Chatter Jee and Shinde. 2005).

In the case of glutathione (GSH), SH is the business part of the molecule, indicating the presence of a sulfhydryl group. Glutathione is a crucial defence mechanism against a range of toxic substances, including pharmaceuticals and carcinogens. Glutathione has other important functions in cells; it participates in the decomposition of toxic hydrogen peroxide and is a crucial intracellular reductant that keeps essential SH groups in the reduced state of enzymes (Guyton & Hall, 2006).

This study aims to investigate the effects of omega-3 on the following:

- Brain function, focusing on the serotonin hormone and GABA, as well as increased blood supply via nitric oxide (nitrate).
- Immunity action through IL-1B, IL-6, TNF-α, oxidants, and antioxidants by estimation of MDA, GSH, and SOD.

2. Materials and Methods

The Sprague-Dawley strain of adult male albino rats weighing 200–230 g was acquired from the Egyptian Organization for Biological Products and Vaccines' laboratory animal farm located in Cairo, Egypt. Throughout the experiment, the rats were housed in a controlled environment with a conventional rat chow diet and unlimited access to water.

Experimental design:

The study included 40 male albino rats; they were divided to two equal groups. The control group (n=20) received placebo for 4 weeks, once daily, Experimental group (n=20) received omega 3, 300 mg /Kg / day for 4 weeks, once daily, by oral gavage

The animals were healthy and free from parasites or skin disease by examination, they were housed in galvanized wire batteries and maintained under hygienic conditions in temperature 22 ± 3.5°c and light (12 L / 12 D)

They were fed on a commercial balanced pelleted ration.

Heparinized and non-heparinized tubes were used to collect the blood when the animals were sacrificed at the conclusion of the experiment:

Kits used in rat studies:

using serum for Rat IL-1B: R, D Systems, RLB00 Rat IL-6: R, D Systems, R6000B
Elabscience
Rat TNFα R, D Systems, RTA00 (Wu et al. 2004)
using Blood for GSH- reduced glutathione Method (Ellman, 1959)
using blood MDA- Malondialdehy Method TBARS of Ohkawa et al (1979)
using Rbclysate for SOD superoxide Dismutase (Marklund and Marklund (1974) Serotonin using plasma
Method http://H.PLC(Vines et al. (2012)
GABA, Gamma aminobutyric acid Reference Rea et al. (2010)
using plasma for GABA Nitrate (NO) level (Miranda et al., 2001)

Statistical analyses

Statistical analyses were made to obtain the standard deviation. The data of the treated rats were compared with data for the control animals by using Student's t-test; all experiments had a significance level of $p<0.05$.

3. Results

Table (1) Omega 3 supplementation indicated that there are decreased levels of IL-1B, IL-6 and TNF-α which indicate immune modulation and reduced inflammation together with elevated SOD and GSH and improved brain performance indicated by elevated serotonin and GABA parameters, and increase Nitric oxide that indicate increase blood supply to important organs like the brain. Decreased MDA indicating suppression of Free radicals and decreased pollution.

Table (1): Effect of omega 3 on IL-1b, IL6, TNF-α GSH Nitrite, SOD, Serotonin, GABA, MDA

Parameter	Control		Treated	
	b	A	b	A
IL-1B (pg/ml)	9.7± 1.7	10.1±1.9	9.6±1.6	↓ 6.3±1.25*
IL-6 (pg / ml)	17.6± 2.3	18.5±2.2	16.9± 2.4	↓ 13.8±1.6*
TNF-α (pg/ml)	6.3±1.3	6.9±1.5	6.7±1.4	↓ 3.2±0.9*
SOD(u/gHb)	1309±31.2	1306±29.9	1309± 30.3	↑ 2036±80.4*
Serotonin(mg / ml)	155.4±40.3	158.6±42.2	161.1±39.2	↑ 261.2±56.2*
GABA(mg / ml)	43.2±12.1	45.3±11.6	42.9±13.2	↑ 76.3±19.1*
GSH(u/g/Hb)	101.4±25.2	106.3±26.3	108.2±27.7	↑ 204.3±53.6*
MDA(nmol/ml)	2.31±0.17	2.40±0.15	2.23±0.14	↓ 1.2±0.11*
Nitrite(nmol/ml)	25.9±2.6	27.1±2.4	26.3± 2.1	↑ 34.1±3.2*

P< 0.05

4. Discussion:

Table (1) Reported that Omega 3 supplementation increase GABA levels which indicate the beneficial effects induced to the CNS of the albino rats due to induction of better Y-maze 70-78% alteration and improves Morris maze.

This was in line with the opinion of Mc Namara et al (2010), they also added that Omega 3 may elevate GABA levels which in turn benefit Y maze and better memory action and increase sleep time GABA inhibits IL-1B, TNF, α and IL-6 release which modulate cytokine storm and stimulate vascular smooth muscle vasodilation.

Additionally, Mc Namara et al (2010) reported different functions of GABA not only as the main inhibitory neurotransmitter in the mammalian CNS, but it has multiple roles beyond just calming the brain, as it has CNS functions in brain and spinal cord and peripheral functions outside brain such as it affect immune system as it inhibits IL-1B, IL-6 and TNF-α, affect pancreas and inhibits glucagon, in case of liver hepatocytes express GABA receptors, inhibits oxidative stress, also in GI tract it regulates motility secretion and GABA in diseases models, affect traumatic brain injury, inflammation and depression and anxiety.

Guyton and Hall (2006) highlighted the functions of specific neurotransmitter substances in the basal ganglia system. For example, GABA always acts as an inhibitory agent, and GABA neurons involved in feedback loops from the cortex to the basal ganglia and back to the cortex create negative feedback loops that stabilize the motor control system.

The data presented in Table 1 indicate that omega-3 leads to increased serotonin concentration. Elevated serotonin levels may activate brain neurons, benefiting the brain's higher centers. This activation can induce a synergistic effect on cognition and memory performance, enhancing observational abilities and accelerating decision-making. This occurs due to the influence on various regions of the cerebral cortex, thalamus, and limbic system, as well as on the brain's intellectual functions.

This is in accordance with the work of Ridley (2001) and Guyton and Hall (2006), They added that serotonin is formed in the body by hydroxylation and decarboxylation of the essential amino acid tryptophan which is related to intelligence and mood stimulation.

According to Blank et al. (2004), hippocampi are one of the most significant output pathways from the limbic system's reward and punishment regions, which supply an individual's motives and background mood and encourage the brain to remember those events and influence memory. Thus, hippocampi support memory, which supports intelligence and learning.

Barret et al. (2000) found that serotonin concentrations are relatively high in some parts of the brain. The brainstem's midline raphe nuclei contain the cell bodies of serotonin-containing neurons, which project to parts of the hypothalamus, limbic system, neocortex, and spinal cord.

They stated that the amygdala, caudate nucleus, putamen, globus pallidus, thalamus, hypothalamus, and substantia nigra may have the highest concentrations of serotonin and dopamine among the different brain regions.

According to Martin (1997), serotonin is linked to intelligence, and the serotonin transporter gene is found on chromosome 11. Manal (2017) and Ridley (2017) are in agreement that leaders, directors, and politicians have higher serotonin levels. The fastest way to increase serotonin concentrations is to ingest tryptophan, which is converted into serotonin in the brain by the pancreas secreting insulin. Ingesting cakes and carbohydrates is the fastest way to stimulate secretions of insulin from the pancreas. Other functions of serotonin include pain reduction, sleep

regulation, circadian rhythm, and excitatory involvement in the regulation of prolactin secretion.

Chatter Jea and Shinde (2006) reported that the functions of serotonin are diverse from acting as potent vasoconstrictor meaning leading to constriction of blood vessels also produces contraction of smooth muscles that is the muscles of internal organs and a stimulator of cerebral activity as it stimulates the brain functions and excitations also of important to say that deficiency of serotonin produces depress ant effect and the increased concentration of serotonin affects mood and wellbeing, they also added that serotonin in the brain is in a bound form and that drugs like reserpine act to release serotonin from its bound form

Guyton and Hall (2006) described how serotonin works to facilitate memory by stimulating the facilitator presynaptic terminal and releasing it at the sensory nerve's surface. Furthermore, serotonin acts on serotonin receptors in the membrane of the sensory terminal; these receptors activate the membrane's adenyl cyclase enzyme, which causes cAMP to form inside the sensory presynaptic terminal. This process, at the end, causes the neurotransmitter serotonin to stimulate memory.

According to Noura (2005), the body uses the amino acid arginine to produce nitric oxide (NO). It is an amazing molecule with a variety of biological functions, including PG synthesis. An enzyme known as "nitric oxide synthase," a cytosolic enzyme, acts on arginine to produce nitric oxide (NO) and citrulline. The following summarizes the functions of nitric oxide:

. It relaxes smooth muscle and serves as a vasodilator.
. It plays a significant role in regulating blood pressure and blood flow.
. It acts as a neurotransmitter in the peripheral autonomic nervous system and the brain.
. It prevents platelets from adhering, activating, and aggregating.
. It may mediate the bactericidal effects of macrophages and could be a component of a primitive immune system.
. It is involved in penile erection
. It is involved in the pathogenesis of atherosclerosis
. It is involved in angiogenesis

Table (1) reported that there is a significant change expressed by decreased Malondialdehyde and significant increased superoxide dismutase meaning that fish oil administration for 4 weeks decreased Free Radicals while increasing

antioxidant and benefit health and immunity of the experimental group. This Result is in accordance with that of Guyton and Hall (2006) that Free Radicals may be harmful to tissues due to stresses, exercises or Radiations

The significant decreased malondialdehyde together with the significant increased antioxidant, superoxide dismutase (SOD) Table (1) indicated that omega 3 consumption for 4 weeks / daily influenced the decreased Free Radicals while increasing (SOD) and benefit health and immunity of the body which is in accordance with Heshmat et al. (2013) they added that free radicals may be harmful to tissues due to stresses, exercise or radiations and the antioxidant (SOD) function is to protect the body against the potential deleterious effects of superoxide anion , also (SOD) uses oxygen as a substrate converting it into H2O2 (Ganong, 2000)

The antioxidant superoxide dismutase can destroy the superoxide ions, and the function of SOD is to protect aerobic organism against the potential deleterious effects of superoxide anion, also SOD uses oxygen as a substrate converting it into H2O2., which can be used by antioxidants such as glutathione peroxidase. This denotes the beneficial action of Fish oil as health promoter and immunity stimulant. Ganong (2000).

Davis et al. (1987) reported the mechanism of action of glutathione peroxidase in protecting red blood cells (RBCs) against hemolysis. Oxidized glutathione (GSSG) is reduced to reduced glutathione (2 G-SH) through the action of glutathione reductase. In turn, glutathione peroxidase, an enzyme that functions as a natural antioxidant, attacks peroxides and hydrogen peroxide (H_2O_2). It catalyzes the removal of H_2O_2 from RBCs using reduced glutathione. Additionally, glutathione peroxidase is associated with the incidence of cancers due to its selenium content.

Studies were conducted using medicinal plants as feed additives instead of antibiotics to reduce antibiotic Resistance (Bean, 2003). IL 6, TNF IL-1B level was significantly increased in case of omega 3 supplementation compared to control group. This is a beneficial effect as it may counteract effect of inflammation through the role played by IL1B, IL-6 through up regulation of peroxisome proliferatory activated receptor-y (PPAR-y) activation (Lee and Vilcek, 1989) which is explained that omega 3 may enhance immunity by stimulating IL 1B, IL6 functions Reporting the most updated studies explaining How omega 3 affect positively rat brain

Fahmi (2021) reported the protective effects of omega 3 and saffron extract on the cerebellum of albino rats.

Radwan et al (2023) stated the protective effect of omega 3 in adult male albino Rats

Mazroa and Mohamed (2023) reported the effect of omega 3 on ameliorated muscle alterations, decreased mast cells activated satellite cells.

Amborkar et al (2019) stated that omega 3 showed significant effect on depressed rats using escitalopram

Conclusion:

It may be concluded that Omega 3 supplementation improves cognitive performance and modulates immune responses through mechanisms involving synaptic plasticity microglia regulation and cytokine suppression

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