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Potential Production of Omega Fatty Acids from *Scenedesmus obliquus* Microalgae: Evaluation of Acute and Chronic Toxicity

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Abstract

Background: Omega-3 fatty acids (O3FA) as dietary supplements for medical use improve health. Methods: Acute and chronic toxicity were done using male and female Wistar rats. Results of acute toxicity showed no behavioral alterations, toxicity, or mortality in treated animals at a dose of 2 g/kg over 24 hours. In chronic study, no significant difference in the serum glucose, ALT, AST, bilirubin, albumin, and creatinine between treated and control rats were detected. However, there was a slight increase in the level of ALP and urea in male rats treated with O3FAs. Chronic administration of O3FAs with a dose of 100 mg/kg b.w. /day for 12 weeks showed insignificant change in hematological parameters. However, the red cell distribution width (RDW-CV) was significantly decreased. Histopathological examinations indicated slight congestion in the glomerular capillaries and peri-tubular capillaries in the renal tissues. Meanwhile, hepatic parenchyma with slight congestion in the central vein and blood sinusoids has been detected. It could be concluded that, the daily use of 100 mg/kg of O3Fas for 12 weeks did not produce marked alterations in biochemical, hematological, and histopathological parameters and it is relatively safe and can be used as an adjuvant for treating various indicated diseases.

Keywords: Omega fatty acids, S. obliquus, hematological parameters, Biochemical parameters, acute toxicity , chronic toxicity , histopathological examination

1. Introduction

Omega-3 fatty acids (O3FAs) are important bioactive nutrients for human health. O3FA is a polyunsaturated fatty acid. Lipid-derived biomolecules such as phospholipids (PLs) and triglycerides (TGs) are detected by the position and number of double bonds [1]. Omega fatty acids are classified into two different classes omega-6 and omega-3 polyunsaturated fatty acids (PUFAs), the latter of which are synthesized from alpha-linolenic acid (ALA; 18: 3), and the former, from linoleic acid (LA, 18: 2) (Deckelbaum et al., 2012). Two very powerful fatty acids are not synthesized in the body and must be obtained from the diet [2] . Omega-6 fatty acids (O6FA) contain linoleic, gamma-linolenic, and arachidonic acids, while O3FAs are made up of DHA, DPA, ALA, stearidonic acid (SDA) and EPA commonly called docosahexaenoic acid, docosapentaenoic acid, stearidonic acid, and stearidonic acid and eicosapentaenoic acid [3]. Studies have shown that DHA and EPA are long-chain omega-3 that contain PUFAs. DHA, produced from microalgae, long-chain polyunsaturated O3FAs are considered important for heart, brain, and eye functioning throughout the life cycle [4] .DHA is useful in the preparation of pregnancy formulas, nutraceuticals, beverages, and food products [5]. According to World Health Organization (WHO) protocol, the consumption of two-thirds of oily fish per week meets the dietary requirements of EPA and DHA (200-500 mg) [4]. Comparable doses have also been approved by other medical organizations around the world [6].

Aquatic organisms of marine origin are the main source of O3FA [7]. The majority of O3FAs are composed of phytoplankton and microalgae, which are then converted into fish and aquatic mammals across the food cycle process [8]. Microalgae, Schizochytrium sp.,

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and Crypthecodinium cohnii (C. cohnii) are the main sources of DHA [9]. Numerous studies have reported on PUFAs and their persistence in fresh fish and seafood with high plenty of these FAs (Rubio-Rodríguez et al., 2010). Because of the existence of heavy metals and toxic metals (mercury, cadmium, copper, and arsenic), their consumption rates have been significantly reduced [10]. In this respect, the majority of fish oils are unpalatable and unacceptable to vegetarians [7]. El-Sheekh et al. [11] concluded that *Scenedesmus obliquus*.was adopted as hopeful microalgae for large-scale derived lipid production due to the high biomass yield resulting in high lipid and fatty acid yields. Darki et al. [12] reported that *S. obliquus* contains high levels of total lipids (32%), polyunsaturated fatty acids (43.7%), and especially α-linolenic acid (28.4%). This study was designed to evaluate the acute and chronic toxicity of omega fatty acids extracted from *S. obliquus.*

2. Materials and Methods

2.1 .*Cultivation of S. obliquus*

Cultivation of *S. obliquus* was carried out in an indoor cultivation unit composed of plastic bottles with a capacity of 17 L containing an actual volume of 15 L of microalgal culture. The plastic bottles were incubated under continuous aeration and continuous white fluorescent light illumination providing a constant light intensity ≈of 2,500 lx for the culture. *S. obliquus* was grown on Bold's Nutrient media [13] containing 0.3 g/L of urea. The culture temperature was 22 ± 3°C. After 10 days of algal growth, the culture was transferred to a fully automated and computer-controlled vertical photobioreactor (PBR) with a capacity of 4000 L. Carbon dioxide was injected into the culture as a carbon source. The culture was left to grow until the biomass reached 2–2.5 g/L. Algal biomass was harvested by centrifugation at 2000 rpm for 15 min using a basket centrifuge. Algal biomass was washed twice with water, dried in an oven at 50 °C, then ground using an electrical grinding mill (FRITSCH Cross Beater Mill PULVERISETTE 16, Germany) to a fine powder. Pulverized algal biomass was stored in a deep freezer until further extraction.

2.2. Preparation of fatty acid extract

The pulverized algal material (25 g) was soaked with Hexane: Ethyl acetate (1:1, v/v) (250 mL, 3 times) in a 1000 mL conical flask and kept on an orbital shaker (Stuart, England) at 160 rpm at room temperature for 24 h.Then, the extract was centrifuged (Sigma 3-18ks Centrifuge, Germany) at 5000 rpm for 20 min at 25°C to separate cell debris from the supernatant. The extraction step was repeated twice and the pooled supernatants were concentrated using a vacuum rotary evaporator (Heidolph Unimax 2010, Germany) at 40°C to dryness giving the omega fatty acid enriched extract. The omega fatty acid-enriched extract was stored in the deep freezer until conducting the toxicity experiment.

2.3. Evaluation of Acute and Chronic toxicity of omega fatty acids-enriched extract of S. obliquus acute toxicity study

The acute toxicity of omega fatty acids-enriched extract of *S. obliquus* was evaluated in mice using the up and down procedure according to the Organization for Economic Cooperation and Development (OECD), guideline no. 423, 2001 (OECD, 2001) as the following; mice of either sex (five females and five males, weight 20-25 gm.) orally received the extracts starting at a dose of 2 g/kg. The animals were observed for toxic symptoms continuously for the first 4 hours after dosing. Each mouse was monitored for any kind of behavioral, physical, and pharmacological toxic effects. Signs that have been recorded during acute toxicity testing were any changes in normality concerning eyes, diarrhea, skin ulcers, hair loss, discoloration, abdominal cramps, lacrimation, salivation, hyper/hypoactivity, lethargy, neurological behavior (changes in motor activity), and/or deaths were recorded [14,15] . Finally, the number of survivors was noted after 24 hours and then maintained for additional 13 days with daily observations.

2.4. Chronic toxicity study

2.4.1. Experimental Animal

Forty Wistar rats weighing 150-200 g from both genders (20 each) were brought from the Animal Colony House of the National Research Centre, Giza, Egypt. The animals were housed in adequate conditions at room temperature 25±1°C with 40% relative humidity. The animals were allowed free access to food and drinking water.

2.4.2. Experimental design and biochemical analysis

Forty albino Wistar rats were randomly divided into four groups of 10 animals per sex. Group 1: normal male control rats received 0.5ml water/ day. Group 2 (tested male) rats received the extract at a dose 100mg/kg b.w. Group 3: normal female control received 0.5ml water /day. Group 4: (tested female) rats received the extract at a dose 100mg/kg b.w. Body weight and the animals were observed for signs of abnormalities throughout the study. Administration of the test doses to the animals in the respective groups is continued for three continuous months, while the control group animals still receive the vehicle.

All animals in the respective groups are carefully observed daily throughout the study. Weight-taking across the groups is also done every two weeks intervals. Mortality and other signs of toxicity are recorded. At the end of the third month of treatment, the animals were fasted overnight, and then lightanesthetized with diethyl ether and sacrificed by collecting blood samples from the venous plexus,

centrifuged at 3000 rpm, and the separated serum stored at -80 ºC for further biochemical examinations.

Hepatic function was evidenced by determining aspartate aminotransferase (AST), alanine aminotransferase (ALT) [16] , alkaline phosphatase (ALP) Belfield and Goldberg [17] , bilirubin, [18] and albumin. Renal function was evidenced by urea and creatinine [19, 20]. Moreover, serum glucose level was assessed according to the method of Teitz [21] . Hematological analysis was performed using an automatic hematological analyzer (Cell Dyn, Abbott). Hematological parameters were Hb, RBCs, HCT MCV, MCH, MCHC, RDW-CV, Platelet count, MPV, and WBCs. Moreover, Differential white cell counts were also determined [22].

Heart, liver, and kidney organs were collected then visually observed their shapes, sizes, and colors , weighed to determine the actual and relative organ weights, and preserved in 10% formalin solution. Tissue slides were stained with hematoxylin and eosin and histopathological examinations were performed. The histology of the test group's organs is compared with the control. Where there is mortality in a test group within the period of assessment, the animal is counted among the sacrificed, and the histology is compared with the control. Signs of organ tissue damage are recorded [23].

2.5. Statistical Analysis

The results are expressed as mean ± SE, and all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The data were analyzed with GraphPad Prism v. 5.0 (GraphPad Software, Inc., CA, USA). The difference was considered significant when the p-value is ≤ 0.05.

3. Results and Discussion

There are two main classes of polyunsaturated fatty acids (PUFAs): omega-6 (ω-6) and omega-3 (ω-3) PUFAs. Both are important components of human nutrition and play important roles in cell signaling, the structure and function of the membrane, and several physiological responses. Consuming omega-3 PUFAs has been shown to improve inflammatory and cardiovascular disease [24]. While, high consumption of ω-6-PUFAs is connected with inflammatory, cardiovascular, Alzheimer's diseases, and more [25]. Our previous results showed that the GC-FID fatty acid profile of *S. obliquus* contains a high percentage of unsaturated fatty acids with a high proportion of omega-3 and omega-9 fatty acids (Data not shown). In this consensus, different authors reported that *S. obliquus* contains high content of ω3 PUFAs, especially α-Linolenic acid along with a high ratio of ω-3/ω-6 PUFAs, which applies it as a suitable dietary supplements source for human health nutrition to reduce the risk of many chronic diseases [12,26] .

3.1. Evaluation of acute and chronic toxicity of O3FAs extracted from S. obliquus microalgae

The acute toxicity of O3FAs extracted from *S. obliquus* microalgae was investigated, and the results demonstrated no general behavioral alterations, toxicity, or mortality in tested animals up to a dose of 2 g/kg over 24 hours, indicating that the extract was non-toxic.

Animals were noticed separately after at least one dose in the first 30 min, periodically for the first 24 h, with particular observation during the first 4 h and daily thereafter, for a total of 14 days, except when they must be discontinued research and is humanely killed for animal welfare reasons or found dead. However, the observation time is not rigidly fixed. It is determined by adverse reactions, time of onset, and length of recovery time, and can therefore be prolonged if necessary. The timing of the appearance and disappearance of signs of toxicity is of great importance, especially if there is a tendency to delay signs of toxicity. All remarks were regularly recorded with individual records kept for each animal. Further observation is required if the animal continues to show signs of toxicity. Observations should include changes in skin and hair, eyes, and mucous membranes, as well as the respiratory, circulatory, autonomic, and central nervous systems, as well as somatomotor activity and behavior. Attention is drawn when tremors, convulsions, salivation, diarrhea, coma, somnolence, and coma are observed. Animals found in ill health and those exhibiting severe pain or showing signs of persistent pain are humanely killed.

Animal body weight was determined immediately before administration of the test substance and at least once a week thereafter. Weight changes were calculated and recorded. At the end of the experiment, the surviving animals were humanely weighed and sacrificed. All examined animals were autopsied by macroscopic methods. All overall pathological alterations were examined for each animal. Microscopic examination of organs showing evidence of gross pathology.

3.1. *Effects of chronic treatment of O3FAs on body weight*

The body weights of males receiving O3FAs at a dose of 100 mg/kg for 12 weeks showed a significant increase in the percentage change in body weight throughout the experiment. On the other hand, the female rats receiving O3FAs at a dose of 100 mg/kg for 12 weeks showed an increase in the percentage change in body weight yet was not significant (Table 1 and Figure 1). In contradictory to the present results García-Cervera et al. [27], declared that the effect produced by a dietary intake of gummies with O3FAs reduced the levels of BMI in fatty girls compared with fatty boys and controls (untreated fatty girls) at several doses. These data were supported by several previously reported studies [28], which indicate that the administration of O3FAs decreased the BMI in girls.

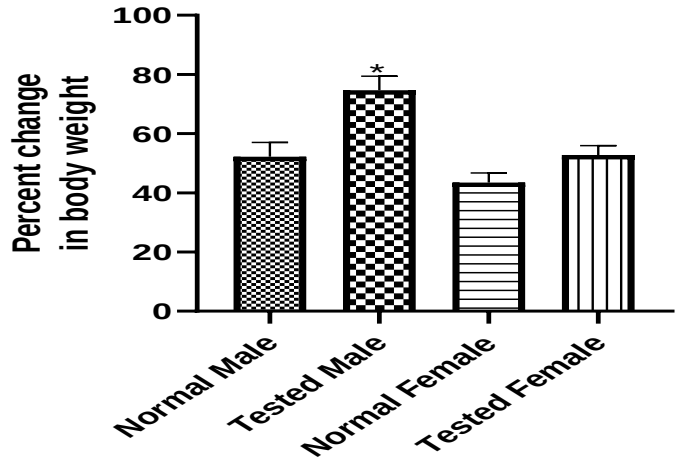

Figure (1): Effects of chronic treatment of O3FAs on percent change in body weight

The results are expressed as mean ± SE, all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.
*Significance difference from corresponding gender normal control group.

3.2. Effects of chronic treatment of O3FAs on serum biochemical parameters

3.2.1. Hepatic Toxicity

In male and female rats, no difference in the serum ALT, AST, bilirubin, and albumin was found between treated and normal rats. However, there was a slight increase in the serum level of ALP observed in male rats treated with O3FAs with a dose of 100mg/kg b.w./day for 12 weeks (Table 2).

3.2.2. Renal Toxicity

In male and female rats, no difference in serum creatinine was found between treated and normal rats. However, there was an elevation in the serum level of urea observed in male rats treated with O3FAs with a dose of 100mg/kg b.w./day for 12 weeks (Table 3).

Table (1): Effects of chronic treatment of O3FAs on percent change in body weight

	Normal Male	Tested Male	Normal Female	Tested Female
Percent change in body weight	**52.24±4.78**	**74.70±4.67***	**44.45±3.29**	**48.80±3.17**

Data are expressed as mean ± SE, all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.
*Significance difference from corresponding gender normal control group

Table (2): Effects of chronic treatment of O3FAs on serum hepatic function parameters.

Hepatic Function Parameters				
	Normal Male	**Tested Male**	**Normal Female**	**Tested Female**
ALT (U/L)	**14.71±1.45**	**16.14±1.49**	**14.42±0.83**	**15.71±1.46**
AST (U/L)	**74±2.76**	**83.42±2.52**	**84.85±3.23**	**86.57±3.13**
ALP (U/L)	**89.89±6.8**	**113.85±5.60***	**31.14±1.63**	**44.85±3.59**
Bilirubin (g/dl)	**0.108±0.022**	**0.135±0.028**	**0.139±0.016**	**0.176±0.02**
Albumin (g/dl)	**3.17±0.04**	**2.95±0.104**	**3.38±0.107**	**3.03±0.138**

The results are expressed as mean ± SE, all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.*Significance difference from corresponding gender normal control group.

Table (3): Effects of chronic treatment of O3FAs on serum renal function parameters

Renal Function Parameters				
	Normal Male	Tested Male	Normal Female	Tested Female
Urea (mg %)	**20±0.94**	**27.14±2.22***	**26.85±1.09**	**28±1.52**
Creatinine (mg%)	**0.54±0.07**	**0.64±0.03**	**0.80±0.05**	**0.72±0.042**

The results are expressed as mean ± SE, all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.
*Significance difference from corresponding gender normal control group.

3.2.3. Metabolic Toxicity

In male and female rats, no difference in the serum glucose levels was found between rats treated with O3FAs with a dose of 100mg/kg b.w./day for 12 weeks and control rats (Table 4). The present results are in agreement with the results of García-Cervera et al. [27] showed that dietary intake of omega-3 polyunsaturated fatty acids did not result in significant differences in glucose levels in either overweight boys or girls. Complex correlations with other lifestyle factors may be to blame. However, other studies indicate that O3FAs ameliorate the metabolism of glucose without significant effects on the degree of obesity in women [29]. The same authors confirmed that the administration of different doses of gummies with O3FAs did not significantly alter insulin levels in boys and girls; these data indicated that O3FAs have no insulin-releasing or synthesizing activity. Although these data are in contrast to other reports indicating that dietary intake of omega-3 fatty acids improve insulin secretion, [30].

3.3. Effects of chronic treatment of O3FAs on hematological parameters

3.3.1. Complete blood count

Chronic administration of O3FAs for male and female rats with a dose 100mg/kg b.w./day for 12 weeks showed no differences in hemoglobin (Hb), RBCs, hematocrit (HCT), MCV, MCH, MCHC, Platelet count, MPV, white blood cells (WBCs) between treated and non-treated male and female rats. However, the red cell distribution width (RDW-CV) in both treated rats was significantly decreased in treated rats (Table 5).

3.3.2. Differential white cell count

Chronic administration of O3FAs for male and female rats with a dose 100mg/kg b.w./day for 12 weeks showed no differences in differential white cell counts evidenced by neutrophilic, lymphocytic, and monocytic counts between treated and non-treated male and female rats (Table 6).

Table (4): Effects of chronic treatment of O3FAs on serum glucose level

Serum Glucose Levels				
	Normal Male	Tested Male	Normal Female	Tested Female
Glucose (mg%)	128±3.15	127±8.42	106.71±5.00	113±9.15

The results are expressed as mean ± SE, all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.

Table (5): Effects of chronic treatment of O3FAs on hematological parameters

	Ref. Range	Normal Male	Tested Male	Normal Female	Tested Female
Hb (g/dl)	13-15.5	12.78±0.34	12.09±0.46	11.59±0.5	13.13±0.34
RBCs (*10^6/cmm)	4.5-5.5	6.55±0.21	6.44±0.19	6.05±0.17	6.68±0.2
HCT (%)	40-50	38.04±1.02	31.66±4.27	34.4±1.50	37.45±1.12
MCV (fl)	82-99	58.29±1.21	57.14±0.98	56.71±1.96	55.71±0.39
MCH (pg)	27-31	19.86±0.13	19.00±0.83	19.29±0.44	19.57±0.28
MCHC (g/dl)	31-36	33.86±0.62	32.71±1.14	34.00±0.97	35.29±0.26
RDW-CV (%)	10-15.9	16.37±0.76	13.86±0.26*	16.9±0.57	14.8±0.17*
Platelet co (*10^3/cmm)	150-450	431.57±47.93	431.43±36.00	416±36.99	521.14±60.96
MPV (fl)	7.4-10.4	7.23±0.15	6.57±0.13	7.44±0.21	7.26±0.12
WBCs (*10^3/cmm)	4000-11000	7.51±0.59	5.51±0.62	9.2±1.78	8.95±0.93

The results are expressed as mean ± SE, and all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.
*Significance difference from corresponding gender normal control group

Table (6): Effects of chronic treatment of O3FAs on differential white cell count

Relative	Ref. Range	Normal Male	Tested Male	Normal Female	Tested Female
Neutrophilic count (*10^3/cm)	40-80	5.67±0.67	4.8±1.2	8.68±1.48	7.6±1.75
Lymphocytic count	20-40	84.44±0.91	86.15±1.8	78.05±2.91	83.08±2.38
Monocytic count	1--2	9.88±0.44	9.04±0.91	11.8±0.95	9.31±0.77

The results are expressed as mean ± SE, all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.

Table (7): Effects of chronic treatment of O3FAs on differential white cell count

Absolute	Normal Male	Tested Male	Normal Female	Tested Female
Neutrophilic count (*10^3/cm)	0.40±0.02	0.26±0.06	0.54±0.07	0.49±0.09
Lymphocytic count	6.3±0.55	4.74±0.53	5.64±0.73	6.46±0.56
Monocytic count	0.73±0.05	0.50±0.07	0.72±0.12	0.69±0.06

The results are expressed as mean ± SE, and all statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The difference was considered significant when the p-value is ≤ 0.05.

3.4. *Effects of chronic treatment of O3FAs on histopathological examination*

Upon gross examination of internal organs, no abnormal signs were observed. Histopathological results indicated slight congestion in the glomerular capillaries (arrow) and peri-tubular capillaries (arrows) (Fig.6; photomicrograph 1) in the renal tissues. Meanwhile, hepatic parenchyma with slight congestion in the central vein (arrow) and blood sinusoids (arrows) has been shown in the hepatic tissue (Fig7; Photomicrograph1).

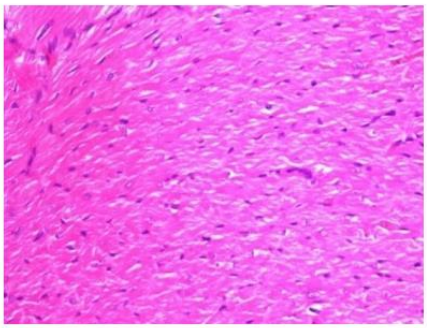

Figure (2): Heart from the normal female group showing normal myocardial muscle bundles with normal nucleation (M), (HE X400).

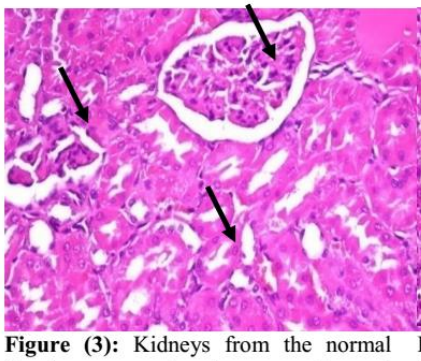

Figure (3): Kidneys from the normal female group showing normal renal parenchyma; note the normal glomeruli (G) and renal tubules (arrows), (HE X400).

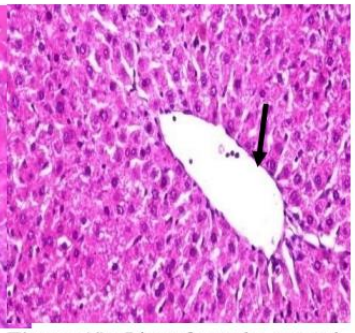

Figure (4): Liver from the normal female group showing normal hepatic parenchyma; note the hepatocytes. blood sinusoids, and central vein (arrows), (HE X400).

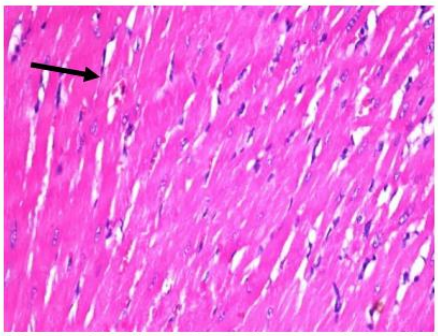

Figure (5): Heart from the treated female group showing healthy myocardial muscle bundles with normal nucleation (M), (HE X400).

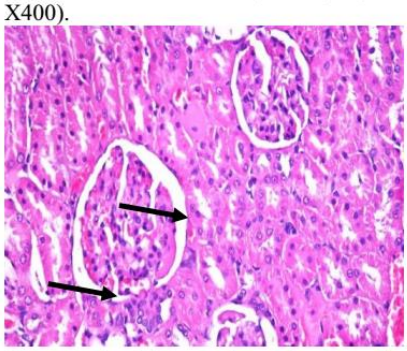

Figure (6): Kidneys from the treated female group showing healthy renal parenchyma with slight congestion in the glomerular capillaries (arrow) and peri-tubular capillaries (arrows), (HE X400).

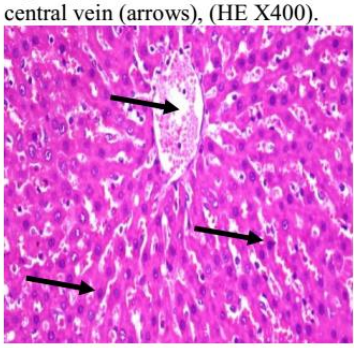

Figure (7): Liver from the treated female group showing healthy hepatic parenchyma with slight congestion in the central vein (arrowhead) and blood sinusoids (arrows), (HE X400).

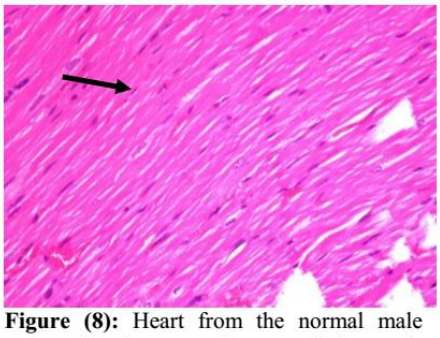

Figure (8): Heart from the normal male group showing normal myocardial muscle bundles with normal nucleation (arrow), (HE X400).

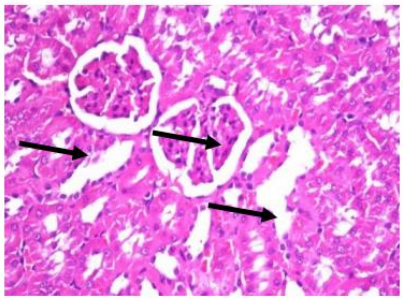

Figure (9): Kidneys from the normal male group showing normal renal parenchyma; note the normal glomeruli (G) and renal tubules (arrows), (HE X400).

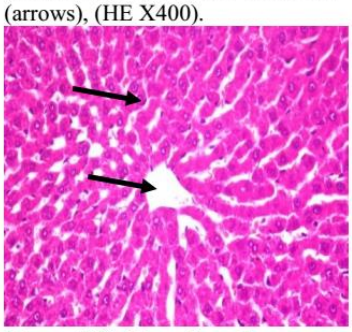

Figure (10): Liver from the normal male group showing normal hepatic parenchyma; note the hepatocytes (H), blood sinusoids, and central vein (arrows), (HE X400).

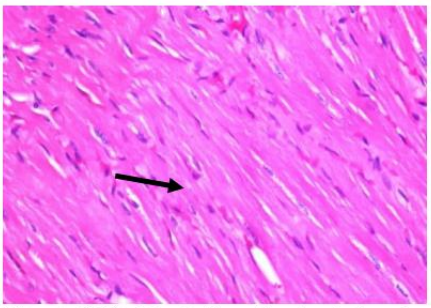

Figure (11): Heart from the treated male group showing healthy myocardial muscle bundles with normal nucleation (arrow), (HE X400).

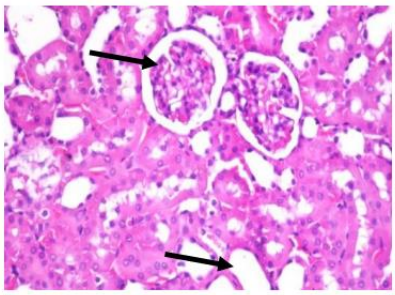

Figure (12): Kidneys from the treated male group showing healthy renal parenchyma; note the normal glomeruli (G) and renal tubules (arrows), (HE X400).

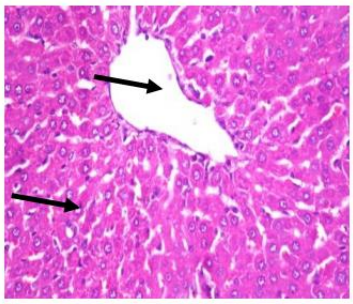

Figure (13): Liver from the treated male group showing healthy hepatic parenchyma; note the hepatocytes (H), blood sinusoids, and central vein (arrows), (HE X400).

Photomicrograph 1: Histopathological alterations of the effect of the O3FAs extracted from *S. obliquus* microalgae on different organs

The present study represents the novel first results deal with the acute and chronic study of the omega 3 fatties extracted from *S. obliquus* microalgae on different biochemical, hematological, and histopathological biomarkers. Polyunsaturated fatty acids (PUFAs) have a promising role in the diet of humans, both for the protection and treatment of various pathologies. Particularly, the effectiveness of O3FAs in improving the lipid pattern and excitability of the myocardium was determined, and hence, their utility in the protection of cardiovascular diseases and arrhythmias after infarction. Since PUFAs are prostaglandins and leukotrienes precursors, which are implicated in phlogosis and immune responses, a diet rich in fish oil that decreased the production of PGE2 is implicated in several cases of phlogosis. In addition, the elevation in the eicosapentaenoic acid (EPA) levels resulted in a decrease in inflammatory cytokines (interleukins 1, 2, 6 and tumor necrosis factor) production; so, it is critical to apply omega-3 in chronic inflammatory diseases, such as rheumatoid arthritis. It appears that omega-3 may protect against the occurrence of hormone-dependent tumors (i.e. breast and prostate cancers).*In vitro* studies revealed that the series 2 PGs, derived from omega-6, have carcinogenic effects; however, the effects of anticancer of omega-3 may be derived from their antagonistic effects on the formation of these PGs; it may therefore be helpful to increase the omega-3/omega-6 ratio in the diet. In addition, the promising effects of omega-3 on the development of the central nervous system and their potential clinical use in certain psychiatric conditions have been established [31] .The current study run in parallel with the results of García-Cervera et al. [27], suggested that O3FAs markedly ameliorate the concentration of total cholesterol in overweight girls compared with overweight boys and controls. However, it is noteworthy that some reports demonstrated that O3FAs, in addition to the reduction in total cholesterol levels, also may produce a lowering in blood pressure level in a dose-dependent manner in overweight girls compared with overweight boys and controls (untreated overweight children) [32] . These data are comparable to other results indicating that O3FAs can induce a reduction in blood pressure in hypertensive patients compared to control (no change in blood pressure level in control subjects [33].

4. Conclusion

Acute toxicity study of omega fatty acids of *S. obliquus* microalgae revealed no significant difference between control and treated mice administered 2g/kg for 24 h. Meanwhile, the results suggested that long-term oral administration of O3FAs at a dose of 100 mg/kg extracted from algae is relatively safe and can be used as an adjuvant for treating various indicated diseases.

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6. Conflict of interest

The authors declare none

7. References

[1] Fahy E., Subramaniam S., Brown H.A., Glass C.K., Merrill A.H. Jr, MurphyR.C., Raetz, C.R., Russell D.W., Seyama Y., Shaw W., Shimizu T., Spener F., van Meer G., Van Nieuwenhze M.S., White S.H., Witztum J.L., Dennis E.A, A comprehensive classification system for lipids. *Journal of Lipid Research*, 46(5), 839-861(2005).

[2] Lobo V., Patil A., Phatak A., Chandra N., Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 4(8), 118-126 (2010).

[3] Simopoulos A.P., An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*, 8(3), 128(2016).

[4] Gupta G., Gupta R., Nutraceutical Status and Scientific Strategies for Enhancing Production of Omega-3 Fatty Acids from Microalgae and their Role in Healthcare. *Current Pharmaceutical Biotechnology*, 21, 1616-1631(2020).

[5] Adarme-Vega T.C., Thomas-Hall S.R., Schenk P.M., Towards sustainable sources for omega-3 fatty acids production. *Curr Opin Biotechnol.* 26, 14-18(2015).

[6] Kris-Etherton P.M., Akabas S.R., Bales C.W., Bistrian B., Braun L., Edwards M.S., Laur, C., Lenders C.M., Levy M.D., Palmer C.A., Pratt C.A., Ray S., Rock C.L., Saltzman E., Seidner D.L., Van Horn L., The need to advance nutrition education in the training of health care professionals and recommended research to evaluate implementation and effectiveness. *Am J Clin Nutr.*, 99(5 Suppl.), 1153S-1166S (2014).

[7] Adarme-Vega T.C., Lim D.K., Timmins M., Vernen F.,Li Y., Schenk P.M., Microalgal biofactories: A promising approach towards sustainable omega-3 fatty acid production. *Microb Cell Fact*. 11, 96(2012).

[8] Martins D.A.,Custódio L., Barreira L., Pereira H., BenHamadou R., Varela J., Abu-Salah K.M., Alternative sources of n-3 long-chain polyunsaturated fatty acids in marine microalgae. *Mar Drugs*, 11(7), 2259-2281(2013).

[9] Senanayake S.P.J.N., Fichtali J., Single-cell oils as sources of nutraceutical and specialty lipids: Processing technologies and applications. Nutraceutical and Specialty Lipids and their Co Products; Shahidi F., Ed.; CRC Press: Boca Raton FL., pp. 251-280(2006).

[10] Jaishankar M., Tseten T., Anbalagan N., Mathew B.B., Beeregowda K.N., Toxicity, mechanism and health effects of some heavy metals. *Interdiscip Toxicol*., 7(2), 60-72(2014).

[11] El-Sheekh M., Abomohra A.E.F. , Hanelt D., Optimization of biomass and fatty acid productivity of Scenedesmus obliquus as a promising microalga for biodiesel production. *World Journal of Microbiology and Biotechnology*, *29*(5), 915-922 (2013).

[12] Darki B.Z., Seyfabadi J., Fayazi S., Effect of nutrients on total lipid content and fatty acids profile of *Scenedesmus obliquus*. *Brazilian Archives of Biology and Technology*, *60* (2017).

[13] Rajendran L., Nagarajan N.G., Karuppan M. Enhanced biomass and lutein production by mixotrophic cultivation of Scenedesmus sp. using crude glycerol in an airlift photobioreactor. *Biochem Eng J.* 161, 107684 (2020).

[14] Botham PA. Acute systemic toxicity—prospects for tiered testing strategies. *Toxicol in Vitro*, 18,227–230(2004).

[15] Saganuwan SA., Toxicity study of drugs and chemicals in animals: An overview. *Bulgarian Journal of Veterinary Medicine*, 20(4), 291-318(2016).

[16] Reitman S., Frankel S., A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology* ,28, 56-63(1957).

[17] Belfield A., Goldberg D.,. Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. *Enzyme* ,12, 561-573(1971).

[18] Walter M., Gerade, H., A colorimetric method for determination bilirubin in serum and plasma. *Micro Chem J*. 15, 231-236 (1970).

[19] Bartels H., Böhmer M., Heierli C., [Serum creatinine determination without protein precipitation]. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 37, 193-197(1972).

[20] Fawcett J.K., Scott J.E.,. A rapid and precise method for the determination of urea. *Journal of clinical pathology* 13, 156-1591(960).

[21] Teitz N., Determination of blood glucose using 4-aminophenazone. Fundamentals of Clinical Chemistry, Philadelphia, WB Saunders Trinder Publications(1970).

[22] Thrall M.A. Veterinary Hematology and Clinical Chemistry. Lippin-cott Williams and Wilkins, Baltimore, Maryland (2004).

[23] Bancroft J., Stevens A. Theory and practice of histological techniques. 4th ed. Churchil Livingstone, Edinburgh, United Kingdom, (1996).

[24] La Rovere M.T., Christensen J.H., The autonomic nervous system and cardiovascular disease: role of n-3 PUFAs. *Vascular Pharmacology*, 71, 1-10(2015).

[25] Patterson E., Wall R., Fitzgerald G.F., Ross R.P., Stanton C., Health implications of high dietary omega-6 polyunsaturated fatty acids. *Journal of Nutrition and Metabolism*, 539426(2012).

[26] Salama E.S., Kim H.C., Abou-Shanab R.A., Ji, M.K., Oh Y.K., Kim S.H., Jeon B.H., Biomass, lipid content, and fatty acid composition of freshwater Chlamydomonas mexicana and Scenedesmus obliquus grown under salt stress. Bioprocess and biosystems engineering, 36(6), 827-833(2013).

[27] García-Cervera E., Figueroa-Valverde L., Gómez E.P., Rosas-Nexticapa M., Virginia M.A., Perla P.G., Regin C.C., Biological activity exerted by omega-3 fatty acids on body mass index, glucose, total cholesterol and blood pressure in obese children. Integr Obesity Diabetes 4: (2018).

[28] Oh S.Y., Ryue J., Hsieh C.H., Bell D.E. Eggs enriched in omega-3 fatty acids and alterations in lipid concentrations in plasma and lipoproteins and in blood pressure. *Am J Clin Nutr*. 54, 689-695(1991).

[29] Baur L., O'Connor J,. Pan D., Storlien L., Relationships between maternal risk of insulin resistance and the child's muscle membrane fatty acid composition. *Diabetes*, 48, 112-116 (1999).

[30] Lardinois C.K., The role of omega 3 fatty acids on insulin secretion and insulin sensitivity. *Med Hypotheses*, 24,243-248 (1987).

[31] La Guardia M. , Giammanco S., Di Majo D., Tabacchi G., Tripoli E., Giammanco M .Omega 3 fatty acids: biological activity and effects on human health. *Panminerva Med*. 47(4), 245-57(2005).

[32] Friday K., Childs M., Tsunehara C., Fujimoto W., Bierman E., Elevated plasma glucose and lowered triglyceride tevels from Omega-3 Fatty Acid supplementation in Type II Diabetes. *Diabetes Care*, 12,276-281 (1989).

[33] Rafraf M., Mohammadi E., Asghari M., Farzadi L. Omega-3 fatty acids improve glucose metabolism without effects on obesity values and serum visfatin levels in women with polycystic ovary syndrome. *J Am Coll Nutr*. 31, 361- 368. (2012)

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