

# Assessment, analysis & evaluation of omega-3-fatty acid concentrate in hypercholesterolemia induced atherosclerosis animal model

## Abstract

Atherosclerosis involves a complex interplay of inflammation, endothelial dysfunction and hyperlipidemia. In the current, epidemiological scenario a vast majority of mortality world-wide is attributable to demolition of vulnerable atherosclerotic plaques. Marine omega-3-fatty acids armed with anti-inflammatory, anti-hypolipidemic and antihypertensive properties are of potential use in clinical management of atherosclerosis. The main purpose of this study was to evaluate the effect of marine omega-3-fatty acid concentrate in atherosclerosis induced animal model. New Zealand white rabbits fed a restricted amount of an atherogenic diet (AD) showed mild and persistent hypercholesterolemia. They developed atherosclerotic lesions 4 weeks after regular exposure to atherogenic risk factors. The levels of cholesterol, triglycerides and apo-lipoprotein were measured and the pharmacokinetic analysis of atherosclerotic lesions in the blood of these animals was performed at the end of the study duration. The omega-3-fatty acid treatment led to a significant dose dependent decrease in blood triglyceride levels, along with decrease in serum apolipoprotein level. Also on performing comparative analysis the bioavailability of omega-3-fatty acids concentrate was found to be more as compared to omega-3-fatty acids administered as marine fish oil. Preliminary studies suggest that the omega-3-fatty acid concentrate demonstrates potential therapeutic efficacy in atherosclerotic animal model.

**Keywords:** atherosclerosis, hyperlipidemia, omega-3-fatty, apolipoprotein, pharmacokinetic

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## Introduction

Atherosclerosis was originated from the Greek root word for "gruel" and "hardening" atherosclerosis or arteriosclerotic valvular disease (ASVD) is popularly envisaged as a multifaceted interaction of inflammation, hyperlipidemia and endothelial dysfunction resulting in accumulation of lipids within the arterial walls leading to a subsequent cascading series of events, that eventually advances to form intimal lesions called the atheromas or atherosclerotic plaque. Eventually these atheromas cause narrowing, hardening, and/or complete occlusion of the affected arteries, in case the plaque ruptures.<sup>1</sup> The modifiable risk factors of the disease include diabetes or impaired glucose tolerance, dys-lipoproteinemia characterized by an LDL: HDL ratio greater than 3:1. Hypertension, hyperlipidemia and cigarette smoking together accounts for approximately sevenfold increase in the risk of atherosclerosis. The non-modifiable defined as advanced age, male gender family history associated with the complication of atherosclerosis and genetic abnormalities viz familial hypercholesterolemia. The risk factors upon aggravation induce endothelial dysfunction consequently initiating atherogenesis.<sup>2</sup> An athermanous lesion begins as a fatty streak, develops into an intermediate lesion, followed by formation of athermanous plaque that is vulnerable to rupture and, finally, enters an advanced obstructive, stage, completely occluding the effected arteries.<sup>3</sup>

According to the medicinal point of view, the scale of coronary episodes follows the atherosclerotic sign estrangement. Sudden mortality to serious illness can occurs due to these coronary episodes

associated with asymptomatic effects. These clinical proceedings associates with noteworthy decline in blood flow supporting myocardium distal to the point of acute plaque rupture, creating disturbed angina.<sup>4</sup> Depending on the site of atherogenesis the cardinal complications of the disease include Myocardial infarction, Stroke, Renal ischemia, intermittent claudication.<sup>5</sup> Omega 3 fatty acid are class of essential fatty acid composing of poly unsaturated fatty acid molecule with carbon-carbon double bond at the 3<sup>rd</sup> carbon atom. These fatty acid composed of 2 ends, the methyl (CH<sub>3</sub>) and the acid (COOH) end. Most popular n- fatty acid comprise eicosapentaenoic acid (EPA, 20 carbon and the 5 double bonds), docosahexaenoic acid (DHA, 22 carbons and 6 double bonds) and  $\alpha$ - linolenic acid (ALA, 18 carbon and 3 double bonds).<sup>6</sup> Cold water fish are the most widely available dietary source of EPA and DHA which includes species like Salmon, Herring, Mackerel and Sardines etc. The oil obtains from these fish's exhibits seven folds more n-3 and n-6 profile. DPA (Docosapentaenoic acid with 22:5 n-3 ratio) is a long chain n-3 PUFA metabolite of EPA is found in marginal amount in fishes.<sup>7</sup>

From the performed literature review it has been elucidated that Omega 3 Fatty acid supplementation possess preventive role in cardiovascular diseases.<sup>8</sup> They have mild antihypertensive effects. Evidently, n-2 fatty acids reduce blood triglycerides levels,<sup>9</sup> thereby providing preventive action in atherogenesis while consistent consumption may decrease the menace of secondary and primary heart attack. The atherosclerosis consist a vital inflammatory element and critical cardiovascular episode can be start by inflammatory mechanism arising in progressive commemoration. The omega 3 fatty

acid impacts inflammation mechanism via diverse process in which many of them initiate or linked with the fatty acid configuration of cell membrane. The human inflammatory cells are rich in n-6 fatty acid arachidonic acid but the extent of arachidonic acid, EPA and DHA can be varied through oral delivery of EPA and DHA. EPA gives rise to Eicosanoids which are having important role in inflammation but biologically they are weak in action. Also EPA and DHA plays vital role in production of resolvins which are anti-inflammatory and inflammation determining molecules. Therefore, the fatty acid pool of human inflammatory cells affects the function of EPA, DHA and arachidonic moieties greatly. Also the PUFA, the marine n-3 polyunsaturated fatty acid subsidizes the protective function towards atherosclerosis and plaque ruptures.<sup>10</sup>

## Material and methods

### Materials

Cod liver oil was purchased from Aventis pharma. limited (Sanofi consumer health care division) Ankleshwar, India. The chemical and reagent are procured from the Hi-media Pvt. ltd. and were of analytical grade.

### Concentration of Omega 3 fatty acid

The preparation of Omega 3 fatty acid concentrates was performed as follows: Saponification was done by mixing 100gm of cod liver oil with 200ml of Sodium hydroxide (NaOH) solution previously prepared by solubilizing 480gm of NaOH and 5gm Na<sub>2</sub>EDTA in 1.6L of ethanol and stirred for 30 min at 60°C and stirred (Figure 1). After that, 25ml hexane was added and the obtained solution was carefully stirred for one hour to extract out fatty acids. The upper layer consist unsaponifiable material which was then eliminated (Figure 2). Hydrochloric acid (HCl) was then added to the lower layer and it was continued stirred till attaining the value *pH*. Finally the 2 layers was formed and the lower layer was eliminated (Figure 3) and the upper layer (hexane layer) was evaporated at 30°C. Further the obtained Omega 3 fatty acid extract was then mixed to the hot solution (temp. 60-65°C) in methanol and stirred at constant rate. The quantity of methanol mixed to 25gm fish oil was 200ml. Heating was done to obtain clear solution. Urea crystal was formed at 10°C which was separated from the parent liquor by mean of filtration and the parted Omega 3 fatty acid in the filtrate was extracted with 1L of hexane and 500ml of conc. HCl solution. The obtained mixture was proceeds for careful agitation for about one. The hexane layer was parted and hexane was evaporated at 30°C to obtain fatty acids.<sup>11</sup>



Figure 1 Saponification achieved on electrical water bath.



Figure 2 Formation of two layers.



Figure 3 Separation of hexane layer.

### Characterization of Omega 3 fatty acid concentrate and Fish oil

The qualitative characterization of the fish oil and the Omega 3 fatty acid concentrate at ideal conditions were assessed according to the Food grade fish oil standard from IFOMA (International association of Fish meal and oil manufacturers).

**Preformulation studies:** The preformulation studies include analysis of those physicochemical properties of active molecule that plays vital role in the therapeutic efficacy and designing of an efficacious dosage along with responsible for the unwanted side effects.<sup>12</sup> The isolated concentrate was imperiled to various preformulation studies.<sup>13</sup> They are as follows:

**Physical appearance:** The obtained Omega-3 fatty acid concentrate was physically and microscopically rationalize for the physical appearance.

**Solubility profile:** Solubility of Omega-3 fatty acid concentrate was resolute in different solvents. Omega-3 fatty acid concentrate (2ml) was deferred in 10-20 ml of different solvents in tightly closed test tubes. These tubes were then carefully shaken for about 24 hrs using wrist action shaker (Yorco, New Delhi) and obtained solubility was recorded.<sup>14</sup>

**Identification test for alkaloids:** Alkaloids test involve mixing about 2ml of Omega-3 fatty acid concentrate to 10ml methanol which was then carefully filtered. In the obtained filtrate 2ml of 1% HCl was added followed by addition of 5-10 drops of Mayer's reagent/Wagner's reagent/Dragendorff's reagent. Appearance of creamish/brown/red/orange color or precipitates indicates the presence of alkaloids in the Omega 3 fatty acid concentrates.<sup>15</sup>

**Identification test for saponins:** In the identification of saponins about 1-3ml of filtered concentrate was added in 10ml of distilled water. The persistence of frothing indicates the presence of Saponins in Omega-3 fatty acid concentrate sample.<sup>16</sup>

**Identification test for tannins:** Analysis of tannins includes addition of 2ml of Omega-3 fatty acid concentrate in 10ml distilled water. The mixture was then filtered by using Whatman's filter paper. After that about 2ml of Ferric chloride solution was added in 5ml of filtered concentrate. The black precipitate indicates the presence of Tannins & Phenols.<sup>17</sup>

**Identification test for flavonoids:** In the analysis of flavonoid contents about 2-5ml of fatty acid concentrate was mixed with about 10ml of ethanol and filtered. After that 2ml filtrate was taken and concentrated HCl solution was added. Formation of Mg ribbon pink or tomato pinkish red color indicates the presence of flavonoids in the fatty acid concentrate sample.<sup>18</sup>

**Identification test for protein:** In the analysis of proteins about 2-5 drops of millions reagent was added in the Omega-3 fatty acid concentrate solution and was mixed thoroughly and heat. Appearance of white precipitates which turns brick red after boiling confirms the presence of protein in the Omega-3 fatty acid concentrate sample.<sup>19</sup>

**Identification test for reducing compound:** In the analysis of reducing compound, the f Omega-3 fatty acid concentrate was treated with about 2-5ml of Fehling A and B solution and was boiled for 5min. the formation of brick red color or precipitates confirms the presence of reducing compound.<sup>20</sup>

**Identification test for cardiac glycosides:** In 2-5ml of Omega-3 fatty acid concentrate 70% of alcohol was added and was boiled for 2-3 minutes and filtered. 5ml filtrate was pipette out and 5-10ml of distilled water was added in it, to this solution 5ml of chloroform was added with vigorous shaking and allowed the separation of 2 layers. Chloroform layer was then pipette out and evaporated in porcelain dish. The residue was dissolved in 3-5ml of glacial acetic acid containing 5% FeCl<sub>3</sub> solution. Then 2ml of conc. sulphuric acid was added along the walls of test tube, reddish brown ring formation at the junction of 2 layers confirms the presence of glycosides.<sup>21</sup>

### Partition coefficient

About 5 of Omega 3 fatty acid concentrate was carefully and precisely weighed and transferred in a clean volumetric flask exhibiting 25ml capacity previously contained 10ml each of immiscible phase, containing 10ml each of two immiscible phases, n-octanol (oil phase) and distilled water (aqueous phase). The flask was shaken using wrist action shaker (York, India) for 24hr. Finally the two phases were separated using a separating funnel and aqueous phase was analyzed spectrophotometrically for the amount of drug after suitable dilution.

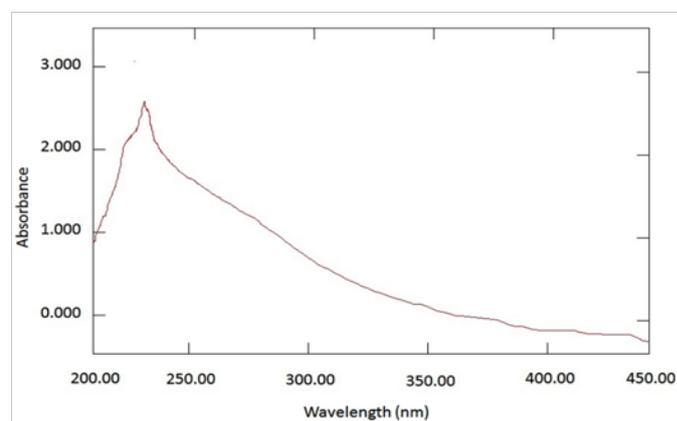
The partition coefficient was calculated using the formula:-

$$P_{O/W} = \frac{C_o}{C_w}$$

Where C<sub>o</sub>=Concentration of drug in n-octanol phase, C<sub>w</sub>= concentration of drug in water, P<sub>o/w</sub>=Partition coefficient.<sup>22</sup>

### Ultra Violet (UV) spectroscopy

The qualitative analysis of the bioactive can be performed by determination of absorption maxima of the compound by mean of ultra-violet (UV) spectroscopy; it estimates the drug concentration in the range between 2- 20µgm/ml in suitable solvent. The absorption maxima are defined as the wavelength corresponding to the maximum absorption and are denoted by λ<sub>max</sub> over which more versatile quantitative results are obtained. 20µg/mL sample of drug in solvent n-hexane was taken in 1cm<sup>2</sup> standard cuvette and scanned in the range of 200–400nm in Shimadzu 1240 UV/visible Spectrophotometer.<sup>22</sup> The wavelength for maximum absorption (λ<sub>max</sub>) was noted and reported in Figure 4.



**Figure 4** Absorption spectrum of omega-3-fatty acid concentrate.

**Preparation of calibration curve:** The stock solution of Omega 3 fatty acid concentrate was prepared by carefully dissolving 10mg of the concentrate in 100ml of n-hexane solution. Standard solutions of the oil were prepared from stock solution in the concentration range of 10-100µg/ml in 10ml volumetric flask using n-hexane as solvent. The absorbance of omega-3-fatty acids standard solutions was measured at 247nm (λ<sub>max</sub> for omega-3-fatty acid), against n-hexane as blank. Calibration curve was plotted between absorbance and concentration (Figure 5 & Table 1). The standard curve was linearly regressed and statistical parameters related to it were derived.<sup>23</sup>

**Table 1** Organoleptic features of the bioactive

| Parameters | Description                            |
|------------|--|
| Colour     | Clear pale yellow viscous liquid (oil) |
| Odour      | Characteristic fishy                   |
| Taste      | Bitter                                 |

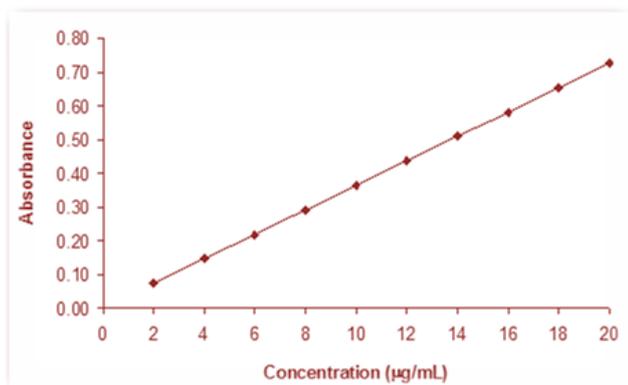


Figure 5 Calibration curve of omega-3-fatty acid concentrate.

### Fourier transformed infra-red (FT-IR) spectroscopy

A Perkin Elmer (Waltham, Massachusetts, USA) was employed to attain FTIR spectra (system 2000) and applied for pure fraction assessment. About 15mg of Omega 3 fatty acid concentrate was mixed thoroughly with potassium bromide (100mg) by triturating in a glass pestle mortal and compressed in to pellets. These pellets were further processed for IR spectra. The IR spectrum obtained (Figure 6) and important bands with interpretation are given in Table 2.

Table 2 Solubility profile of the bioactive

| S No | Solvent         | Solubility profile |
|------|-----------------|--------------------|
| 1    | Distilled water | -                  |
| 2    | Methanol        | -                  |
| 3    | Ethanol         | -                  |
| 4    | Chloroform      | -                  |
| 5    | Benzene         | ++++               |
| 6    | n-Hexane        | ++++               |
| 7    | Petroleum ether | ++                 |

Keys:

++++: Freely soluble = 1-10 part of solvent.

++: Sparingly soluble = 30-100 part of solvent.

-: Insoluble = <10,000 part of solvent.

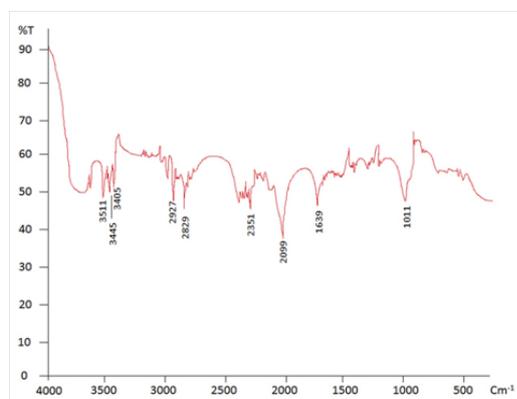


Figure 6 FT-IR Spectra of omega-3-fatty acid concentrate.

### Preparation of KBr (potassium bromide) pellet for IR assessment

- Dry Infrared grade of Potassium bromide (KBr) in a drying oven for at least 1hr.
- Grind approx. 15mg of Omega 3 fatty acid concentrate dried crystal in an agate mortar and pestle for about 20mins.
- Kbr-Omega 3 fatty acid concentrate mixture was placed in an evacuable die on a hydraulic laboratory press. Press *in vacuo* at about 15000 pounds of pressure for about 5minutes.
- The applied pressure was then releases and the die from press was removed, disassemble and the KBr pellets was eliminated. KBr pellets were then placed in a receptacle and finally drove into the sample beam of an Infrared spectrophotometer.
- The spectrum was then operated between 4000 to 6000cm<sup>-1</sup>.<sup>24</sup>

### Animal study protocol

#### Animal model

A total of 24 New Zealand white rabbits of 1.0-2.0kg of body weight, were conscripted and alienated into 4 groups having 6 animals in each test groups. Animal experimentation was conducted only after getting approval from Sagar Institute of Pharmaceutical Sciences, Sagar Institutional animal ethics committee, which is registered for the purpose of control and supervision of experiments on animals (CPCSEA), government of India. The animals were housed in rabbit cages and kept at temperature (18±1 °C) and humidity (65%) with 12 hours natural light-dark cycles. Water for drinking was available ad libitum throughout the protocol study. The diet used in the study were as follows: 97% standard chow diet, 3% sunflower oil (standard diet); 96% standard chow diet and 1.3% of cholesterol of lard to induce atherosclerosis (Atherogenic diet).<sup>25</sup> Animals of the normal control group were maintained on standard diet throughout the study protocol while in the animals of the other 3 groups were maintained on atherogenic diet for 2 months for inducing hypercholesterolemia in them. After 2 months, except for the animals of the normal control group rest animals were exposed to all the risk factors that tend to initiate atherosclerosis in conjugation with hypercholesterolemia. The susceptible animals were introduced to regular high calorie sugar intake and daily 30-45mins of cigarette smoke along with the prescribed dietary and treatment intervention for next 2 months.<sup>26</sup>

#### Acute toxicity studies for dose determination

The acute oral toxicity parameter was performed according to the OECD 123 guidelines. Acute toxicity testing on omega-3-fatty acid concentrate revealed that the dose selected for the study is 200mg/kg OD, administered via oral route.<sup>27</sup> Physiological, biochemical and pathological observations were determined before and after performing the experimental protocol.

#### Experimental design

The New Zealand white rabbits of either gender were arbitrarily alienated into 4 postulated study groups (n=6 rabbits per group) as described below:

**Group I (Normal control or Negative control):** Composed of healthy animal treated with normal saline only.

**Group II (Negative control):** Atherosclerosis induced animals treated group.

**Group III (Concentrate treated):** Oral administered Omega 3 fatty acid concentrate treated group.

**Group IV (Treatment group):** Normal fish oil treated group.

### Sample collection

Prior to sample collection the animals were subjected to overnight fasting. About 3ml of blood was collected at two-week intervals from the marginal ear vein of each animal after overnight fasting.<sup>28</sup>

### Determination of physiological and biochemical parameters

The body weight of the experimental animals were measured prior to initiating the experiment, two months after initiating atherogenic diet and finally at the end of the study protocol. Determination of blood lipids includes measurement of the concentration of total cholesterol, LDL and HDL respectively. The results are expressed in mg/dl. The plasma lipid profile of the experimental animals was measured at the end of the study protocol. The measurement of Apo-A and Apo-B are useful in identifying the extent and progression of atherosclerosis. Determination of serum apolipoprotein content was done from Hemant pathology (Sagar MP India). At the end phase of study the blood was collected from the marginal ear vein of the animal by after overnight fasting and the sample was subjected to determination of clotting time by routine capillary method.<sup>29</sup>

### Statistical analysis

The data was expressed  $\pm$ S.E.M. The data of anti-atherosclerotic activity were analyzed by one way ANOVA followed by Dunnet's T- test. The ANOVA procedure performs analysis of variance for balanced data from a wide variety of experimental design.<sup>30</sup>

## Results and discussion

The omega-3-fatty acid concentrate was isolated from the bioactive by urea crystallization method. The fish oil and the Omega 3 fatty acid concentrate at optimal state were evaluated according to the Food grade fish oil standards from International association of Fish meal and oil manufacturers (IFOMA). The total content of Omega 3 fatty acid was found to enhance from 31.44 %w/w in cod liver oil to 89.64 %w/w in the isolate. The preformulation studies were executed under the qualitative analysis, quantitative analysis, solubility and partition coefficient analysis heads. The organoleptic characteristics as resolute from qualitative investigation showed the isolate to be pale yellow colored viscous liquid with a typical fishy smell and bitter taste (Table 1). Solubility profile reveals that the extracted compound is freely soluble in benzene and n-hexane, sparingly soluble in petroleum ether, insoluble in polar solvents (Table 2). The partition coefficient ( $K_{ow}$ ) of the concentrate as calculated from mentioned formula was found to be 4.743 (Table 3) and since chemicals with  $K_{ow}$  of 2-6 are said to be lipophilic (Oliver and Charlton, 1984), it tends to exhibit that omega-3-fatty acid concentrate is a lipophilic compound owing to its low bioavailability.

**Table 3** Partition coefficient of the bioactive

| Solvent system  | $C_o(\mu\text{g/ml})$ | $C_w(\mu\text{g/ml})$ | $K_o/w$ |
|-----------------|-----------------------|-----------------------|---------|
| n-octanol:Water | 1.106                 | 0.231                 | 4.743   |

The phytochemical analysis profile of omega-3-fatty acid concentrate reveals that the isolated concentrate is rich in proteins and saponins with peripherally existence of cardiac glycosides (Table 4). Ultra violet spectrophotometric analysis of the isolate exhibited it to possess absorption maxima of 247nm which also confirmed it to be an Omega 3 fatty acid concentrate. For quantitative analysis a standard curve was prepared exhibits linear shape which concludes that the isolated compound obeys Beer Lamberts law within the concentration range of 2-20 $\mu\text{g/ml}$  at the  $\lambda_{max}$  of 247nm (Table 5). Further subjected to qualitative analysis of Omega 3 fatty acid, FT-IR spectra was performed in the range between 4000 $\text{cm}^{-1}$  to 500 $\text{cm}^{-1}$  shows P-O Stretch at 1011.22, C=C Stretch at 1639.12, Alkynes ( $\text{RHC}=\text{CH}_2$ ) at 2099.54, O- $\text{CH}_3$  at 2829.75, Alkanes ( $-\text{CH}_3$ ) at 2927.88, N-H Stretch at 3405.18, Primary free N-H at 3399.16 & 3445.12, N-H Stretch at 3511.10 (Table 6). Physiological, biochemical and pathological observations were determined at the end of the experimental protocol. The data obtained was subjected to statistical analysis to determine the level of significance. The weight of animal enhance upon feeding of the diets did not diverge significantly among the experimental groups at the end of 2 or 4 month. The body weight of animal increased consistently in all the experimental group animals. The given diets were well tolerated and all the animals completed the studies without any mortality. Serum total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides levels were found to be remarkably more in the atherogenic and other group animals as equated to normal control groups animals.

**Table 4** Phytochemical profile of drug extract

| S No | Constituents       | Observation |
|------|--------------------|-------------|
| 1    | Tannin             | -           |
| 2    | Saponin            | ++          |
| 3    | Alkaloids          | +           |
| 4    | Flavonoids         | -           |
| 5    | Proteins           | +++         |
| 6    | Reducing Sugar     | -           |
| 7    | Cardiac Glycosides | +           |

Keys:

+++ Abundant,

++ Moderately presence

+ Present

- Absent

Concurrent administration of omega-3-fatty acid containing concentrate or cod liver oil with atherogenic diet caused a significant improvement in the lipid profile. But the improvement in the lipid profile group of the oil treated group was found to be lower than that observed in the concentrate treated groups (Figure 7). A significant reduction in serum Apo-A was observed after 2 months treatment with the concentrate of Omega 3 fatty acid and cod liver oil treated animal. Similarly, a significant increase in serum Apo-B was observed after 2 months treatment with the omega 3 fatty acid concentrate, and the plain cod liver oil treated animals. The improvement in apolipoprotein profile was observed after the treatment period. Apolipoprotein B level decreases in concentrate treated and in oil treated animal were observed, however the decrease in Apo-B was found to be enhance in the Omega 3 fatty acid concentrate treated group compare to the

plain oil treated group. There was also an increase of in Apo-A in the Omega 3 fatty acid concentrate treated group than in the oil treated group (Figure 8). Clotting time was reduced significantly in atherogenic control group animals when equated to the normal control group. After the treatment with omega 3 fatty acid concentrate clotting time levels increased to near normal (Figure 9).

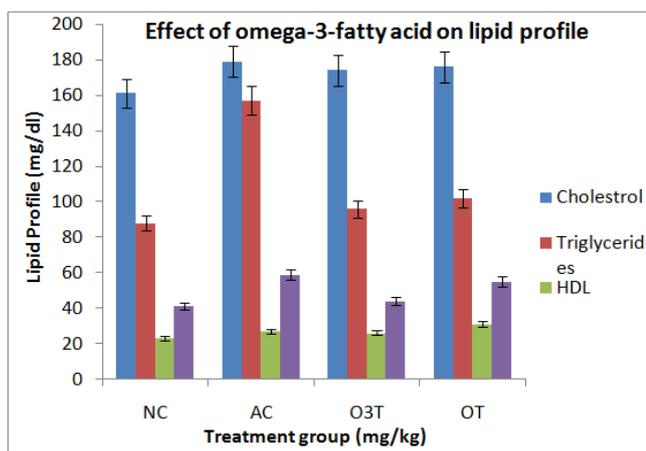


Figure 7 Effect of omega-3-fatty acid on lipid profile.

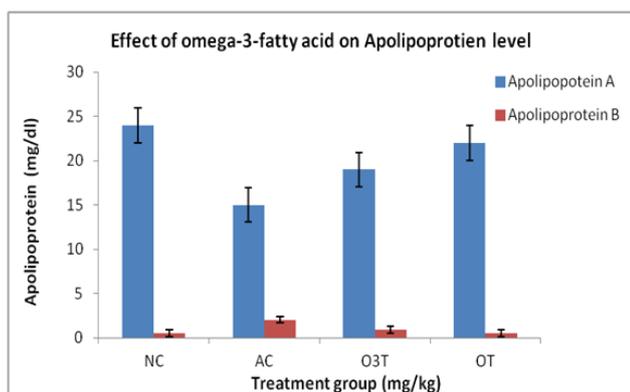


Figure 8 Effect of omega-3-fatty acid on Apolipoprotein level.

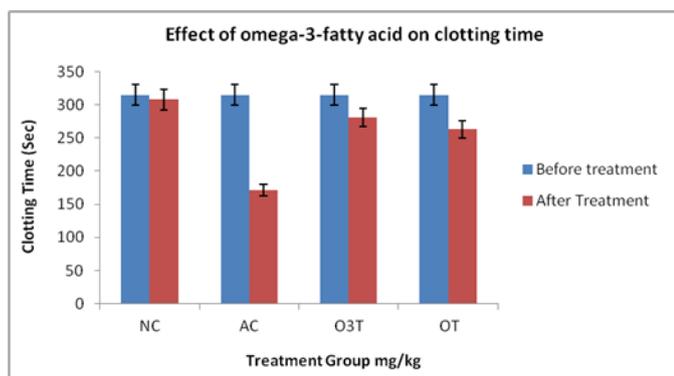


Figure 9 Effect of omega-3-fatty acid on clotting time.

Table 5 Absorption v/s concentration values

| S No | Concentration (µg/mL) | Absorbance | Regressed absorbance | Statistical parameters |
|------|-----------------------|------------|----------------------|------------------------|
| 1    | 10                    | 0.0793     | 0.075                |                        |
| 2    | 20                    | 0.1448     | 0.1474               |                        |
| 3    | 30                    | 0.2123     | 0.2198               |                        |
| 4    | 40                    | 0.3081     | 0.2922               |                        |
| 5    | 50                    | 0.3769     | 0.3646               | Y=0.0362x + 0.0026     |
| 6    | 60                    | 0.4529     | 0.437                | R <sup>2</sup> =0.9983 |
| 7    | 70                    | 0.5236     | 0.5094               |                        |
| 8    | 80                    | 0.5903     | 0.5818               |                        |
| 9    | 90                    | 0.6534     | 0.6542               |                        |
| 10   | 100                   | 0.7206     | 0.7266               |                        |

Table 6 Important band frequencies in FT-IR spectrum of omega 3 fatty acid concentrate

| S No | Wave number (CM) | Functionality  |
|------|------------------|--|
| 1    | 3511.10          | Intermolecular hydrogen bonding                                  |
| 2    | 3445.12          | N-H stretching vibration   |
| 3    | 3405.18          | N-H stretching vibration   |
| 4    | 3399.16          | Primary free N-H stretching                                      |
| 5    | 2927.88          | C-H stretching (Alkanes)   |
| 6    | 2829.75          | C-H stretching vibration (Aromatic methylene di-oxy compounds)   |
| 7    | 2099.54          | Alkynes C=C Stretching Vibration                                 |
| 8    | 1639.12          | C=C stretching vibration   |
| 9    | 1011.22          | C-O stretching vibration (P-Aromatic methylene di-oxy compounds) |

## Conclusion

The current study reveals the significant anti-atherosclerosis property of Omega 3 fatty acid concentrate isolate from cod liver oil when administered orally. The ω-3 fatty acid concentrate at a dose of 200 milligram per Kilogram of body weight exhibit noteworthy anti atherosclerosis potential however the activity was observed to enhance on administering the ω-3 fatty acid concentrate orally leading to the patient compliance. The pharmacokinetic parameter like Apo-lipoprotein A & B, level of HDL, LDL, triglycerides and total cholesterol evident the remarkable impending of omega 3 fatty acid concentrate in the atherosclerosis condition. Thus from this venture it can be established that omega-3-fatty acid concentrate can be employed as an efficient treatment intervention against atherosclerosis and associated diseases.

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## Conflict of interest

The author declares that there is no conflict of interest.

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