

BIOCHEMICAL STUDIES ON SOME EGYPTIAN FISH

By

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ABSTRACT

The present investigation was undertaken to study the biological effects of fish oil (FO) of sardine feeding on protecting experimental of hypercholesterolemic animals and the biological effects on curating as well as lowering hypercholesterolemia , which induced diseases such as coronary heart diseases (CHD), cardiovascular diseases (CVD) and atherosclerosis which are the most common heart diseases. Fish oil was extracted from sardine fish and analysed to know its fatty acids' content. This extracted oil was used for the present study. Total of 72 male albino rats weighing from 144 ± 6 g were used in the present work. Rats divided into two main groups for two experiments as follows: First experiment: studying the protective effects of fish oil on animals fed on hypercholesterolemia-induced diets (HCD) containing 0, 3, 7 and 10% fish oil. Second experiment: studying the curative effect of fish oil to decrease the hypercholesterolemia in hypercholesterolemic animals (fed on hypercholesterolemic- diets for 15 days before the second experiment). The feeding experiments continued for three weeks on basal diets containing Lipostat drug as well as other treatment with 0, 3, 7, 10% fish oil. At the end of experiments, the animals were decapitated, then plasma was prepared as well as livers and brains were excised, rinsed and kept frozen at -20C. Total lipids, triglycerides, cholesterol, LDL-c, vLDL-c, HDL-c and glucose, contents as well as AST, ALT and ALP activity were investigated in plasma. In addition to total lipids, triglycerides and cholesterol contents, AST and ALT activities (in liver), and malondialdehyde in liver and brain were measured. The results of the first experiment showed that fish oil prevented the increase of hypercholesterolemia. In which 3% FO diet kept triglycerides while 10% FO diet kept vLDL-cholesterol at about normal level; while the 10% and 7% FO diets were able to prevent the increase in plasma total lipids and total cholesterol. 10% FO diet prevented the decrease of plasma LDL-cholesterol, while the 3% kept them at normal state. On the other hand, hepatic triglycerides still in the normal level at 3% concentrations of fish oil diet. While hepatic total lipids and cholesterol were still more than that in normal rats. Also, it can be said that 3%, 7% and 10% fish oil diets kept MDA of brain and liver less than that in HCD. Hepatic transaminases activity was still in the normal level; while plasma transaminases activity markedly increased. On the other hand, ALP activity still in the normal state at different concentrations of fish oil diet. Also, it could be shown that the results of the second experiment, fish oil at different concentrations diet as well as Lipostat drug, decreased plasma total lipids, triglycerides and cholesterol, as well as vLDL-cholesterol and increased LDL-cholesterol and risk of coronary heart disease compared with hypercholesterolemic rats fed on basal diet only; but the effects of fish oil were more better than the effect of Lipostat drug. On the other hand, fish oil significantly decreased hepatic total lipids, triglycerides, cholesterol, while no alteration was recorded by Lipostat drug. Also, it can be shown that fish oil at different concentrations return hepatic and plasma transaminases activity to the normal state, while no effect of Lipostat drug on these transaminases activity. Also, it must be noticed that Lipostat caused an increase in blood glucose. It can be said that using fish oil may be protected and curated obesity and heart diseases

Keywords: Fish oil, hypercholesterolemia, Lipostat sardine.

LIST OF ABBREVIATIONS

4-AAP	4-aminoantipyrine
ALA	Alfa-linolenic acid.
ALP	Alkaline phosphatase.
ALT	Alanine amino transferase.
AMD	Age related macular degeneration.
AST	Aspartate amino transferase.
BHT	Butylated hydroxyl –toluene.
CAT	Catalase.
CHD	Coronary heart diseases.
Ch E	cholesterol esterase
Ch O	cholesterol oxidase
CVD	Cardovascular diseases.
DHA	Dcosaheaxaenoic acid.
DNA	Deoxyribonucleic acid.
DPA	Docosapentaenoic acid.
EPA	Eicosapentaenoic acid.
FO	Fish oil.
GLC	Gas liquid chromatography.
GK	glycerol kinase
GPO	glycerol phosphate oxidase
GO	glucose oxidase
HCD	Hypercholesterolemia –induced diets.
HDL	High density lipoprotein.
LA	Linolenic acid.
LDL	Low density lipoprotein.

LPL	lipoprotein lipase
MDA	Malondialdehyde.
PUFA	Poly unsaturated fatty acid.
POD	peroxidase
S.O.	Sardine oil.
TAC	Total antioxidant capacity.
TBA	Thiobarbituric acid.
TG	triglycerides
vLDL	Very low density lipoprotein.

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INTRODUCTION

Fish is a very tasty, nutritive and easily digestible source of food found abundantly in all parts of the world. In recent times, fish cultivation is being taken up on large scale to meet the growing consumption of human ever increasing population. Nowadays, even more people are turning to fish as a healthy alternative to red meat.

Fish, especially sardine is an excellent source of protein and minerals. It also contains copper, sulphur, phosphorous, iron, iodine and glycogen. Fish oils rich in n-3 polyunsaturated fatty acids (EPA and DHA), vitamin A and D. Fish has amino acids that help in building the body tissues. The nutritive value of fish is equal to meat **(IFG, 1997)**. Egypt is endowed with fishery resources comprising the river Nile and its tributaries, artificial Lake Nasser (fresh water), lakes Manzala, Burulus, Iduko, Maryut (brackish water), lake Bardaweel as well as the Mediterranean and Red seas (salt water) **(Farah, 1995)**. There are many kinds of fish products, caned, smoked, salted fish and fish oil. Some of these products are good and some may be bad that may be due to unsuitable conditions of processing and storage. The present investigation was undertaken to study fish oil. Sardine was found to be the best source of n-3 fatty acids **(Zlatanov and Laskaridis, 2007)**. Several studies have suggested that ω 3-fats of marine origin may have a protective role in heart disease. Suggest that dietary ω 3-fatty acids

incorporated into a low- rather than a high-fat diet have a wider spectrum of more favorable effects on cardiovascular risk factors (**Mori *et al.*, 1997**). Fishes are rich sources of structurally diverse bioactive compounds. In recent years, much attention has been paid to the existence of peptides with biological activities and proteins derived from foods that might have beneficial effects for humans. Antioxidant and antimicrobial peptides isolated from fish sources may be used as functional ingredients in food formulations to promote consumer health and improve the shelf life of food products (**Najafian and Babji, 2012**).

The objective of the present investigation was to study the biological effects of fish sardine oil (S.O) feeding in protecting experimental animals of hypercholesterolemia. Another objective is to study the biological effects of sardine oil in curate and lowering hypercholesterolemic agents in the experimental rats. In addition the present study aimed to investigate how much sardine oil is needed for protecting and curate of hypercholesterolemia diseases (CHD and CVD) which are the principle cause of heart diseases as atherosclerosis. It also aimed to suggest fish oil mechanisms of action for reducing heart diseases.

REVIEW OF LITERATURE

Composition of fish

The fish has a skeletal or cartilaginous structure which provides support for the body. The muscles which form the edible part account for most of the weight of the fish. The skin forms a cover, often with an outer layer of scales, and secretes a slimy mucus, which lubricates the fish and seals the surface. The gills are the main part of the breathing mechanism and take up oxygen from the water. The organs in the body cavity, including the stomach, intestine and liver are known as the guts. The protein content is usually in the region of 15-20 percent, whereas the fat content varies widely from species to species and from season to season. Water is the main constituent, with considerable variations, typically 80 percent in lean fish and 70 percent in fatty fish. Carbohydrates, minerals, vitamins and some water extractable components are examples of other minor substances present **(FAO, 1994)**.

The composition of fish is determined by the fat content variations in season, sex and stage of maturity. The fat content of the fish is located in the liver and viscera. Fat is also found in muscle tissue, skin, milt and roe. Fish fat contains cholesterol, lecithin, waxes and fatty alcohols. Glycerides of fatty acids is the principal component of fish oils **(Zuraini *et al.*, 2006)**.

Fish can also be classified on the basis of their fat content as: lean, fish having less than two percent fat; medium, fish having two to five percent fat; fat, fish having more than five percent fat **(IFG, 1997)**.

Apart from their nutritional importance, morphological features of sardines would be important to consider; they have a large, conspicuous mouth with a protruded snout. Their body sizes usually do not reach more than 15 centimetres. They can be found in the Mediterranean and temperate seas (both Southern and Northern). Additionally, these oily and dark colored fishes are abundant in the intertidal zone as well as in estuaries. According to some of the evaluations, there are five genera with 21 species of sardines, and most of those are commercially significant. **(Samonte et al., 2009)**

Fish product

The Egyptian Mediterranean coast is about 1100 km extending from El-Sallum in the West to El-Arish in the East and yielding about 55 thousand ton annually (GAFRD annual reports, 1995-2009). The fishing grounds along the Egyptian Mediterranean coast are divided into four regions; Western region

(Alexandria and ElMex, Abu-Qir, Rasheed, El-Maadiya and Mersa Matruh), Eastern region (Port Said and El-Arish), Demietta region and Nile Delta region. El-Arish fishing ground constitutes 6% of the total fish production in the Egyptian Mediterranean (GAFRD annual reports, 1995-2009). The round sardinella, *Sardinella aurita* (family: Clupeidae) is a marine pelagic fish that is widely distributed throughout tropical and subtropical seas including the entire Mediterranean and black seas. It is one of the most important fishes in the Egyptian sector of the Mediterranean-sea contributing about 30% of the total fish production from Egyptian Mediterranean waters. In El-Arish waters, it is the most important fish group and represents about 85% of the total fish (**Mehanna and Salem, 2011**).

Fish meal is used as a human and animal feeding agent .Salted fish and fish oil also used for feeding and industrial purposes. There are two kinds of separated fish oil namely liver oil and fish body oils. They contain vitamin A and vitamin D. Those parts of fish that are not consumed by humans as used as animal and poultry feed (**IFG, 1997**)