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The Effect of *Foeniculum vulgare* Seeds on Lipidemia, Glycemia and Liver Enzymes.

by

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ABSTRACT

The Effect of *Foeniculum vulgare* Seeds on Lipidemia, Glycemia and Liver Enzymes.

by Rana Bitar –Shamas

Foeniculum vulgare plant that is commonly known as Fennel or "Shumar" in Lebanon has been proved to have a wide range of biological activities. But there is no study that mentions its hypolipidemic effects; therefore, the present study explores the effects of the powdered and ethanol extract of *Foeniculum vulgare* seeds upon rat blood lipid profile, glycemia, and liver enzymes.

After two weeks of chronic intake of *F. vulgare* powder seeds 10%w/w or *F. vulgare* ethanol extracts 0.6%, 1.2%, 2.4 % or 4.8% w/w mixed with food, rat serums showed a slightly decrease in total cholesterol , HDL-cholesterol and LDL-cholesterol when the rats were put on high fat diet. They also showed a significant decrease in triglyceride. Liver enzymes activities ALT and ALP decreased in the group that was treated with 1.2% *F. vulgare* extract as compared to the control and in the group that was given high lipid diet and treated with 1.2% *F. vulgare* extract ALP enzyme as compared to the experiment group. While AST enzyme activity increased in groups that treated with 0.6%, 2.4 % and 4.8% w/w *F. vulgare* extract, ALT enzyme increased in groups that treated with 2.4 % and 4.8% w/w *F. vulgare* extract, ALP enzyme increased in groups that treated with 0.6% and 2.4% w/w *F. vulgare* extract and the LDH enzyme activity decreased in groups that treated with 0.6 and 2.4 % w/w *F. vulgare* extract and increased in the group that treated with 4.8% w/w *F. vulgare* extract. On the other hand, the insulin level increased in the group that was given high lipid diet and in the group that was given high lipid diet and treated with 1.2% *F. vulgare* extract as compared to the experiment group. In conclusion, *F. vulgare* oil showed

a mild hypocholesteremia on a high fat diet and a hypo triglyceremia. *F. vulgare* oil causes a slight increased in the enzyme liver but it doesn't cause a serious damage. Finally it doesn't have any glycemic effect

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GLOSSARY

ADP	Adenosine 5' – diphosphate
Apo	Apolipoprotein
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
CETP	Cholesteryl ester transfer protein
CHE	Cholesterol esterase
CHO	Cholesterol oxidase
CM	Chylomicrons
ELISA	Enzyme Linked Immuno – Sorbent Assay
FEO	Fennel essential oil
FV	*Feoniculum vulgare*
FVO	Fennel volatile oil
G	Gram
GSH	Glutathione
GST	Glutathione S-transferases
GK	Glycerolkinase
GOD	Glucose oxidase
GPO	Glycerol phosphate dehydrogenase
HDL	High Density Lipoprotein
HP	*Helicobacter pylori*
IDL	Intermediate Density Liproprotein
LCAT	Lecithin cholesterol acyltranferasae
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LPL	Lipoprotein lipase

MDA	Malondialdehyde
NAD^+	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NF-αB	Nuclear factor
P	Probability
POD	Peroxidase
PGE_2	Prostaglandin E_2
PRP	Platelet rich plasma
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
TNF	Tumor necrosis factor
SEM	Standard Error Mean
TAG	Triacylglycerol or triglyceride
TBS	Tris Buffered Saline
TCA	Trichloroacetic acid
VLDL	Very Low Density Lipoprotein

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Chapter 1
INTRODUCTION

Herbal medicine is an important part of folk medicine. It refers to mostly unrecorded and orally transmitted traditional procedures used for treatment of different diseases and disorders. In different cultures, herbs were used to develop medicines depending on personal experience and passed on from generation to generation by word of mouth. They consist of gathered plants used most of the time as infusions or ointments of one type of plant or combination of plants (Steiner, 1986).

Herbal medicine may include spices which have medicinal properties because of phenolics and a reported ability to heal various physical, mental and emotional problems (Singh et al, 2004).

In many countries, herbal medicine assumes higher interest in the health care of persons and communities and the international trade in herbal medicine products have increased. In the Mediterranean region, culinary medicine is in use since earlier time up till now. People utilize it since it is less expensive than modern medicine. Herbal medicines as well as modern drugs are simply coexisting nowadays, giving individual patients the freedom to choose between the two or take both simultaneously. However, troubles may occur due to intrinsic toxicity, lack of proper criteria and possible contamination. In order to solve these problems ethnobotanists, biologists, pharmacologists, and chemists are increasingly working together to benefit the most from the pharmacy of nature (Subehan et al, 2006).

1.1 *Foeniculum vulgare* Taxonomy and Distribution

Fennel (*Foeniculum vulgare* Miller) is a plant belonging to the subfamily *Apioideae* of the family *Apiaceae* (*Umbelliferae* Jussieu). The subfamily is characterized by more or less finely divided leaves, compound umbels, free bifid carpophores, and mericarps attached at the apex and not lignified. The genus *Foeniculum* (Miller) is monotypic (Tutin,1968), and is a glabrous, glaucous perennial or biennial plant up to 250 cm high with three to four pinnate leaves, more or less triangular in outline, usually 5—50 mm long, and filiform, with acuminate lobes cartilaginous at the apex. Sepals are absent, petals are yellow, oblong, and narrow only slightly at the involute apex. There are 4 to 30 inflorescent rays. Bracts and bracteoles are

usually absent. The fruit is 4—10.5 mm, ovoid-oblong, scarcely compressed with lateral ridge that are lightly more prominent than dorsal (Fig 1.1).

Figure 1.1 *Foeniculum vulgare* plant (alveo-a-stefaniak.ws/deskription.html).

There are two subspecies, *vulgare* and *piperitum* (Conforti et al, 2006). The first is widely cultivated for flavoring or as a vegetable because of its large tuberous stock and common in most of Europe (except the north) but it is probably native only in the south and southwest. The second is a perennial plant with rigid and rather fleshy leaf lobes, terminal umbels often overtopped by lateral ones, usually 4—10 rays and sharp-tasting fruit and is typical in dry, rocky places in the Mediterranean region (Tutin, 1968). In Italy, both subspecies are often found together but *piperitum* is more common in the south (especially on clay or in rocky calcareous sites) and subspecies *vulgare* is accepted as naturalized (Conforti et al, 2006).

1.2 Foeniculum Vulgare usage

Foeniculum vulgare is commonly cultivated throughout the temperate and subtropical regions of the world for its aromatic fruits which are used as a culinary spice. In the tradition of Calabria cooking subsp. *piperitum* collected in the wild is the most often used; the young plants are eaten as a vegetable or used as a garnish on raw or cooked dishes, particularly with oily foods. It is also used as a pot herb. (Singh & Kale, 2008). Other uses are in sausage preparation for flavor improvement, and in the preparation of olives as a flavoring and

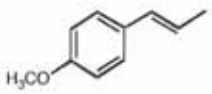
preservative agent (Singh & Kale, 2008). Bread is usually flavoured by the fennel seeds and sweets known as mustaccioli and susumelle (Conforti et al, 2006).

1.3 Foeniculum Vulgare Plant constituents and their medical effects

Foeniculum Vulgare essential oil has been reported to have antimicrobial and insecticidal activities. Essential oil extracted from leaves, flowers and roots of fennel offers protection against the mosquito *Culex pipiens molestus* bites (Traoulsi et al. 2005). While the essential oil extracted from fennel seeds (FS) exhibited antibacterial activity (Mohsenzadeh , 2007; Lo Cantor *et al.*, 2004; Singh *et al.*, 2002). It has been demonstrated to have wide antibacterial activities against twenty seven pathogenic bacterial species. Therefore it can be used as natural bactericide for controlling bacterial disease of plant and seed treatment (Lo Cantor *et al.*, 2004). Singh et al. (2002) reported that fennel essential oil has a moderate antibacterial activity against eight pathogenic bacteria such as *Streptococcus haemolyticus, Staphylococcus aureus Bacillus subtilis,* and Proteus *vulgaris.* In addition, ether extracted oil from fennel exhibited high antimicrobial effect on *Candida albicans, Salmonella typhimurium and Shigella dysenteria (*Khaldun, 2006). Since fennel oil is characterized by having a high level of antifungal activity, it may be used in the food industry for eliminating fungal infections of the skin, hair and nails which are mainly due to dermatophytes (Mimica-Dukić et al., 2003).

The seeds of fennel plant (Fig 1.2) contain 1%-3% of volatile oil such as limonene and ß-myrcene, 50%-85% of anethole and about 20% of d-fenchone (Simándi et al., 1999).

Fennel
(Foeniculum vulgare)



Anethole

Figure 1.2 Fennel seed and the active ingredient anethole (Aggraval et al., 2008).

Trans-anethole, [1-methoxy-4-(1-propenyl) benzene], the active ingredient of fennel oil, has been shown to have estrogenic activity by a sensitive and specific bioassay using recombinant yeast cells expressing the human estrogen receptor (Howes *et al.*, 2002). It has

been shown to regulate the menstrual cycle and increase the symptoms of female climacteric syndrome (Albert-Puleo, 1980).

One study indicated that the ethanol extract of FS reduced the hair diameter in women suffering from idiopathic hirsutism (Javidnia *et al.*, 2003). Mammary glands weight is increased when gentle and mild doses are given; whereas the oviduct, endometrium, myometrium, cervix and vagina weights are increased when elevated doses are administered (Annusuya *et al*, 1988).

In Lebanon, fennel which is called "Shumar" or "Shumra" in the native language, is used for its beneficial effects on the digestive system. It is also used for pain relieve and for its diuretic properties (Jeambey et al., 2009). A noted enhancement in specific activities of superoxide dismutase (SOD) and catalase is shown with anethole. Also there is a significant drop in the high density lipoprotein–cholesterol concentrations along with an abate in the peroxidative damage (Choi and Hwang, 2004).

Choi and Hwang, 2004 showed that the methanolic extract of *Foeniculum vulgare* fruits showed inhibitory effects against acute and sub-acute inflammatory diseases and central analgesic effects. These effects may be mediated by anethole, the major component of fennel (Chio and Hang, 2004). Anethole block NF-αB (nuclear factor) therefore it blocked TNF (tumor necrosis factor) signaling. Thus, the inhibitory effects of anethole on TNF-induced cellular responses could be due to its role in suppression of inflammation (Aggarwal et al., 2008).

A strong antioxidant activity is shown with the water and ethanol extracts of (FS). This antioxidant property displayed a dose dependent effect (Oktay et al., 2003).

Carcinogenesis might be inhibited by anethole (Aggrawal et al., 2008). This compound has striking metabolic effects. It increases the intracellular levels of glutathione (GSH) and glutathione S-transferase (GST) (Aggrawal et al., 2008). The (GST) represent a major group of detoxification enzymes in the liver (Hayes and Pulford, 1995). Water extracts of *F. vulgare* (FV) infusion in rats along with trichloroacetic acid (TCA) significantly declined the GST activity in the brain; kidney and liver of these rats in comparison to those of TCA exposed and controlled rats (Celik and Islik, 2008).

Anethole acts as hydroxyl radical scavenger. (Conforti *et al.*, 2006) showed that ethanol fennel seed extracts exhibited expressive antioxidant capacity in the β -carotene–linoleic acid

test system. Impeded breakdown of lipid hydroperoxides to unrequired volatile products allowed the determination of secondary antioxidants in certain mechanisms of relation (Conforti et al., 2006). Even more, other studies showed that fennel seeds extract along with other plants extracts inhibit the growth of prostate cancer (Fig et al., 2003).

Carbon tetrachloride (CCl_4) induced liver injury was used to study the hepatoprotective activity of (FEO) in the rat model (Ozek et al., 2003). The hepatotoxicity produced by acute CCl_4 administration indicated that FEO has a potent hepatoprotective action against CCl_4-induced hepatic damage in rats (Ozek et al., 2003).

Reports showed that FVO is used as a remedy for pediatric paroxysmal pains and some respiratory disorders due to its anti-spasmodic effects (Alexnderovich et al. 2003). Another study also reported that the daily average crying time was reduced by 85.4% for colic baby group treated with *Foeniculum vulgare* and 48.9 % for colic baby group treated with placebo after one week. (Savino *et al.*, 2005).

F. vulgare is used as a pharmaceutical and fragrant herb since ancient time. FV fruit is used as a natural remedy for disorders of the digestive system. Ethanol-induced gastric damage can be significantly decreased as reported by certain studies. Also, pretreatment with FVE significantly lowered the blood malondialdehyde (MDA) (Birdane et al., 2007).

The gram-negative bacterium *Helicobacter pylori* (HP), identified in 1982, is now recognized as the primary etiological factor associated with the development of gastritis and peptic ulcer disease. Methanol extract of *F. vulgare* seed exhibited inhibitory effect on the growth of *Helicobacter pylori* in vitro (Mahady et al., 2005).

F. vulgare has a potent effect on mental and emotional problems. Administration of the methanolic extracts of the entire plant of fennel for a period of four days ameliorated the amnesic effect of scopolamine (0.4 mg/kg) and aging induced memory deficits in mice. This indicates that the plant can be a possible treatment of cognitive disorders as dementia and Alzhemier's disease (Joshi, 2006).

Inhaling the aroma of essential oils such as fennel oil together with other essential oils, resulted in 1.5- to 2.5-fold increase in relative sympathetic activity, representing low frequency amplitude of systolic blood pressure (SBP-LF amplitude), in comparison to the inhalation of odorless solvent such as triethyl citrate(Haze et al., 2002).

1.4 Lipids

1.4.1. Introduction

Lipids are essential components of all living organisms. They are water-insoluble organic compounds composed of many types of molecules such as fatty acids, steroids, lipid vitamins, terpenes (Horton et al., 2002), glycerides, complex lipids such as lipoprotein and glycolipids, and waxes (Vance and Vance, 2004). The physiological importance of lipids for humans has four major functions. Lipids act as structural constituents of biological membranes, supply energy stores mainly in the form of triacylglycerols, and act as vitamins, hormones and lipophilic bile acids (Vance and Vance, 2004).

1.4.2. Triacylglycerols

Triglycerides are the main storage forms of lipids that constitute about 95% of fatty tissues (Loeb, 1994). They can be stored and secreted from the cells. The storage occurs in most cells, while triglycerides are only secreted by certain organs such as the liver, intestine and mammary gland (Olofsson et al., 2008). Dietary triglycerides pass through the stomach to the gut where they are emulisified in the presence of the bile salts. Pancreatic lipase is secreted into the gut where it hydrolyses triglycerides to fatty acids and glycerol. The fatty acids and glycerol are absorbed by intestinal cells and re-esterified into triglycerides again (Salway et al., 2006). They are found in the bloodstream with normal blood levels between 40 to 160 mg/dl in adult men and 35 to 135 mg/dl in adult women. Elevations of triglyceride level have been correlated with overweight, development of atherosclerosis, and cardiovascular diseases. Accumulation of triglycerides mostly in liver and muscles is very much associated with the development of insulin resistance and type 2 diabetes (Loeb, 1994, Olofsson et al., 2008).

1.4.3. Cholesterol

Cholesterol is an inevitable component of almost all phospholipid membranes in the human organism (Olofsson et al., 2008).Very high or too low membrane cholesterol contents are detrimental to cell function. When cholesterol is present in surplus amounts in cells, it becomes toxic (David and Cohen, 2008). Cholesterol occurs in both the free and ester form of cholesterol and fatty acids. In plasma, about one third of cholesterol is free, and two thirds exist as esters containing linoleic and oleic acids. Intracellularly, the stock

pool of cholesterol is formed by its esters with oleic, palmitic and linoleic acids in some cells. Cholesterol in the organism originates both from the external environment by absorption from the digestive tract and by synthesis de novo from acetyl-CoA in the liver and body cells. Under normal circumstances, a significant portion of the required amount of cholesterol is obtained from food (Olofsson et al., 2008).High serum cholesterol level can be deposited in the arterial walls causing atherosclerosis. Cholesterol can also be deposited as yellow deposites in soft tissues causing tendon xanthomata (Salway et al., 2006).

1.4.4. Blood Lipoprotein

Since both triglycerides and cholesterol are packaged into complexes called lipoproteins. Lipoprotein particles are composed of a non polar core of triglycerides and cholesteryl esters. They are spherical and enveloped by an amphipathic layer of phospholipids, free cholesterols and special proteins called apolipoproteins. Five major groups exist which are: high density lipoprotein (HDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL), very low density lipoprotein (VLDL), and chylomicrons (Vaiser, 2009). These lipoproteins differ by their size, density and function. They are divided into two groups according to their functions. The first group carries triglycerides from liver and intestine to peripheral tissues and includes: chylomicrons, VLDL, IDL, and LDL. While the second group (HDL) carries triglycerides from peripheral tissues to the liver. Lipoproteins are synthesized in the intestine and the liver, arised from metabolic changes of precursor lipoproteins, or assembled at the cell membranes from cellular lipoprotein or apolipoproteins. In the circulation, lipoproteins undergo enzymatic reactions of their lipid components, transfer of soluble apolipoproteins and conformational changes of the apolipoprotein in response to the compositional changes. In the final stages, lipoproteins are taken up and metabolized in the kidney, liver, and peripheral tissues (Vances and Vances, 2004).

1.4.4.1 Chylomicron

Chylomicrons (CM) are the lowest density lipoprotein. First triglycerides and cholesteryl ester are enveloped by a coat of phospholipids and apoB48 to form nascent

chylomicrons. The chylomicrons are secreted by enterocytes into the lymphatic system, and then joined blood stream where they acquire apoE and apoC2 from HDL to form mature chylomicrons (Salway et al., 2006). Once mature chylomicrons arrive to the liver, fat cells and skeletal muscles, they bound to lipoprotein lipase. Lipoprotein lipase is activated by apoC2 where it hydrolysis triacylglycerol of chylomicrons to produce fatty acids, glycerol and denser smaller particles called chylomicron remnants (Loeb, 1994; Cooper, 1997; Salway, 2006). Eventually chylomicron remnants can deliver fatty acids to be oxidized in muscle to generate energy or stored in adipose tissues. Moreover, chylomicron remnants can be used in hepatic VLDL synthesis; therefore, they are removed from the circulation by the liver. This up-take is mediated by apolipoprotein E (apo E) and the low density lipoprotein receptor-related protein (LRP), which has an important role in mediating this up take (Kowal et al., 1989 & Cooper 1997). The apolipoproteins that are predominant before entering the circulation are apo B-48 and apoA-I, A-II and A-IV. ApoB-48 is only combined with chylomicrons (Vance and Vance, 2004).

1.4.4.2. Very Low Density Lipoprotein (VLDL)

Very low density lipoproteins are similar to chylomicrons and contain high amount of triglycerides. They are produced from free fatty acids which are formed during chylomicron breakdown in the liver or from triglycerides that are synthesized endogenously by the liver or intestine (Kingsbury and Bondy, 2003). The nascent VLDLs are liver-derived lipoproteins that have apoB100 as their structural protein. One apoB100 is associated with one VLDL particle (Cabezas, 2003).

These nascent VLDLs become mature VLDLs after receiving from high density lipoproteins (HDLs) the apolipoproteins apoCII and apoE. In the capillaries of the target tissues, the apolipoproteins apoB100 and apoE bind to the VLDL receptor while apoCII activates lipoprotein lipase (LPL), which is further stimulated by insulin. LPL hydrolyses the TGS contained in the VLDLs producing fatty acids as well as glycerol. For the hydrolysis of TG by LPL, apoCII present on the surface of VLDL is a necessary cofactor. ApoCIII, regulates the hydrolysis of TGs by impeding the binding to LPL (Cabezas, 2003). In summary, VLDLs are the molecules created to transport triacylglycerols which are endogenously derived to extra-hepatic tissues. Even more, these particles include cholesterol, cholesteryl

esters and the apolipoproteins: apoB-100, apoC-I, apoC-II, apoC-III and apoE (Vance and Vance, 2004).

1.4.4.3. Intermediate Density Lipoprotein

When the VLDL is hydrolyzed and fatty acids are released, the remaining lipid portion is called intermediate density lipoprotein (IDL), a smaller and denser lipoprotein. This particle is short lived and contains equal amounts of cholesterol and triacylglycerol, and smaller amounts of phospholipids and protein (Loeb, 1994). IDLs, the VLDL remnants, are rich with apoE. The LDL receptor binds both the C-terminal part of apoB100 and the apoE on IDL. Binding to apoE results in rapid internalization of particles, in contrast with apoB100 binding, which is a slow catabolic step. IDL is either transported through these receptors into the liver, or is degraded to LDL by enzymes contained in the liver sinusoids, hepatic TG lipase, when more triacylglycerols are removed (Ger and Van der Vusse, 2004 & Cabezas, 2003).

1.4.4.4. Low Density Lipoprotein

Low density lipoproteins are often called bad cholesterol. They are made up of half cholesterol and half protein, triglycerides and phospholipids. In addition, LDL is produced mainly through the process of catabolic breakdown of VLDL and IDL and by liver secretion (Loeb, 2004). VLDLs are converted to LDL by the action of lipoprotein lipase in the cicrulation (Mangiapane and salter, 2001). Apo B, the major apolipoprotein of LDL, has an essential role in LDL cholesterol metabolism. It is produced by the hepatic cells to be secreted in VLDL. Apo B secretion or degradation is regulated by the lipid availability. In addition, apo B production is associated with LDL cholesterol level. LDL receptors mediate the clearance of LDL cholesterol from plasma. A part of those LDLs is also removed by scavenger receptors (Vance and Vance, 2004).

1.4.4.5. High Density Lipoprotein

HDL, the smallest lipoprotein, has the lowest lipid content. It contains a lipid core made of cholesteryl esters (CE) and TG. It is surrounded by phospholipids and apolipoproteins (Breslow, 1991). Apolipoprotein AI (apoAI) is synthesized in the liver and small intestine

through the action of ATP-binding cassette transporter AI (ABCA1). It is secreted in the form of immature HDL (imm HDL) particles containing higher amounts of protein in comparison to low amounts of free cholesterol (Hahn et al., 2008). Free cholesterol and phospholipids are donated by the macrophages and peripheral tissues to apoAI for the synthesis of additional immature HDL particles. Lecithin cholesterol acyltransferase (LCAT) adds esterified cholesterol to the core of HDLs. This action leads to mature HDL particles having apoA1 as a major component, phospholipids, as well as cholesterol esters which are shuttled to apoB-rich low-density lipoproteins (LDLs) and very-low-density lipoproteins (VLDLs) mediated by the actions of cholesterol ester transfer protein (CETP). Phospholipids are then transported from LDLs/VLDLs to HDLs by phospholipid transfer protein (PLTP). Cholesterol can be recycled or exocytosed into the bile to be disposed (Fig 1.3) (Hahn et al., 2008 &Vance and Vance, 2004).

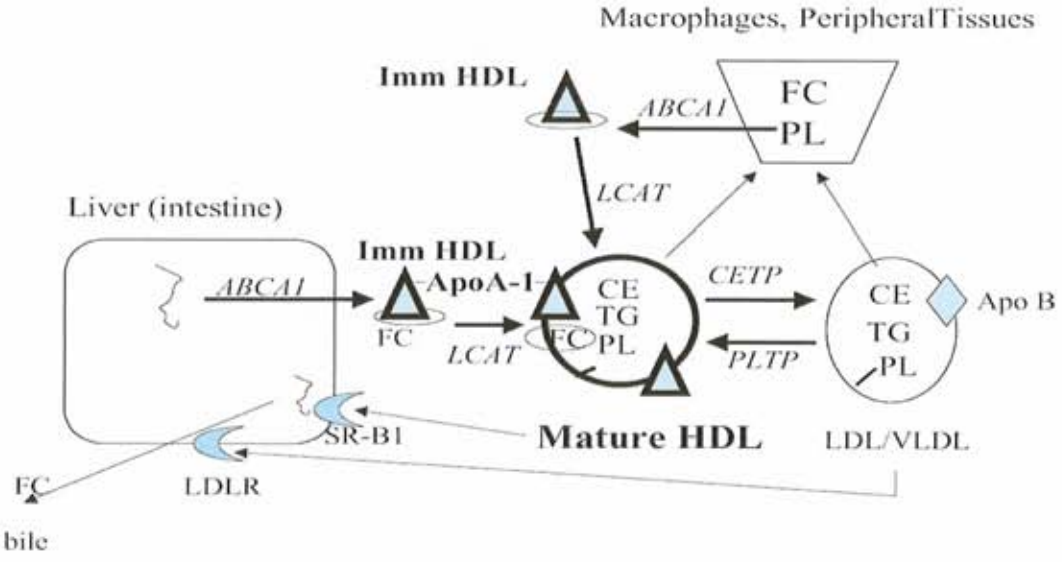


Figure 1.3 This figure represents the over review formation and clearance of HDL triangles = apoA1; diamond = apoB. CE, cholesterol esters; FC, free cholesterol; PL, phospholipids; TG, triglycerides (Hahn et al., 2008)

1.5 The activity of hepatic enzymes

The liver which has a vital role in metabolism of fat, carbohydrates as well as protein metabolism is a large and complex organ. Waste products detoxification of metabolism occurs in the liver, such as through the process of deamination of amino acid, which

produces urea. Bilirubin, resulting from the breakdown of the hemoglobin in the red blood cells, is excreted into bile by hepatocytes. The liver is responsible for the synthesis and secretion of bile as well as for the formation of lipoproteins and plasma proteins. Glucose levels are kept within a normal range by taking it up by the liver which is then stored as glycogen through a process called glycogenesis, and when needed, glycogen is hydrolyzed down through glycogenolysis to form glucose from non carbohydrate sources of glucose may come from compounds such as amino acids (gluconeogenesis). In addition, the liver stores fats, iron, copper, and many vitamins including vitamins A, D, K, and B12 (Norton et al., 2008).

Herbal drugs are becoming very popular and their use is extensive. Herbal products' licensing regulations and pharmaceutical clear cut benifits are still incomplete and have no proof of their efficacy in hepatic disorders. Besides their therapeutic properties, many observations are reported pertaining to liver injury after the intake of herbal drugs, including those which are publicized as treatments for liver diseases. (Stickel and Schuppen, 2007).

Laboratory evaluation of serum levels of hepatic transaminases, alkaline phosphatase, bilirubin levels, γ-glutamyl tranaminase (GGT), and ammonia are in practical use to assess liver functions. Hepatic transaminases included asparatate aminotransferase (AST)/glutamate oxaloacetate transaminase (SGOT), and alanine aminotransferase (ALT)/ glutamate pyruvates transaminase (SGPT). These enzymes are produced by hepatocytes. An increase of the serum of aminotransamiase levels may indicate hepatocytes malfunction or liver disorder (Norton et al., 2008). There are high concentrations of aminotransferases (in both forms) in the liver. They are also found in the heart, skeletal muscle, kidneys, brain and red blood cells. In skeletal muscle and kidney, they are found in low concentrations. Increased ALT serum levels are more specific for liver damage. However, liver toxicity is suitably estimated by the increases of aminotransferases levels and slight increases in bilirubin levels. It is to be noted that AST levels reach a peak level before ALT levels (Giannini et al., 2005).

Alkaline phosphatase *(ALP): It* is an enzyme which acts as carrier of metabolic byproducts across cell membranes. Hepatic parenchymal disorder and bone diseases are mainly the major causes of pathological boosting of ALP levels. (Norton et al., 2008 &Giannini et al.,

2005). Liver injury which is drug induced may show conditions of hepatic stability pattern (preferential increase in ALP) (Giannini et al., 2005).

Lactate Dehydrogenase *(LDH)* catalyzes the reversible conversion of muscle lactic acid into pyruvic acid. LDH is almost found in all cells of the body (Loeb, 1994). Lactate dehydrogenase (LDH) which is a marker of ischemic damage, can reach very high concentrations. This is shown in 80% of arterial blockage injury patients (Giannini et al., 2005).

1.6 The purpose of the project

Extensive studies on *F.vulgare* showed a wide range of beneficial pharmacological properties such as antibacterial, antiviral, antitumor, anti-inflammatory, antioxidant, analgesic, spasmolytic, expectorant, estrogenic, hypotensive and hypoglycemic effects.

Thus, the present investigation was under taken to evaluate the effects of of *F. vulgare* seeds (powedered and ethanol extract) upon:

- Blood lipid profile (TAG, total cholesterol, LDL-cholesterol and HDL-Cholesterol)
- Glycemic profile (glucose and insulin)
- Liver enzyme activities (GOT, GPT, ALP, LDH) in rats put on lipid diet.

Chapter 2

MATERIAL AND METHOD

2.1 Seed collection, feed and oil extract preparation

Dried plant samples were purchased from Bekaa valley specifically from Hermel, a town in the north of the Bekaa. The fennel *(Foeniculum vulgare* subsp. *Piperitum)* seeds were pulverized with the help of a blender and were mixed with water and standard feed pellet, according to the concentration 10 %(w/w), the prepared feed was then dried in the incubator at 50°C over night. The taxonomic identification of the seed material was confirmed through the botanic and plant taxonomy books (Tutin, 1968 and Conforti, 2006).

100 g of pulverized fennel seeds were mixed with 300 ml of 98% ethanol for two day, and this procedure repeated twice. The respective extract was filtered in a vaccum filter apparatus using Whatman No.1 paper and evaporated to dryness under reduced pressure. The crude extracts were stored for later use. All these processes were done in complete darkness to prevent fennel oil oxidation since it rapidly oxidized in light (Misharina, 2005). The crude extracts were isolated according to the following concentrations: 0.6%, 1.2%, 2.4% and 4.8% w/w were mixed with standard rat diet.

2.2 Animals

Sixty four Sprague-Dawley rats weighting about 225-300 g were housed in groups of eight at an ambient temperature of 22 ± 1° C and 60 % relative humidity with a lighting cycle from 7:00 a.m. to 7:00 p.m. The rats had access to water *ad libidum* and had received isocaloric diet of 6.5 g of food per 100 g body weight. The investigation was conducted in accordance with the Guide for the Care and Use of laboratory Animals published by the US National Institutes of Health (NIH publication no.85-23 revised 1996).

2.3 Experiment procedure

The experiment was divided into three stages. Each stage lasted for two weeks. In the first stage, three groups of rats were taken (A, B, C). Group A (the control) was given only chow food, group B was given chow food mixed with 10%(w/w) fennel seed powder, group C was given chow food mixed with fennel seed oil extract 1.2% (w /w). In the second stage of

the experiment, another three groups of rats were taken (D, E, F). Groups D, E and F were given chow food mixed with fennel oil extract 0.6 %, 2.4 %, 4.8 %(w/w) respectively. In the third stage of the experiment, two groups of rats were taken (G, H). Group G (the experimental control) was given chow food mixed with 4.4mg/kg of cholesterol in form of coconut oil and egg yolk while group H was given chow food mixed with 4.4mg/kgof cholesterol in form of coconut oil and egg yolk and fennel oil extract 1.2% (w/w).

2.4. Serum assays

Samples preparation:

Venous blood (8 ml) from fasted animals was drawn, put into glass tubes and allowed to clot for 30 to 45 minutes at room temperature. Blood was then centrifuged for 15 minutes at 3000 rpm at 4°C. The supernatant serum was transferred into eppendorf tubes and put directly on ice water. This serum was used to assess the liver toxicity by measuring the activity of the liver enzymes (SGOT, SGPT, LDH and ALP), lipid profile (TAG, total cholesterol, HDL cholesterol, LDL cholesterol) as well as glucose levels and insulin content, in the control and treatment groups.

2.4.1. Determination of serum lipid profile

2.4.1.1. Determination of Triglyceride

Principle: The lipoprotein lipase (LPL) is an enzyme that rapidly and completely hydrolyzes TAG to glycerol and free fatty acids. This hydrolysis is followed by oxidation of glycerol to dihydroxyacetone phosphate and hydrogen preroxide by glycerol kinase (GK) and glycerol phosphate dehydrogenase (GPO). The hydrogen peroxide produced then reacts with 4-aminophenzone and chlorophenol, catalyzed be peroxidase (POD) and produces quinine, a red colored product. The color intensity is directly proportional to the concentration of TAG and the absorbance can be determined by the spectrophotometer at a wavelength of λ = 505nm.

$$TAG + H_2O \xrightarrow{LPL} Glycerol + Fatty\ acid$$

$$Glycerol + ATP \xrightarrow{GK} Glycerol\text{-}3\text{-}phosphate + ADP$$

Glycerol-3-P+O_2 ⟶ Dihydroxyacetone-P +H_2O

Peroxidase

H_2O_2 + 4-aminophenazone + P-chlorophenol ⟶ quinine +H_2O

.**Procedure:** 10 µl of the standard or serum sample was mixed with 1 ml of the working reagent (buffer solution and enzymes: LPL, GK, GPO and POD) and incubated 10 min at room temperature. The absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ= 505nm using a Heluis spectrophotometer.

Calculation:

$$\text{Concentration of TAG in the unknown sample (mg/dl)} = \frac{\text{Abs. of unknown} \times \text{Conc. Of standard}}{\text{Abs. of standard}}$$

The Concentration of the standard is 200mg/dl according to SPINREACT kit

2.4.1.2. Determination of total cholesterol

Principle: Cholesterol is formed enzymatically by cholesterol esterase (CHE) in the following reaction in which cholesterol esters yield free cholesterol and fatty acids.

CHE

Cholesterol Esters +H_2O ⟶ Cholesterol + Fatty acids

Cholesterol, in the presence of oxygen and with the aid of cholesterol oxidase, is then converted to 4-cholestenona and hydrogen peroxide.

CHOD

Cholesterol + O_2 ⟶ 4-cholestenona + H_2O_2

The hydrogen peroxide produced reacts with 4-aminophenazone and phenol, catalyzed by peroxidase, all present in the working reagent, and forms quinonimine, a red dye.

$2H_2O_2$ + 4-aminophenazone ⟶ quinonimine + $4H_2O$

The color intensity is directly proportional to the concentration of cholesterol and the absorbance can be determined by the spectrophotometer at a wavelength of λ= 505nm.

Procedure: 10 µl of the standard or serum sample was mixed with 1 ml of the working reagent (solution and enzymes: CHOD, CHE and POD) and incubated 10 min at room temperature. ` absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ= 505nm using a Heluis spectrophotometer.

Calculation:

$$\text{Concentration of cholesterol in the unknown sample (mg/dl)} = \frac{\text{Abs. of unknown} \times \text{Conc. Of the standard}}{\text{Abs. of standard}}$$

The concentration of the standard is 200 mg/dl according to SPINREACT kit

2.4.1.3. Determination of HDL-Cholesterol

Principle: Low density and very low density lipoproteins (LDL and VLDL) are specifically precipitated by phosphotungstic acid and magnesium ions and after centrifugation, high density lipoproteins (HDL) remain in the supernatant.

Procedure:1 ml of each serum sample was mixed with 100µl of the precipitating reagent (phosphotungstic acid and magnesium chloride) and allowed to stand for 10 minutes at room temperature. Then they were centrifuged at 4000 rmp for 20 minute (Spinreact). The supernatant collected was used for HDL cholesterol determination using the cholesterol assay kit as described previously for the total cholesterol

Calculation:

$$\text{Concentration of cholesterol In the unknown sample (mg/dl)} = \frac{\text{Abs. of unknown} \times \text{Conc. Of the standard}}{\text{Abs. of standard}}$$

2.4.1.4. Determination of LDL-Cholesterol

In order to determine the LDL-cholesterol concentration, no kit was used. The LDLc was calculated using the Friedewald Formula: (Spinreact)

LDL cholesterol= total cholesterol – $\frac{\text{triglycerides}}{5}$-HDL Cholesterol

2.4.2. Determination of liver enzyme

2.4.2.1. Determination of Serum Glutamate Oxaloacetate Transaminase (SGOT)

Principle: Aspartate aminotransferase (AST) (glutamate oxaloacetate transminase) is a hepatic enzyme that catalyses the transfer of an amino acid from aspartate to α-ketoglutarate.

$$\alpha\text{- ketoglutarate + Aspartate} \xrightarrow{\text{AST}} \text{Glutamate + Oxaloacetate}$$

The oxaloacetate in the presence of malate dehydrogenase and NADH produces malate and NAD^+

$$\text{Oxaloacetate +NADH +H}^+ \xrightarrow{\text{Malate dehydrogenase}} \text{Malate +NAD}^+$$

The photometerically determined decrease of NADH rate is directly proportional to the rate of formation of oxaloacetate and thus the AST catalytic activity.

Procedure: 100 µl of each serum samples were mixed with 1 ml of the working reagent(Tris buffer pH = 7.8, aspartate, NADH, malate dehydrogenase and a- ketoglutarate). After 1 minute of incubation at room temperature, the absorbance was read at one minute interval for 3 minutes against distilled water using Heluis spectrophotometer at a wavelength of 340 nm. The difference between absorbance and the average absorbance differences per minute were calculated.

Calculation:

$$\text{SGOT activity (U/L)} = \Delta E/(\text{min}) \times 1750$$

2.4.2.2. Determination of Serum Glutamate Pyruvate Transaminase (SGPT)

Principle: Alanine aminotransferasae (ALT) (glutamate pyruvate transaminase) is a hepatic enzyme that catalyses the transfer of amino acids from alanine to α- ketoglutarate.

$$\alpha\text{- ketoglutarate + Alanine} \xrightarrow{\text{ALT}} \text{Glutamate + Pyruvate}$$

The Pyruvate in the presence of lactate dehydrogenase and NADH produces lactate and NAD^+

$$\text{Pyruvate + NADH + } H^+ \longrightarrow \text{L-Lactate + } NAD^+$$

The photometrically determined decrease of NADH rate is directly proportional to the rate of formation of oxaloacetate and thus the ALT catalytic activity.

Procedure: 100 µl of each serum samples were mixed with 1 ml of the working reagent (Tris buffer pH = 7.8, L-alanine, NADH, lactate dehydrogenase and a- ketoglutarate). After 1 minute of incubation at room temperature, the absorbance was read at one minute interval for 3 minutes against distilled water using Jenway 6501 U.K spectrophotometer at a wavelength of 340 nm. The difference between absorbance and the average absorbance differences per minute were calculated.

Calculation:

$$\text{SGPT activity(U/L)} = \Delta E/(\text{min}) \times 1750$$

According to Spinreact procedure.

2.4.2.3. Determination of Lactate Dehydrogenase

Principle : Lactate dehydrogenase (LDH) catalyses the conversion of pyruvate to L-lactate with the oxidation of NADH. The rate of decrease in NADH (measured photometrically) is directly proportional to the rate for formation of lactate, and thus the LDH catalytic activity.

$$\text{Pyruvate + NADH + } H^+ \xrightarrow{\text{LDH}} \text{Lactate + } NAD^+$$

Procedure: 100 µl of each serum samples were mixed with 1 ml of the working reagent (Tris buffer pH = 7.8, NADH and pyruvate). After 1 minute of incubation at room temperature 25-30° C, the absorbance was read at one minute interval for 3 minutes against distilled

water using Heluis spectrophotometer at a wavelength of 340 nm. The difference between absorbance and the average absorbance differences per minute were calculated.

Calculation:

$$\text{LDH activity (U/L)} = \Delta E(\text{min}) \times 4925$$

2.4.2.4. Determination of Alkanline Phosphatase

Principle: Alkaline phosphatase (ALP) catalyses the breakdown of p-nitrophenyl -phosphate to p-nitrophenol and phosphate. The photometrically determined increase of phosphate rate is directly proportional to the ALP catalytic activity.

$$\text{P-nitrophenyl -phosphate} \xrightarrow{\text{ALP}} \text{P-nitrophenol + phosphate}$$

Procedure: 50 µl of each serum samples were mixed with 3 ml of the working reagent (diethanolamine buffer pH 10.4, magnesium chloride and p-nitrophenylphosphate). After 1 minute of incubation at room temperature 25- 30° C, the absorbance was read at one minute interval for 3 minutes against distilled water using Heluis spectrophotometer at a wavelength of 405 nm. The difference between absorbance and the average absorbance differences per minute were calculated.

Calculation:

$$\text{ALP activity (U/L)} = \Delta E\ (\text{min}) \times 3300$$

According to Spinreact procedure

2.4.3. Glycemic Assay

2.4.3.1. Determination of glucose

Principle : Glucose is oxidized by glucose oxidase (GOD) to gluconic acid in the presence of atmospheric oxygen. The resultant hydrogen peroxide oxidizes aminophenazone and phenol to 4-(P-benzo-quinone-monoimino)-phenazone in the presence of peroxidase (POD). The color intensity of the red-violet dye is directly proportional to the glucose concentration and can be measured spectrophotometrically at a wavelength of 505nm. The enzymatic reaction used in the assay is as follows:

GOD

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{POD}} H_2O_2 + \text{Gluconic acid}$$

$$H_2O_2 + \text{phenol} + \text{aminophenazone} \longrightarrow \text{quinine} + H_2O$$

Procedure: 10 µl of the standard and of each serum samples were mixed with 1 ml of the working reagent (buffer solution and enzymes) and incubated 10 min at room temperature. The absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ= 505nm using a Heluis spectrophotometer.

Calculation:

Concentration of glucose

In the unknown sample (mg/dl) $= \frac{\text{Abs. of unknown} \times \text{Conc. Of standard}}{\text{Abs. of the standard}}$

2.4.3.2. Determination of serum insulin

Principle: insulin in rat serum was determined using the Rat/mouse Insulin ELISA (Enzyme Linked Immuno-Sorbent Assay) kit (LINCO-USA). The Rat insulin ELISA is based on the direct insulin sandwich technique in which a micro titer plate is used. This plate is coated with mouse monoclonal anti-rat insulin antibodies. During incubation, insulin in the samples reacts with monoclonal anti-rat insulin antibodies and then biotinylated anti-insulin antibodies directed against antigenic insulin molecules will also bind. The streptavidin-horseradish peroxidase once added, binds to biotinylated anti-insulin antibody. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with the substrate 3, 3', 5, 5'-tetramethylbenzidine. The reaction is stopped by adding acid to give colorimetric endpoint result that is read spectrophotometrically by the increased absorb at 450 nm, corrected from the absorbance at 590 nm. The increase in the absorbency is directly proportional to the amount of captured insulin in the unknown sample; subsequently the latter can be derived by interpolation from a reference curve generated with reference standards of known concentrations of rat insulin.

Procedure: The serum samples and the standards are dispensed in the appropriate wells. The anti-insulin- HRP conjugate is added into the wells and incubated in a horizontal shaker at room temperature for 120 minutes. The liquid is removed and the washing solution is added into each well and then aspirated. This step is repeated 5 times after which the plate should be inverted firmly against an absorbent paper. The peroxidase substrate is dispensed into the wells and incubated for 15 minutes at room temperature, avoiding any direct light. The stopping reagent is finally added and the absorbance is read at 450 nm using the Spectra Max Plus ELISA reader.

Calculation: A standard curve is constructed using all standard points for which absorbances are < 1.5 OD unit. The insulin concentrations of the samples are determined using a computerized data reduction of absorbance for the standards versus the concentration using a cubic regression performed with E-LizaMat 300 reader and the DGR ELISA regression program.

Chapter 3

RESULTS

The rats were put on diet containing *F. vulgare* seed powder or *F. vulgare* seed extract oil in the three experiment stages for two weeks. Animals from the three stages of the experiment were scarified, blood collected and serum was used for screening tests including: Triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol. The animals were also screened for SGOT, SGPT, LDH, ALP liver enzymes. In addition, the rat blood serum was used for screening glucose and insulin.

3.1 Serum lipid profile

The tables 3.1 summarize the mean serum concentration in mg/dl of total cholesterol, TAG, LDL-cholesterol, and HDL-cholesterol in control and treatment groups.

3.1.1. Total cholestrol

The total cholesterol levels showed a significant increase in cholesterol level in groups D, G and H as compared to control (group A) (tables 3.1). In addition, there was a significant decrease in cholesterol level in group H as compared to the experiment control (group G) table 3.1.

3.1.2. TAG

The triglyceride levels showed a significant decrease in all groups that treated with fennel with respect to control regardless of the fat content in the diet (tables 3.1).

3.1.3. LDL-cholesterol

The LDL-cholesterol level showed a significant increase in LDL-cholesterol level in groups D and F with respect to control (tables 3.1). However, a significant decrease in LDL-cholesterol level in group H was observed as compared to the experimental control (group G) (table3.1).

3.1.4. HDL-cholesterol

The HDL-cholesterol showed a significant decrease in HDL-cholesterol level in groups F and H with respect to control groups A and G respectively (table3.1).

3.1.5. Cholesterol/HDL and LDL/HDL

The ratios of total cholesterol/HDL of the groups G and H showed a significant increase with respect to control (A) (figure3.1). The LDL/HDL of the groups D, G and H showed a significant increase with respect to control (A) (figure3.1).

Table 3.1 cholesterol (mg/dl), triglyceride (mg/dl), LDL-cholesterol (mg/dl) and HDL-cholesterol (mg/dl) measured after two weeks in the serum of groups A, B, C, D, E, F, G and H respectively. Group A: control group, Group B: treated with 10% w/w fennel seed powder Groups D, C, E and F: treated with 0.6%, 1.2%, 2.4%, and 4.8% w/w fennel extract oil in rat diet respectively, Group G: and H: were given high lipid diet with group H treated with 1.2% fennel extract oil in the rat diet. Values are expressed as ± SEM (n=8)

Group	A	B	C	D	E	F	G	H
Total Cholesterol	50.62±2	53.2±2.31	56.65±2.25[a]	50.65±3.33	50.24±2.12	46.87±3.85	156.4±5.49[a]	118.92±4.12[ab]
Triglyceride	60.52±2.54	53.75±1.57[a]	41.04±1.15[a]	47.12±1.25[a]	51.78±1.36[a]	46.86±1.7[a]	83.6±2.8[a]	61.93±1.88[ab]
HDL Cholesterol	28.25±1.32	30.5±2.37	28.43±0.93	31.64±1.37	28.9±1.66	23.75±1.14[a]	58.54±2.4[a]	44.71±2.55[ab]
LDL Cholesterol	10.25±1.25	11.5±1.09	19.47±3.3[a]	9.32±1.38	11.2±1.07	14.2±2[a]	80.9±5.5[a]	62.02±8.94[ab]

[a]P< 0.05 with respect to control
[b]P<0.05 with respect to control on high fat diet

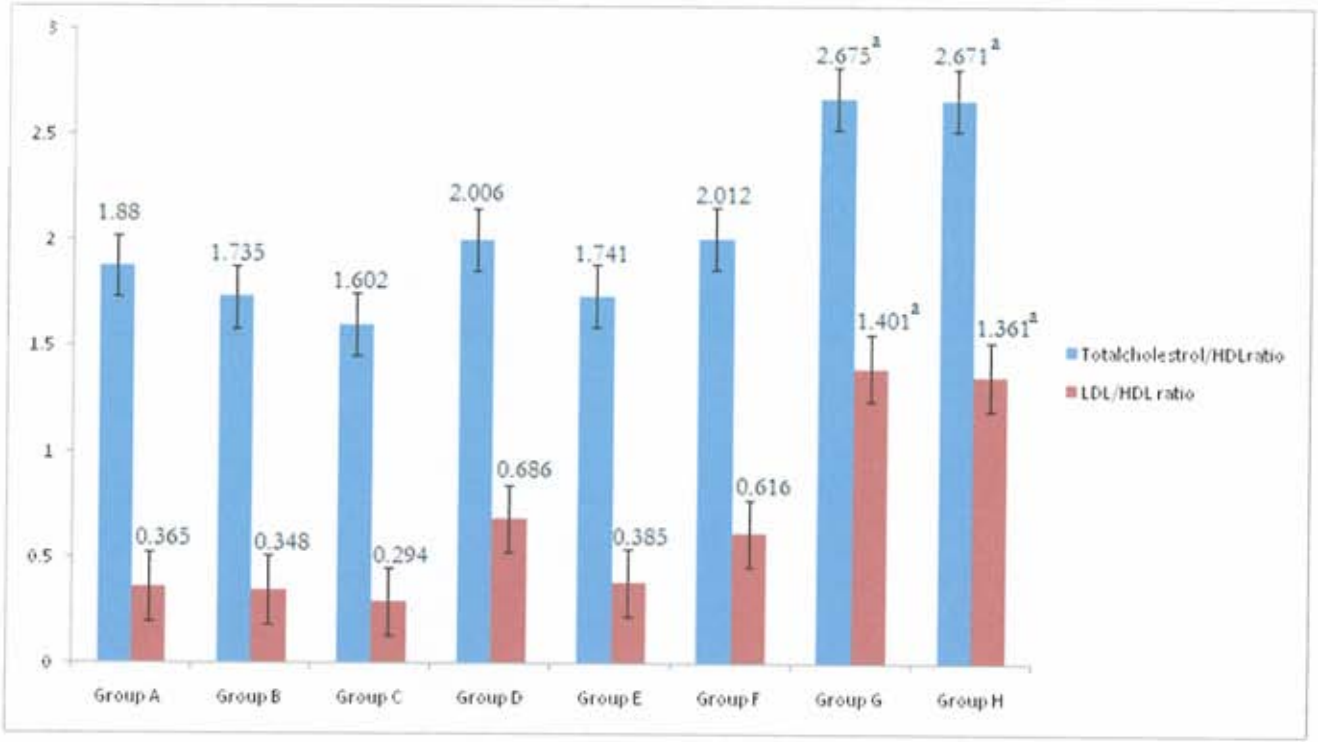


Figure 3.1: The ratio of Total cholesterol/HDL and LDL/HDL of groups A, B,C, D, E, F, G and H. Group A: control group, Group B: treated with 10% w/w fennel seed powder Groups D, C, E and F: treated with 0.6%, 1.2%, 2.4%, and 4.8% w/w fennel extract oil in rat diet respectively, Group G: and H: were given high lipid diet with group H treated with 1.2% fennel extract oil in the rat diet.
[a]$P < 0.05$ with respect to control

3.2 The effect of Foeniculum vulgare on the liver enzymes

The activity of liver enzymes was computed from the extinction decrease measured by spectrophotometer for 3 minutes at an interval of 1 minute. These results were obtained after two weeks of the treatment administration. (Table3.2)

3.2.1 SGOT/AST

The groups D, E and F showed a significant increase in the SGOT activities with respect to the control. However, the groups C showed a significant decrease in the SGOT activities with respect to the control.

3.2.2 SGPT/ALT

The groups E and F showed a significant increase in the SGPT activities with respect to the control.

3.2.3 ALP

The groups D, E and G showed a significant increase in the ALP activities with respect to the control. While, the groups C and H showed a significant decrease in the ALP activities with respect to the control.

3.2.4 LDH

The groups B, D and E showed a significant decrease in the LDH activities in with respect to the control. However, group F showed a high significant increase in the LDH activities with respect to the control.

3.2.5 SGOT/SGPT

The group E exhibited a significant increase in the calculated AST: ALT ratio with respect to the control.

Table 3.2 SGOT, SGPT, LDH ALP (U/L) and SGOT/SGPT ratio measured after two weeks in the serum of groups A, B, C, D, E, F, G and H respectively. Group A: control group, Group B: treated with 10% w/w fennel seed powder Groups D, C, E and F: treated with 0.6%, 1.2%, 2.4%, and 4.8% w/w fennel extract oil in rat diet respectively, Group G: and H: were given high lipid diet with group H treated with 1.2% fennel extract oil in the rat diet. Values are expressed as ± SEM (n=8)

Group	A	B	C	D	E	F	G	H
GOT	29.25±1.29	30.68±1.69	25.96±0.8[a]	35.88±1.6[a]	41.175±1.83[a]	41.98±1.24[a]	29.86±1.57	25.75±1.93[a]
GPT	14.31±0.98	13.5±1.39	13.24±1.22	16.49±1.54	17.66±2.13[a]	20.45±1.39[a]	14.72±0.25	13.33±1.25
ALP	116.87±2.66	114.75±1.41	97.75±1.98[a]	124.37±1.64[a]	127.25±2.78[a]	120.25±1.66	130.75±1.9[a]	88.25±1.31[ab]
LDH	58.5±1.01	51±1.9[a]	55.75±2.24	48.09±1.35[a]	51.55±1.32[a]	85.37±1.84[a]		
GOT/GPT	2.03±0.16	2.3±0.25	2.17±0.23	2.01±0.22	2.42±0.27[a]	2.03±0.16	2.02±0.11	2.09±0.25

[a]P< 0.05 with respect to control
[b]P<0.05 with respect to control on high fat diet

3.3. Serum glycemic profile

3.3.1. Insulin

Both insulin serum concentrations in the groups G and H showed significant increase with respect to the control (figure3.2).

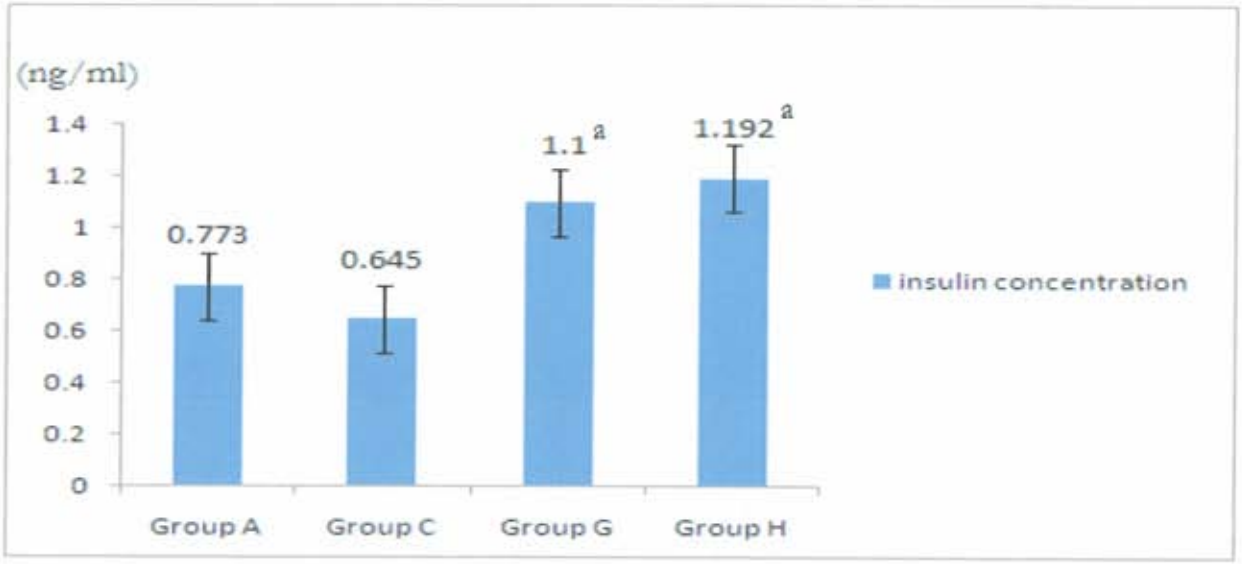


Figure 3.2 Insulin serum concentrations (ng/ml) in the groups A, C, G and H Group A: control group, Group C: treated with 1.2% w/w fennel extract oil in rat diet Group G: and H: were given high lipid diet with group H treated with 1.2% fennel extract oil in the rat diet. [a]$P < 0.05$ with respect to control

3.3.2. Glucose

The fasting serum concentrations of glucose significant increase in the groups B and F with respect to the control (figure 3.3).

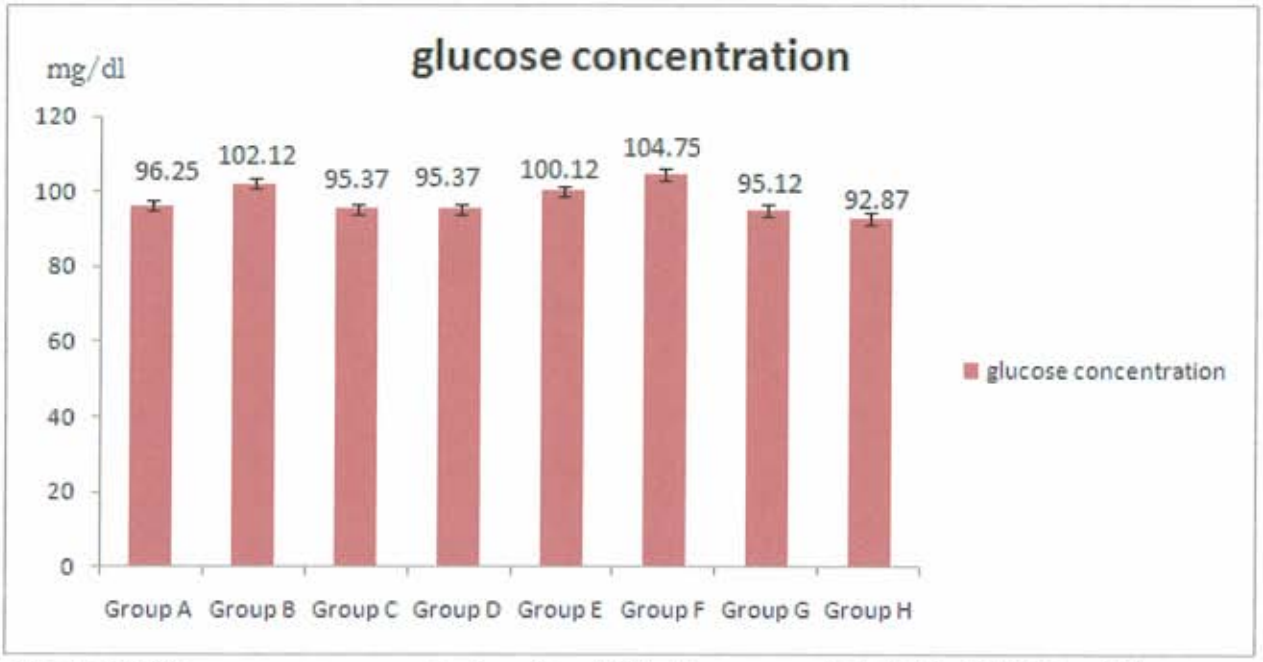


Figure3.3 Glucose serum concentrations (mg/dl) in the groups A, B, C, D, E, F, G and G. Group A: control group, Group B: treated with 10% w/w fennel seed powder Groups D, C, E and F: treated with 0.6%, 1.2%, 2.4%, and 4.8% w/w fennel extract oil in rat diet respectively, Group G: and H: were given high lipid diet with group H treated with 1.2% fennel extract oil in the rat diet.

Chapter4

DICUSSION

The increased use of naturally occuring products instead of synthetic biochemicals has again renewed the focus and diligence on plants as a source of flavoring and biologically active compounds (Stefanini, 2006). Fennel is being widely used for cooking purposes which makes it part of the regular dietary regime. It holds an abundance of medicinal attributes (Singh and Kale, 2008). There are many reports about the anti-inflammatory, analgesic, and antioxidant potentials of the fennel fruit (Choi and Hwang, 2004; Ozbek et al., 2003; Ozbek, 2005; Singh et al., 2006 Birdane, 2007 and Aggarwal et al., 2008). It has been also reported that fennel has anti-carcinogenesis in vitro and in vivo (Aggarwal et al., 2008 and Singh and Kale, 2008), hepatoprotective ability (Ozbek et al., 2003, Ozbek et al., 2004 and Celik and Islik, 2008), and hypoglycemic activity (Ozbek , 2002 and Javadi et al., 2008). It was also proved to have estrogenic and phytoestrogenic effect (Malini, 1985; Annusuya et al, 1988; Howes et al., 2002 and Javidnia et al., 2003). With this at hand, the present study was under taken to evaluate the effects of the seed powder and seed ethanol extract of *F. vulgare* on hyperlipidemia, glycemia and liver enzymes in vivo as indicative factors of general health.

Serum cholesterol changes and states of low-density lipoproteins are mainly associated with various disorder conditions. Hypercholesterolemia and atherosclerosis are correlated with an augmentation in the serum levels of cholesterol and low-density lipoproteins respectively. (Skoumas et al., 2003 and Oboh, 2006). The five main variables: total cholesterol, plasma triglycerides, HDL-cholesterol, LDL- cholesterol and cholesterol/HDL and LDL/HDL ratios are so important to be assessed in order to construe the effect of ethanol extract on hyperlipidemia. These parameters were measured after two weeks of *F. vulgare* seeds or ethanol extract intake mixed with regular food. The present study showed a significant decrease in the triglyceride levels in the all groups that were treated with fennel seed or ethanol extract regardless of the fat content in the rat diet. These results indicate that *F. vulgare* has a hypotriglyceridemic activity (table 3.1). Hypotriglycerimia may be attributed to flavonoids (rutin, quercetin and kaempferol glycosides) and sterols in fennel as has been reported as constituents (Birdane, 2007 and Javadi et al., 2008). Flavonoids could modulate lipoprotein and lipid metabolism directly in the liver. This maybe suggested due to the elevated flavonoids levels as well as their metabolites in hamsters' livers. Flavonoids decrease

the secretion of apo B (Kurowska et al., 2004). The present study also showed a significant decrease in total cholesterol, plasma triglycerides, LDL- cholesterol in the group that was given high fat diet and treated with 1.2% w/w ethanol extract with respect to experimental control. These results showed that *F. vulgare* may have a hypocholesterimia activity only when a high lipid diet is given (table 3.1). This activity may be due to anethole, the active ingredient of fennel that proved to have estrogenic activity (Howes et al., 2002). Higher level of estrogen is associated with lower levels of cholesterol (Szafran & Smielak-Korombel, 1998). One possible explanation proposed for estrogenic effect is through up regulation of LDL receptor (George, 2002). On the other hand, this study also showed a significant decrease in HDL- cholesterol in the group that was given high fat diet and treated with 1.2% w/w fennel ethanol extract with respect to experimental control and the group that was given 4.8%w/w fennel ethanol extract with respect to control. It also showed a significant increase in total cholesterol, LDL- cholesterol in the group that was given 4.8%w/w fennel ethanol extract with respect to control (table 3.1). These are drawbacks due to the use of fennel oil extract. It is very important to calculate the total cholesterol/HDL and LDL/HDL ratios as an additional understanding of the effect of *F. vulgare* on the distribution of lipoproteins. It can be noted that total cholesterol/HDL and LDL/HDL ratios of the group G and H significantly increased with respect to control. Knowing that these groups were given high fat diet (figure 3.1).

It is popular to believe that herbal products are the best remedies that can be used and the safest, especially in developing countries. Because herbal remedies are effective and inexpensive, consumers are becoming more proactive in self-treating (Pak et al., 2004). Herbal remedies have been used worldwide for hundreds of years. However, hepatotoxicities of medicinal plants have increasingly been reported recently (Aithal , 2005). The liver is a main target of drug-related pathologies since it is the chief site for drug catabolism. The action of drug-metabolizing enzymes together with microsomal cytochrome P450 enzymes biotransform exogenous compounds (Stickela and Patsenkerb, 2005). To test effects of *F. vulgare* seeds (powder and ethanol extracts) on the liver, the activities of transaminases (SGOT and SGPT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in serum of rats were measured after two weeks of seed plant extract intake. This study showed that the group that was given 10%w/w fennel powder showed a significant decrease in LDH level as compared to the control and the group that was given 1.2%w/w fennel ethanol

extract showed a significant decrease in GOT and ALP levels as compared to the control. The group that was given high fat diet and treated with 1.2% w/w fennel ethanol extract showed a significant decrease in ALP levels with respect to control and experimental control. This study showed that 1.2%w/w fennel ethanol extract doesn't affect the liver regardless of fat content in the diet but instead it may have a mild protective effect possibly due to activities of d-limonene and ß-myrcene. It has been reported that volatile components of fennel seed extracts contain d-limonene and ß-myrcene which proved to affect liver function. The concentration of reduced glutathione (GSH) in the liver is increased by D-limonene (Reicks & Crankshaw 1993). Glutathione is a necessity for by several enzymes. ß-myrcene inhances the levels of apoproteins CYP2B1 and CYP2B2, which are subvariations of the P450 enzyme system (De-Oliveira 1997) the present and Özbek et al. (2003) studies revealed that FEO has a potent hepatoprotective action.

On the other hand, the group that was given 0.6% fennel ethanol extract showed a significant increase in GOT and ALP levels and a significant decrease in LDH levels as compared to the control. The group that was given 2.4% fennel ethanol extract showed a significant increase in GOT, GPT, and ALP levels and a significant decrease in LDH levels as compared to the control. The group that was given 4.8% fennel ethanol extract showed a significant increase in GOT, GPT and LDH levels as compared to the control. Taking these concentrations of fennel ethanol extract may affect the liver but it doesn't induce a serious damage because transaminase enzymes level must exceed 250-350(U/L) in order to talk about serious damage (Titcomb et al., 2001). This activity of fennel ethanol extract may be due to presence of flavonoids in fennel oil (Sheikh et al., 1997). These results are consistent with studies done by Subehan et al. (2006) who proved that methanol extracts of *F. vulgare* inhibit the activity of CYP3A4 in a periodic-time related and concentration manners. (Cytochrome CYP3A4 is a sub family of cytochrome P450 (CYP) that catalyzes the oxidative metabolism of a broad variety of chemicals of external regions such as drugs, toxins, carcinogens, and endogenous constituents like steroids, fatty acids, and prostaglandins) (Subehan et al. 2006).

The effect of ethanol extract of *F. vulgare* seed on the glycemic profile in the fasted state was studied. In this study fennel doesn't show any effect on glycemic profile although it causes a significant increase in insulin levels in the groups that were given high fat diet. An increase in insulin level was observed in all groups receiving high fat diet revealing a direct

correlation between high lipid diet and serum insulin concentration. These results contradict with the study done by Javadi et al., (2008) who showed that *F. vulgare* (250mg/kg) essential oil reduces blood glucose.

Finally, more precise studies are suggested in order to specify the exact activities of Flavonoids , Anethole and phenol. Moreover, it is suggested that if the experiment was implemented for longer periods, the results may be clearer and more definitive. Other further studies must be done to identify the active ingredients that are present in *F. vulgare* oils that affect the liver enzymes. In a conclusion, *F. vulgare* oil showed a mild hypocholesteremia on a high fat diet and a hypo triglyceremia. *F. vulgare* oil causes a slight increased in the enzyme liver but it doesn't cause a serious damage. Finally it did not reveal any clear glycemic effect. In conclusion, *F. vulgare* seed exhibited a hypotriglyceridemic effect regardless of the fat content in the diet. However, *F. vulgare* ethanol extract showed a significant decrease in all cholesterol types only when the diet was high in lipid. No severe liver damage was observed with *F. vulgare* intake.

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Appendix I

Classification of *Foeniculum vulgare*

Kingdom Phylum/Division Class Order Family Genus Species

Plants(*plantae)*

Kingdom

Flowering Plants (*Magnoliophyta*)

Division

Dicotyledons | Dicots (*Magnoliopsida)*

Class

Order *Apiales*-Ginseng family, Carrot family

Order

Carrot Family (*Apiaceae)*

Family

Fennel (*Foeniculum*)

Genus

Sweet Fennel (Vulgare Vulgare)

Species

Appendix II

Botanical species, Botanical synonyms and Common names

Botanical species . and variety

Foeniculum vulgare Miller subsp

vulgare var. *vulgare* (bitter fennel)

Foeniculum vulgare Miller subsp.

vulgare var. *dulce* (Miller) Thellung(sweet fennel)

Botanical synonyms

Anethum foeniculum L.

Foeniculum capillaceum Gilib.

Foeniculum officinale All.

Common names

Shumar(Arabic)

Fennel (English)

Venkel (Dutch)

Fenkoli (Finnish)

Fenouil (French)

Fenchel (German)

Finocchio amaro (o selvatico)

dolce (o romano) (Italian)

Fennikel (Norwegian)

Hinojo (Spanish)

Fankal (Swedish)

Part of the plant Fruit (whole cremocarp and mericarp)

Appendix III

Foeniculum vulgare habit and seeds

alveo-a-stefaniak.ws/deskription.html

Fennel
(Foeniculum vulgare)

(Aggraval et al., 2008).