THE EFFECT OF *INULA VISCOSA* EXTRACT ON INFLAMMATION, MICROBIAL GROWTH, GLYCEMIA, AND BLOOD LIPID PROFILE

by

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ABSTRACT

The present study evaluates the effects of a one month period of chronic consumption of water, methanol, and petroleum ether extracts of *Inula viscosa* on fasting blood lipid profile, sugar level, and liver enzymes in rats receiving a regular fat diet. The present investigation also included the antimicrobial and anti-inflammatory effect of the plant extract.

A general fasting hypoglycemic effect was only observed with chronic intake of water extract. This hypoglycemic effect was accompanied with a significant increase in serum insulin concentration. Both methanol and petroleum ether extracts of *Inula viscosa*, showed a potential antimicrobial activity on gram-positive and gram-negative bacteria as well as antifungal effects. A dose dependent antimicrobial inhibition trend in both methanol and petroleum ether extracts was observed.

In the acute inflammation induced by carrageenan, *Inula viscosa* water extract showed an anti-inflammatory effect with the 500 and 1000mg/kg B.W. doses, in a dose dependent manner. The 1000 mg/kg B.W. dose and diclofenac showed similar inflammatory inhibition (36% *vs* 39%). In the chronic inflammatory study, *Inula viscosa* water extract showed a consistent dose dependant anti-inflammatory effect on formalin induced paw-edema. The 1000mg/kg B.W. dose had similar anti-inflammatory effect (44%) compared with diclofenac (44%).

Water, methanol, and petroleum ether extracts contributed to substantial changes in the blood lipid profile. Although no significant changes in the concentration of circulating TAG was observed among the different groups, all extracts reduced significantly serum total cholesterol, LDL cholesterol and LDL/HDL cholesterol ratio with respect to the control group. Only the methanol group showed a significant decrease in the concentration of HDL-cholesterol with respect to all other groups. VLDL apolipoprotein B100 (apo B100) showed no significant differences among the different groups.

Determination of SGOT, SGPT and LDH activities showed similar values in all groups except with LDH where the activities were significantly lower than the control group.

In conclusion, *Inula viscosa* methanol and petroleum ether extracts appeared to have important anti-bacterial and anti-fungal activities. Water extract seems to be a very effective anti-inflammatory drug that may be used for both acute and chronic inflammation. The water extract is also effective in reducing serum glucose level via possibly promoting insulin secretion by the pancreas. Both water and petroleum ether extracts appear to be effective in reducing blood cholesterol level through enhanced lipoprotein metabolism and clearance. No liver damage has occurred during the study period with all extract used.

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I would also like to thank God, that finaly it is over.

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GLOSSARY

Abs: Absorbance
ad lib: ad libitum.

ALT: Alanine Transaminase
Apo: Apolipoprotein

APS: ammonium persulfate solution

AST: Aspartate Transaminase
BW: body weight
CETP: Cholesteryl ester transfer protein

CHD: coronary heart disease

Chol: Cholesterol

CM: Chylomicron

Conc: concentration

DTT: Dithiothreitol

EDTA: Ethylene-diamine-tetraacetic acid

ELISA: Enzyme Linked ImmunoSorbent Assay

Ext: Extract

FA: Fatty acid

FFA: free fatty acids

GK: Glycerol kinase

GPO: Glycerol-3-phosphate oxidase

HDL: High-density lipoprotein

HL: Hepatic lipase

IDL: Intermediate-density lipoprotein

LCAT: Lecithin cholesterol acyltransferase

LDH: lactate dehydrogenase

LDL: Low Density Lipoprotein

LPL: Lipoprotein lipase

MAG: Monoacylglycerol

MDH: malate dehydrogenase
NaN_3: Sodium azide

O.D: Optical Density
PAFAH: platelet activating factor acetylhydrolase

PMSF: Phenylmethylsulfonylfluoride

PON: Paroxonase

SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SEM: Standard error from the mean

Sf: Svedberg flotation rate

sGOT: Serum Glutamic Oxaloacetic Transaminase

sGPT: Serum Glutamic Pyruvic Transaminase

TAG: Triacylglycerol

TBS: Tris Buffer Solution

TEMED: Tetramethylethylenediamine

I.viscosa: inula viscosa

VLDL: Very Low Density Lipoprotein

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION AND AIM OF THE PROJECT

Inula viscosa *(I.viscosa)* is a perennial weed native of the Mediterranean Basin. It has sticky (viscous) leaves with a typical odor. Normally, no pests harbor on this plant nor animals feed on it (Cohen et al ., 2002). *I.viscosa* is a well-known medicinal herb that grows wild in turkey, Spain, Italy, Portugal, Bulgaria and the middle east (Baytop,1984; AL-Eisawi, 1998), and contains some pharmacologically active compounds (Bohlmann et al., 1978; Ulubelen et al., 1987; Wollenweber et al., 1991).

In the Mediterranean area, inula viscosa was used for years in folk medicine for its anti-inflammatory (Barbetti et al., 1985) antipyretic, antiseptic, and antiphlogistic activities (Yaniv et al.,1987). In Jordan, traditional medicine ascribes several uses to *I. viscosa*: anthelmintic, expectorant, diuretic, treatment of bronchitis, tuberculosis, anemia and as cataplasm for rheumatic pain (Karim and Quraan, 1986; Karim et al.,1990; Al-Khalil et al., 1992). Also, the plant has been used in Spanish folk, medicine for treating gastroduodenal disorders (Lastra et al., 1993). Extracts of *I.viscosa* are used to heal inflammations and its dry shoots to repel insects. Extensive chemical analyses of *I.viscosa* revealed a series of terpenoid compounds, with some exhibiting anti fungal activity (Cohen et al ., 2002).

It is also known, in folk-medicine of different cultures, for its hypoglycemic properties (i.e. decreasing the blood-sugar concentration and therefore can be used for treatment of diabetes) (Lewis and Elvin-Lewis, 1977; Lauro and Rolih,1990). It can be used as diuretic, topical

antipruritic, by being boiled in oil for myalgia, and as haemostatic on surface wounds (Trease and Evans, 2002)

Scientific studies (Trease and Evans, 2002) revealed some interesting pharmacological effects of the plant, such as gastro-protection and prostaglandin E2 generation in rats by its flavonoid extracts, and its role in the treatment of diabetes. Aromatic plants have been known since antiquity to possess biological activity, notably antibacterial and antifungal properties (Deans and Svoboda, 1990).

No reports have dealt with the effect of *I.viscosa* extracts upon blood lipids taking into consideration lipoprotein secretion and metabolism. Apolipoprotein B (Apo B), a surface marker of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), has been shown to be a better predictor of coronary heart disease (CHD) than other markers, such as LDL and total cholesterol, especially in individuals with low or normal LDL-cholesterol (Sniderman et al., 2001).

1.2 Lipids of Physiologic Significance:

The lipids are a heterogeneous group of compounds related, either actually or potentially, to the fatty acids (Bogdanov, 1999). Lipids are important dietary constituents not only because of their high energy value but also because of the fat-soluble vitamins and the essential fatty acids contained in the fat of natural foods (Broun et al, 1999).

1.2.1 Biomedical Importance

In the body, fat serves as an efficient source of energy- both directly and potentially when stored in adipose tissue. it serves as a thermal insulator in the subcutaneous tissues and around certain organs, and nonpolar lipids act as electrical insulators allowing rapid propagation

of depolarization waves along myelinated nerves(champ and Harvey,1994).

Fat absorbed from the diet and lipids synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage (Broun et al,1999). Since lipids are insoluble in water, the problem arises of how to transport them in an aqueous environment-the blood plasma. This is solved by associating nonpolar lipids (TAG and cholesteryl esters) with amphipathic lipids (phospholipids and cholesterol) and proteins to make water-miscible lipoproteins.

Combination of fat and protein (lipoproteins) are important cellular constituents, occurring both in the cell membrane and in the mitochondria within the cytoplasm, and serving also as the means of transporting lipids in the blood (Champ and Harvey,1994). A knowledge of lipid biochemistry is important in understanding many current biomedical areas of interest for example, obesity, atherosclerosis, and the role of various polyunsaturated fatty acids in nutrition and health.

In a meal-eating omnivore such as the human, excess calories are ingested in the anabolic phase of the feeding cycle, followed by a period of negative caloric balance when the organism draws upon its carbohydrate and fat stores. Lipoproteins mediate this cycle by transporting lipids from the intestines as chylomcrons, and from the liver as very low density lipoproteins (VLDL), to most tissues for oxidation and to adipose tissue for storage (Gotto and pownall, 1999).

Lipid is mobilized from adipose tissue as free fatty acids (FFA) attached to serum albumin. Abnormalities of lipoprotein metabolism occur at the sites of production or utilization of lipoproteins, causing various hypo- or hyper-lipoproteinemias (Gurr and Harwood,1991). The most common of these is diabetes mellitus, where insulin deficiency causes excessive mobilization of FFA and underutilizationof

chylomicrons and VLDL, leading to hypertriglyceridemia. Most other pathologic conditions affecting lipid transport are due primarily to inherited defects in synthesis of the apoprotein portion of the lipoprotein, of key enzymes, or of lipoprotein receptors. Some of these defects cause hypercholesterolemia and premature atherosclerosis. Excessive fat deposits constitute obesity (Lardy,1990).

1.2.2 Digestion, Absorption, Secretion, and Utilization of Dietary Lipids

An adult ingests about 60 to 150 g of lipids per day, of which more than 90% is normally TAG. The remainder of the dietary lipid is made up of cholesterol, cholesteryl esters, phospholipids, and unesterified "free" fatty acids (champ and Harvey,1994).

The digestion of lipids begins in the stomach, catalyzed by an acid-stable lipase. However, the rate of hydrolysis is slow because the lipid is not yet emulsified (Broun et al, 1999). In the duodenum, the critical process of emulsification of the dietary lipids occurs. Emulsification increases the surface area of the lipid droplets, so that the digestive enzymes can act effectively. Emulsification is accomplished by two complementary mechanisms: use of the detergent properties of the bile salts and mechanical mixing due to peristalsis.

1.2.2.1 Absorption of Lipids by Intestinal Mucosal Cells

Free fatty acids, free cholesterol, and 2-monoacylglycerol are the primary products of dietary lipid degradation in the jejunum. These, together with bile salts, form mixed micelles-clusters of amphipathic lipids that coalesce with their hydrophobic groups on the inside and their hydrophilic groups on the outside of the cluster, and which are soluble in the aqueous environment of the intestinal lumen. The mixed micelles approach the primary site of lipid absorption, where

the component lipids pass through the unstirred water layer and are absorbed (champ and Harvey, 1994).

Fatty acids are converted into their activated form by fatty acylCoA synthetase (thiokinase). Using the fatty acylCoA derivatives, the 2-monoacylglycerols absorbed into the intestinal mucosal cells are converted to TAG by a family of acyltransferases that recognize fatty acyl CoAs of specific chain length (Duplus et al,2000).

2-monoacylglycerol + 2 fatty acylCoA $\longrightarrow$ TAG + 2 CoA

1.2.3 Lipids are Transported in the Plasma as Lipoproteins:

Four major groups of lipoproteins have been identified and are important physiologically and in clinical diagnosis. These are (1) chylomicrons, derived from intestinal absorption of TAG; (2) very low density lipoproteins (VLDL, or pre-β-lipoproteins),derived from the liver for the export of TAG; (3) low density lipoproteins (LDL, or β-lipoproteins), representing a final stage in the catabolism of VLDL; and (4) high-density lipoproteins (HDL, or α-lipoproteins) as shown in Table 1.1 (champ and Harvey,1994).

In addition to the use of techniques depending on their density, lipoproteins may be separated according to their electrophoretic properties into α-, β, and pre-β-lipoproteins (figure1.1) and may be identified more accurately by means of immunoelectrophoresis (Vance and vance,2002).

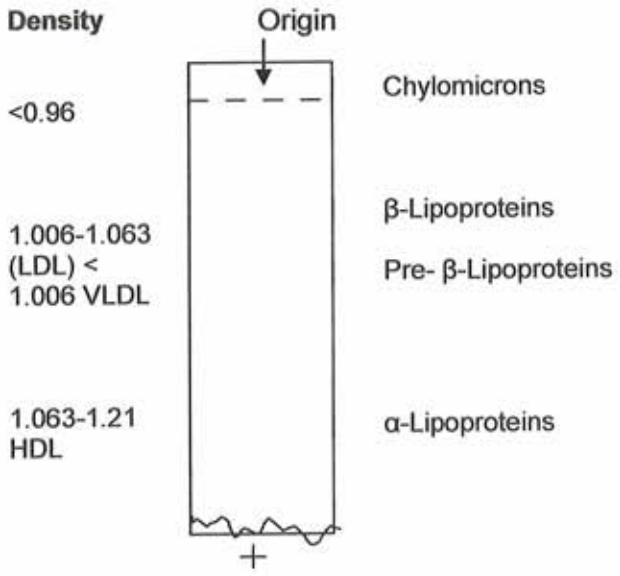

Figure 1.1 Separation of plasma lipoproteins by electrophoresis on agarose gel (Champ and Harvey, 1994).

Table 1.1: Composition of the lipoproteins in plasma of humans (Champ and Harvey, 1994)

Fraction	Source	Diameter (nm)	Density	COMPOSITON						
				Protein %	Total Lipid %	PERCENTAGE OF TOTAL LIPID				
						TAG	PhosPholipid	Cholesteryl Ester	Cholesterol (Free)	Free Fatty Acids
Chylomicrons	Intestine	90-1000	<0.95	1-2	98-99	88	8	3	1	...
Very-low density lipoprotein (VLDL)	Liver (Intestine)	30-90	0.95-1.006	7-10	90-93	56	20	15	8	1
Intermediate density lipoproteins (LDL)	VLDL	25-30	1.006-1.019	11	89	29	26	34	9	1
Low-density lipoprotein (LDL)	VLDL	20-25	1.019-1.063	21	79	13	28	48	10	1
High-density lipoproteins	Liver and intestine VLDL Chylomicron	10-20	1.063-1.125	33	67	16	43	31	10	...

1.2.3.1 Plasma Lipoproteins:

The plasma lipoproteins are molecular complexes of lipids and specific proteins called apolipoproteins. These dynamic particles are in a constant state of synthesis, degradation, and removal from the plasma (champ and Harvey,1994). Lipoproteins function both to keep lipids soluble as they transport them in the plasma, and to provide an efficient mechanism for delivering their lipid contents to the tissues (Anderson et al, 1998). In humans, the delivery system is less perfect than in other animals, and, as a result, humans experience a gradual deposition of lipid (especially cholesterol) in tissues. This is a potentially life-threatening occurrence when the lipid deposition contributes to plaque formation, causing the narrowing of blood vessels- a condition known as atherosclerosis (Murray et al, 1993).

1.2.3.2 Composition of Plasma Lipoproteins

The principal lipids carried by lipoprotein particles are TAG and cholesterol (free or esterified), obtained either from the diet or de novo synthesis (Gurr and Harwood,1991). Lipoproteins are composed of a neutral lipid core (containing TAG and cholesteryl esters) surrounded by a shell of apolipoproteins (apoproteins), phospholipids, and nonesterified cholesterol all oriented so that their polar portions are exposed on the surface of the lipoprotein, thus making the particle soluble in aqueous solution (champ and Harvey, 1994).

1.2.3.3 Size and Density of Lipoprotein Particles:

The chylomicrons are the lipoprotein particles lowest in density and largest in size, and contain the most lipid and the smallest percentage of protein (Champ and Harvey, 1994). VLDLs and LDLs are successively more dense, having a higher content of protein and a lower content of lipid. HDL particles are the most dense of the plasma lipoproteins.

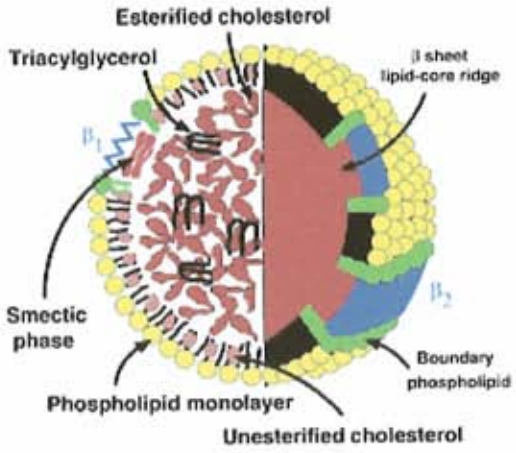

Figure1. 2 general structure of lipoproteins)(Mathews,1999)

1.2.3.4 Metabolism of Chylomicrons:

Chylomicrons are assembled in intestinal mucosal cells and carry dietary TAG, cholesterol, and cholesteryl esters to the peripheral tissues (Champ and Harvey, 1994). Assembly of the apolipoproteins and lipid into chylomicrons occurs during transition from the ER to the Golgi, where they are packaged in secretory vesicles that are exported from the cell into the lymphatic system by exocytosis (Mathews, 1999).

1.2.3.5 Modification of Nascent Chylomicron Particles:

The particle released by the intestinal mucosal cell is called a "nascent" chylomicron and contains apolipoprotein B-48 (apoB-48); it is so named because it constitutes the N-terminal 48% of the protein coded for by the apoB gene (Champ and Harvey, 1994). ApoB-100, synthesized by the liver and found in VLDL and LDL, represents the entire protein coded for by the apoB gene (Murray et al, 1993). When it reaches the plasma, the nascent chylomicron is rapidly modified, receiving apoE (which, in conjunction with apo B-48, is recognized by hepatic receptors) and C apolipoproteins (including apoC-II, necessary for the activation of lipoprotein lipase, the enzyme that degrades the

TAG contained in the chylomicron). The source of these apolipoproteins is circulating HDL (Vance and vance,2002)

1.2.3.6 Degradation of TAG by Lipoprotein Lipase

Lipoprotein lipase is a negatively charged extracellular enzyme that resides on the capillary walls of most tissues but is found predominantly in the capillaries of the adipose tissue and cardiac and skeletal muscle (Murray et al,1993). Lipoprotein lipase, activated by apoC-II on circulating lipoprotein particles, hydrolyzes the TAG contained in these particles to yield monacylglycerol, fatty acids, and glycerol. Patients with a deficiency of lipoprotein lipase or apoC-II show a dramatic accumulation of TAG-rich lipoproteins in the plasma.

1.2.3.7 Formation of chylomicron remnants:

As the chylomicron circulates and the TAG in its core is degraded by lipoprotein lipase, the particle decreases in size and increases in density. In addition, the C apolipoproteins are returned to the HDLs. The remaining particle is called a "remnant" (Mathews, 1999). In humans, these chylomicron remnants are removed from the circulation by the liver. Hepatocyte membranes contain lipoprotein receptors that recognize the combination of apolipoprteins B-48 and E. Chylomicron remnants bind to these receptors and are taken into the cells by endocytosis. The endocytosed vesicle then fuses with a lysosome, and the apolipoproteins, cholesteryl esters, and other components of the remnant are hydrolytically degraded, releasing amino acids, free cholesterol, and fatty acids (Liscum and Munn,1999).

1.2.3.8 Metabolism of Very Low Density Lipoproteins

VLDLs are produced in the liver. They are composed predominantly of TAG, and their function is to carry this lipid from the liver to the peripheral tissues (Murray et al, 1993). There, the TAG is degraded by

lipoprotein lipase. Fatty liver occurs in conditions in which there is an imbalance between hepatic TAG synthesis and the secretion of VLDL. Diseases such as hepatitis, uncontrolled diabetes mellitus, and chronic ethanol ingestion can cause fatty liver.

VLDLs are released from the liver as nascent VLDL particles containing apolipoproteins B-100 and A-I. They must obtain apoC-II and apoE from circulating HDL (Champ and Harvey, 1994).

1.2.3.9 Modification of Circulating VLDL:

As VLDLs pass through the circulation their structure is altered. TAG is removed by lipoprotein lipase, causing the VLDL to decrease in size and become more dense. Surface components, including the C and E apolipoproteins (that originally had been donated to the VLDL from HDL), are transferred to HDL. Finally, cholesteryl esters are transferred from HDL to VLDL in an exchange reaction that transfers TAG or phospholipids from VLDL to the HDL. This exchange is accomplished by cholesteryl ester transfer protein (Vance and Vance, 1991).

1.2.3.10 Production of LDL from VLDL in Plasma:

After these modifications, the VLDL has been converted in the plasma to LDL. An intermediate-sized particle, the intermediate density lipoprotein (IDL) is observed during the transition from VLDL to LDL in the plasma. IDLs can also be taken up by cells through receptor-mediated endocytosis (Murray et al, 1993).

LDL particles retain apoB-100, but lose their other apolipoproteins to HDL. They contain much less TAG than their VLDL predecessors, and have a high concentration of cholesterol and cholesteryl esters (Champ and Harvey, 1994).

1.2.3.11 Metabolism of High Density Lipoproteins (HDL)

HDL particles are synthesized in the liver and are released into the bloodstream by exocytosis (Vance and Vance, 1991. They perform a number of important functions, including serving as a circulating reservoir of apoC-II; removing free cholesterol from extrahepatic tissues and esterifying it, using the plasma enzyme phosphatidylcholine cholesterol acyltransferase; transferring cholesteryl esters to VLDL and LDL in exchange for TAG; and carrying cholesteryl esters to the liver, where the HDL is degraded and cholesterol released (Murray et al, 1993).

1.2.3.12 HDL as a Reservoir of Apolipoproteins:

HDL particles not only serve as the source of apolipoprotein required for the proper metabolism of other plasma lipoproteins, but also take back most of these proteins before the chylomicron remnants and LDLs bind to their cell-surface receptors and are endocytosed (Champ and Harvey, 1994).

1.2.4 Apolipoproteins:

The apolipoproteins associated with lipoprotein particles have a number of diverse functions, including serving as structural components of the particles, providing recognition sites for cell-surface receptors, and serving as activators or coenzymes for enzymes involved in lipoprotein metabolism (Mathews et al,2000).
Apolipoproteins are divided by structure and function into classes A to H, with most classes having subclasses, apoA-I and apoC-II (Murray et al, 1993).

1.2.4.1 The Distribution of Apolipoproteins Characterizes the Lipoprotein:

One or more apolipoproteins (proteins or polypeptides) are present in each lipoprotein (Table 1.2). Apolipoproteins carry out several roles: (1) they are enzyme cofactors, (2) they can act as lipid transfer proteins, (3) they act as ligands for interaction with lipoprotein receptors in tissues (Vance and Vance, 1991).

Table 1.2. Apoproteins of human plasma lipoproteins (Murray et al, 1993)

Apolipoprotein	lipoprotein	Molecular mass (Da)	Additional remarks
A-I	HDL,chylomicron	28,000	Activator of lecithin: cholesterol acyltransferase(LCAT)
A-II	HDL,chylomicron	17,000	Structure is 2 identical monomers joined by a disulfide bridge. Inhibitor of LCAT
A-IV	Secrete with chylomicrons but transfers to HDL	46,000	Associated with the formation of triacylglycerol-rich lipoproeins. Function unknown.
B-100	LDL, VLDL, IDL	550,000	Synthesised in liver. Ligand for LDL receptor.
B-48	Chylomicrons,chylomicron remnants	260,000	Synthesized in intestine.
C-I	VLDL, HDL, chylomicrons	7600	Possible activator of LCAT.
C-II	VLDL, HDL, chylomicrons	8916	Activator of extrahepatic lipoprotein lipase.
C-III	VLDL, HDL, chylomicrons	8750	Several polymorphic forms depending on content of sialic acids.
D	Subfraction of HDL	20,000	Function unknown.
E	VLDL, HDL, chylomicrons, chylomicron remnants	34,000	Present in excess in the β-VLDL of patients with type III hyperlipoproteinemia.

1.3 Inula viscosa

Family: Compositeae

Genus: Inula

Species: viscosa

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1.3.1Common names: Arabic: Tayoun. Maltese: Tulliera komuni, English: sticky Fleabane, also known as dittrichia viscose or Cupularia viscose.

1.3.2 Known Plant Constituents: (Trease and Evans, 2002)

- Terpene derivatives
- Azulenes
- Hispidulin
- Psi-Taraxasterol acetate
- Camphor
- Thymo carvacrol
- Flavonoids
- Sesquiterpenes
- Aromatic compounds (inulin, resins)

1.3.3 Appearance:

A perennial or annual with unstalked, narrow, hairy, gland-dotted and highly aromatic leaves. The plant forms a dense mass branching from the ground. Tiny closely packed yellow flowers appear from August to November in the Mediterranean area.

1.3.4 Habitat

Grows from sea-level to 5,000 ft in moist situations by roadsides, riversides and on bare ground. It is often the first plant to colonize recently cleared ground. The following areas are considered to be this plants natural range: Europe - Mediterranean. Introduced and naturalized in a few localities in Britain.

1.3.5 Uses and Properties

The plant has been used in the past for treating enteritis and gonorrhea. Powdered leaves were employed to treat burns. The roots which are rich in inulin, resins and other aromatic compounds are employed as powders or infusions for their expectorant, diuretic and tonic properties. The plant is an efficient insect repellent and is often rubbed on window shutters in villages and used to repel fleas by striking mattresses with fresh stems.

1.3.6 Flowers and Foliage:

- The flowers are classified as hermaphrodite.
- Insects are responsible for pollinating this variety.

1.3.7 Landscaping and Planting:

- This plant variety generally cannot be successfully grown in areas where the soil quality is of a poor standard, i.e. lacking in sufficient nutrients.
- This plant variety does not tolerate heavy clay soils.
- This variety can be grown in anything from a light to a heavy soil mixture.
- A well drained soil is not required to successfully grow this variety.
- This variety prefers a position within full sun.
- It is preferable to plant this variety in a moist position.

1.3.8 Medicinal uses:

The plant possesses anti-implantational and luteoltic effects (Al-Dissi et al, 2001). According to Dafni et al (1984), women are seated briefly above a bath of the plant three days after copulation or are administered the ground leaves through anus for sterility purpose. Many plants of the compositae family have a folk reputation as an abortion promoter, but few have this abortive property confirmed experimentally (Farnsworth et al., 1975). It is known that progesterone is an important factor in the implantation processes during pregnancy. It is also known that corpora lutea are major sites for progesterone synthesis during early pregnancy. Therefore, luteolysis and the decrease in the blood levels of progesterone may contribute to the antifertility activity of *I.viscosa*, such as abortion and anti-implantation (Al-Dissi et al, 2001).

Repeated pretreatment with *I.viscosa* plant extracts induced a significant protective effect in variable degrees against ethanol-induced gastric mucosal damage. When I.viscosa extracts and other plant extracts are arranged regardless of the petechial lesions (minute hemorage in the skin), the ethanolic extract of I.viscosa comes at the top (Alkofahi and atta, 1999)

1.3.8.1 Hypoglycemic effect:

In an extensive ethno-botanical survey of the medicinal plants of Palastine, 16 species were found to be used for hypoglycemic treatments. The list included *I.viscosa* (L) (Yaniv et al, 1986). According to this article tisane is prepared from the leaves of I.viscosa and taken daily.

1.3.8.2 Anti-inflammatory Effect:

Some extracts from *I.viscosa* were examined for acute anti-inflammatory activity in vivo. Rhamnocitrin, 7-O-methylaromendrin, and 3-O-acetylpadmatin (as flavonoids) and sesquiterpene lacton, (inviscolide), sesquiterpene acid (ilicic acid), and a digalactosyl-diacylglycerol (inugalactolipid A) were isolated from the dichloromethane (CH2CL2) extract, identified by spectroscopic methods, and characterized as the topical anti-inflammatory principles of this species (Manez et al, 1999). On the basis of the reported results, inuviscolide is the main anti-inflamatory sesquiterpenoid from inula viscose, and may act by interfering with leukotriene synthesis and phospholipase A(2)-induced mastocyte release of inflammatory mediators (Hernandez et al, 2001).

1.3.8.3 Antioxidant Activity of Anti-inflammatory Plant Extracts

There is an increasing interest in the antioxidant effects of derived herbal compounds and their role in health and disease. In addition to the protective effects of the endogenous antioxidant defense system, natural products with antioxidant activity could retard the oxidative damage of a tissue by increasing those defenses (Aruoma, 1996; Duthie et al, 1996; Keli et al, 1996; Schinella et al, 2002). It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events (Halliwel and Gutteridge, 1989; Aruma, 1998; Feher et al, 1987). It has been shown

that *I.viscosa* has the ability to protect against enzymatic and non-enzymatic lipid peroxidation in model membranes and exhibited scavenging activity on the superoxide radicals (Schinella et al, 2000).

1.3.8.4 Antimicrobial Activity

All the extracts proved to have a significant antifungal activity against dermatophytes even at low concentrations (0.01 mg/ml). The leaf extracts exhibited the greatest antifungal efficacy. The high concentration of the sesquiterpene (carboxyeudesmadiene), occurring in the leaf extracts, may explain its greater antifungal activity (Cafarchia, 2002).

Extracts derived from *I.viscosa* with organic solvents are shown to contain antifungal compounds. Water extracts showed a poor yield of such compound suggesting that those fungicidal materials are mostly lipophilic. In vitro and in plant studies revealed broad-spectrum efficacy of these extracts in controlling various diseases including Oomycetes, Ascomycetes, Basidiomycetes and fungi imperfecti (Cohen et al, 2002)

Fungitoxicants from higher plants may have a better future than synthetic fungicides largely due to their non-phytotoxic, systemic, and easily biodegradable nature (Fawcett and Spencer, 1970; Beye, 1978). *I.viscosa* extract cause a decline in chitin content in dermatophytes and C.albicans is of a great importance since chitin is a component of the fungi wall that does not exist in human cells (Maoz and Neman, 2000).

1.4 Aim of the Project

The present work covers the chronic effect of water, petroleum ether and methanol extracts of *I.viscosa* upon the blood lipid profile. The study is further extended to cover the anti-inflammatory, antibacterial, antifungal and glycemic effects of the plant extracts. Blood lipid profile and glycemia were investigated in the rat model after a period of one month of chronic consumption of either water, methanol, or petroleum ether extracts of *I.viscosa*.

The study aimed at covering the following parameters:

- Plasma cholesterol, high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol,
- Triacylglycerol (TAG) and glucose .
- Serum sGOT, sGPT, and LDH activity.
- VLDL Apo B100
- Anti-Inflammatory effect.
- Antibacterial and antifungal effect.
- Serum Insulin.

Chapter 2

MATERIALS AND METHODS

2.1 Animal Treatment

Male Sprague-Dawley (*Rattus norvegicus*) rats weighing between 200 to 250g (Lebanese American University stock), are used in all experiments. Animals were maintained and experimental protocols complied with the Guide for the Care and Use of Laboratory Animals (National Research Council of the United States 1985). All animals were sacrificed using diethyl ether, at the end of the procedures described, without recovery from anaesthesia.

2.1.1 Plant Material

Leaves of Inula viscosa *(I.viscosa)* were collected from the stony hillslopes, and the road sides surrounding LAU, Blat-Byblos-Lebanon during flowering season (Spring 2004). The leaves were dried for two weeks in a shaded, well-ventilated area, and ground into fine powder using a kitchen blender.

2.1.2 Preparation of Water Extracts

Water extract was prepared by soaking 2.8g of plant powder in 1L of pre-boiled hot water with occasional stirring for 15 min. Then the filtrates were introduced to rats *ad libitum* instead of water.

2.1.3 Preparation of Petroleum Ether Extracts

I.viscosa (120g) dried powdered leaves were extracted with 1200ml Petroleum ether (PE) for 24 hours with continuous shaking (Innova™ Bronswick, New Jersey, USA). Suction filtration was used to collect the filtrates. Oil extracts of *I.viscosa* were reduced in volume to thick syrup in rotary vaccum evaporator (BUTCHI Waterbath B-480, Rotarvapor R-124, USA), at 40°C. Extracts were dissolved in 400ml

absolute ethanol, which was then sprayed on food as 20ml extract 1kg food (v/w).

2.1.4 Preparation of Methanol Extracts

Details of methanol extraction are the same as the petroleum ether extraction procedure mentiond above, using methanol instead of petroleum ether.

2.1.5 Assigning Animal Groups

In the process of studying the effect of a one month period of ingestion of water, petroleum ether and methanol extracts of *I.viscosa* upon blood lipid profile, 36 rats were randomly assigned into 4 groups of 8 rats each. Animals were maintained under natural lighting of 12 hours photoperiod and at a temperature of 20 - 22°C, during the whole study. Group subdivisions are shown in table 2.1.

Table 2.1: Assigning animals into the different groups. Each group incuded 8 rats.

Group	Control (A)	Water extract (B)	Petroleum ether extract (C)	Methanol extract (D)
Food	7 g/kg BW rat chow diet	7g/kg BW rat chow diet	7g/kg BW rat chow diet containing petroleum ether extract.	7g/kg BW chow diet containing methanol extract.
Drink	Plain water	Water extract	Plain water	Plain water

2.2 Sample Collection & Blood Study

After one month of treatment, fasted rats (18 hrs) anesthesized using diethyl ether. A midline abdominal incision was made for about two-thirds of the length of the abdomen and the inferior vena cava was exposed by exteriorizing the intestine. Using 10 mL syringes, about 8 mL of blood were withdrawn from the inferior vena cava and transferred into 2 tubes, one containing disodium ethylene-diamine-tetraacetic acid (Na_2EDTA 1mg/mL blood) for collection of plasma, and the other being plain for collection of serum. To collect serum, tubes were left at room temperature for 30 minutes to clot, then centrifuged (Sigma Laborzentrifugen 2K15, Germany) at a speed of 3000rpm for 20 minutes at a temperature of 10°C. Plasma was collected by immediate centrifugation at 4°C. 2 mL of plasma were used to isolate lipoproteins as described in the following section. 400 µL of serum were placed in Eppendorf tubes for direct enzyme analysis. Remaining samples were collected in Eppendorf tubes and stored in deep freeze for subsequent analyses

2.3 Isolation of Lipoproteins

Each 2 mL of the collected plasma was placed in the bottom of a 10 mL polycarbonate ultracentrifuge tube (Sorvall, Kendro Laboratory products) on ice. Crystalline NaCl (0.14g/ml) was added to the plasma samples and vortex mixed. Proteolytic degradation was minimized by adding a preservative solution containing aprotonin, 2mg/mL, and phenylmethylsulfonylfluoride (PMSF), 10mM dissolved in 2-propanol, at concentrations of 5 µL/mL plasma and 10 µL/mL plasma respectively. A 6.5ml NaCl solution (density= 1.006g/ml) 0.01% (w/v) Na_2EDTA and 0.02% sodium azide (NaN_3) with the pH adjusted to 7.4 was layered on top of the plasma in the ultracentrifuge tube using the peristaltic pump machine (Watson-Marlow Limited, England).

The tubes were then centrifuged for 20 hours at 15°C (Sorvall RC 28S centrifuge; Supraspeed F-28/13 fixed angle rotor) at 28000rpm. After which, the upper 400µl layer, containing the lipoproteins Sf > 20, are carefully aspirated and placed in Eppendorf tubes to be used as, described later, for gel electrophoresis.

2.4 Preparation of Samples for VLDL apo B100 Analysis

100 µl from the samples, containing lipoproteins, were mixed with 300 µl of sample buffer (ratio 3:1) as described below:

Sample buffer is a solution containing 0.15M sodium phosphate, 12.5% (v/v) glycerol, 2% (w/v) sodium dodecyl sulfate (SDS), 5% (v/v) mercaptoethanol, 0.001% (w/v) bromophenol blue, adjusted to pH=6.8.

The Eppendorf tubes containing the lipoprotein samples and the sample buffer were denatured at 90 °C for 4 min using Eppendorf tube heater, and then centrifuged (Heraeus Sepatech BIOFUGE 13R) for 5 min at 13000rpm. Samples were then stored at -20°C for Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis.

2.5 Sodium Dodecyl-Sulfate-Polyacrylamide Gel Electrophoresis

2.5.1. Principle

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is a low-cost, reproducible, and rapid method for quantifying, comparing, and characterizing proteins. This method separates proteins based primarily on their molecular weights (Laemmli, 1970).

SDS binds to hydrophobic portions of a protein, disrupting its folded structure and allowing it to exist stably in solution in an extended conformation. As a result, the length of the SDS- protein complex is

proportional to its molecular weight. The ease of execution and wide application of SDS-PAGE have made it an important analytical technique in many fields of research (blackshear,1984).

2.5.2 Stock solutions

0.5 M Tris buffer (pH 6.8): 6.02 g Tris was dissolved in approximately 80 ml distilled water, pH adjusted to 6.8 with 12 M HCl. Then distilled water was added to a final volume of 100 ml. This solution is stable for several weeks at 4°C.

3M Tris buffer (pH 8.8): 36.6 g Tris was dissolved in about 80 ml distilled water and pH adjusted to 8.8 with 12 M HCl. Distilled water was added to a final volume of 100 ml. This solution is stable for several weeks at 4°C.

1.5 % ammonium persulfate solution (APS): 0.15 g of ammonium persulfate was dissolved in 10 ml of distilled water.

Protogel: 30 g acrylamide and 0.8 g N, N'-bis-methylene acrylamide were dissolved in approximately 70 ml of distilled water; the final volume was adjusted to 100 ml.

Urea/SDS/dithiothreitol solution: 19.2 g urea, 12.3 mg dithiothreitol and 0.08 g lauryl sulfate SDS were dissolved in 30 ml of distilled water and the final volume was made up to 40 ml.

Electrode buffer (pH 8.3): 99.4 g glycine and 21.4 g Tris were dissolved in 2 L of distilled water, then 7 g of SDS were added to the Tris/Glycine solution and the final volume made up to 7 L with the addition distilled water.

Water-saturated n-butanol: 90 ml n-butanol + 10 ml water.

2.5.3 SDS-PAGE Preparation

2.5.3.1 Preparation and Pouring of Resolving and Stacking Gels

Following the method of Hames (1990) according to the original method of Laemmli (1970), linear gradient (5-20%) polyacrylamide gels were prepared to allow an increased protein sieving effects and a better separation of a larger range of protein molecular weights. A 5 % acrylamide resolving gel, a 20 % acrylamide resolving gel and a stacking gel , to be layered on top of the resolving gels, were prepared as shown in Table 2.2.

Table 2.2: Gel mixtures for 5-20% gradient gels (4 gels)

	Resolving Gel		Stacking Gel
	5%	20%	
Protogel	10ml	40ml	3.7ml
Urea/SDS/DTT	6ml	600µl	0.6ml
1.5%APS	1.4ml	1.4ml	2.3ml
Sucrose	-	9g	-
Water	40.4ml	5.4ml	15ml
0.5M Tris	-	-	7.5ml
3M Tris	7.4ml	7.4ml	-
TEMED	46 µl	46 µl	22.5 µl

The electrophoresis apparatus (Hoefer SE 600, Amersham Pharmacia Biotech) was assembled using the side spacers and clamps according to the manufacturer's instructions. Clamped glass plates were tested to ensure they are water tight and thus prevented leakage. Immediately before pouring the resolving gels, 46 µl tetramethylethylenediamine (TEMED) was added to each of the gels to initiate polymerization. The 20% gel was placed in the mixing chamber and the 5% gel in the reservoir chamber of a gradient forming apparatus (Coleparmer, USA). Using a peristaltic pump, the gels were poured into the glass chambers while stirring of the 20% chamber.

After the resolving gel has been poured, it was overlayered with water-saturated butanol and left to polymerize. After about 45 min, the water-saturated butanol was discarded and the gel top was rinsed several times with distilled water. The remaining water droplets were removed with a filter paper. A fifteen-well comb was inserted between the glass plates leaving about 0.5 cm between the bottom of the combs and the resolving gel. Before overlayering the polymerized resolving gel with the stacking gel, 22.5 µl of TEMED were added to the stacking gel. The poured stacking gel was left for about 30 min to polymerize then the comb was carefully withdrawn. Electrode buffer was added at this stage to the wells of the gel in order to avoid any later disturbance in the wells. Protein samples were loaded in the wells along with the molecular weight marker and the protein standard, after which the upper and lower electrode chambers were filled with electrode buffer.

2.5.3.2 Loading of Samples

Samples stored at -20°C were thawed in preparation for loading. Molecular weight markers, (Sigma Chemical Co.Ltd) were dissolved and denatured according to the supplier's instructions. Using disposable gel loading tips, 10 µl of molecular weight marker solution was loaded into the first gel lane, 5 µl, 15 µl and 40 µl of standard solution were added respectively into lanes 2, 3 and 4 of the gel. The remaining lanes were loaded with 100 µl of the lipoprotein samples under examination.

2.5.3.3 Electrophoresis

After loading all samples, electrodes of the upper chamber lid were properly attached, and the water-cooling system and power supply (Consort E455, Belgium) were then switched on. Electrophoresis was carried out at 220V, 120mA, 20W for both gel chambers. Four hours

later, the power is switched off, the water-cooling system disconnected and the gel carefully removed for the staining-destaining steps.

2.5.4 Staining Gel & Quantification of Proteins

2.5.4.1 Stock solutions

Coomassie Gel Stain

1.0g Coomassie blue R-250
450 ml methanol
450 ml H2O
100 ml glacial acetic acid

Coomassie Gel Destain

800ml water
100ml methanol
100ml glacial acetic acid

2.5.4.2 Staining and Destaining of Gels

Staining and destaining were carried out in an automated gel stainer-destainer (Hoefer processor plus, Amersham Pharmacia Biotech) for about 17 hours. Then, the gel slabs were carefully removed, placed between two transparencies, labelled and scanned.
The image saved was used for quantitation of apo B100 bands using 1-D Advanced software (Advanced American Biotechnology, 1166E Valencia Dr, #6C, Fullerton CA. 92831). A standard curve was then constructed for every gel slab and the concentrations of apo B100 were estimated accordingly.

2.6 Serum assays

2.6.1 Determination of Serum Glucose

Principle

The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a colored compound.

Procedure

Samples, run in duplicates, were mixed with the working reagent, and incubated 10 minutes at 37°C. The absorbances of the unknown and the standard samples were measured against the Blank reagent at λ = 520 nm (Hελios-γ spectrophotometer, UVG 101103, UK).

Calculation

Concentration of unknown (mg/dl) =

(Abs. of unknown/ Abs. of standard) x conc. of standard

Glucose standard is 100 mg/dl concentration according to the manufacturer's leaflet (SGMitalia).

2.6.2 Determination of Serum TAG

Principle

The TAG assay kit (Spinreact, S.A, Espana), used is based upon a colorimetric method. The TAG are enzymatically hydrolyzed to glycerol and free fatty acids. The glycerol liberated reacts with Glycerol Kinase (GK) and Glycerol-3-Phosphate Oxidase (GPO) yielding H_2O_2. The H_2O_2 concentration is determined through the Trinder's reaction. The enzymatic reaction sequence employed in the assay was as follows:

Triacylglycerol + H_2O $\xrightarrow{\text{LPL}}$ Glycerol + Fatty Acids
Glycerol +ATP $\xrightarrow{\text{GK}}$ Glycerol-3-phosphate + ADP
Glycerol-3-phosphate + O2 $\xrightarrow{\text{GPO}}$ Dihydrooxyacetone-P + H_2O_2
H_2O_2 + 4-AP + p-Chlorophenol $\xrightarrow{\text{POD}}$ Quinonimine(red) + H_2O

Procedure

Samples, along with standards, were run in duplicates, and were mixed with the working reagent and incubated 10 minutes at room temperature. The absorbances (Abs.) of the unknown and the standard samples were measured against Blank reagent at λ = 505 nm (Helios-γ spectrophotometer, UVG 101103). Color of the mixtures was varying from light pink to dark red, and it was stable for about 30 mn.

Calculation

Sample conc. (mg/dl) =

(Abs. of unknown/ Abs. of standard) x conc. of standard

TAG standard is 200 mg/dl concentration according to the manufacturer's leaflet (SPINREACT).

2.6.3 Determination of Serum Cholesterol

Principle

The cholesterol assay kit (Spinreact, S.A, Espana) used was based upon a colorimetric method. Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters and H_2O_2 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol-oxidase according to the following equation:

Cholesterol Esters + H_2O $\xrightarrow{\text{CHE}}$ Cholesterol + Fatty Acids
Cholesterol + O_2 $\xrightarrow{\text{CHOD}}$ Cholest-4-en-one + H_2O_2
H_2O_2 + 4 AP + Phenol $\xrightarrow{\text{POD}}$ Quinonimine + H_2O

Procedure

Samples, along with standards, were run in duplicates, and were mixed with the working reagent and incubated for 10 minutes at room temperature. The absorbances of the samples (unknown) and the standard samples were measured against Blank reagent at λ = 505 nm. Color of the mixtures was varying from light pink to dark red, and it was stable for about 30 mn.

Calculation

Cholesterol conc. (mg/dl) =

(Abs. of unknown/ Abs. of standard) x conc. of standard

Cholesterol standard is 200 mg/dl concentration according to the manufacturer's leaflet (SPINREACT).

2.6.4 Determination of Serum HDL-Cholesterol

Principle

LDL and VLDL are specifically precipitated by phosphotungstic acid and magnesium ions and can then be removed by centrifugation. HDL remain in the supernatant. Determination of HDL cholesterol is performed using the clear supernatant.

Procedure

Samples, run in duplicates, were mixed with the precipitating reagent (Phosphotungstic Acid 14 mmol/L, Magnesium chloride 2 mmol/L), allowed to stand for 10 minutes at room temperature and centrifuged at 12000 rpm for 4 minutes. The clear supernatant was used for

cholesterol determination using the cholesterol assay kit as described in the previous section.

Calculation

HDL-cholesterol concentration was estimated according to the following equation:

HDL-cholesterol (mg/dl) = Absorbance of sample x 320 at λ = 505 nm

2.6.5 Determination of Serum LDH

Principle

Using the LDH assay kit (SPINREACT). Optimized test according to the recommendation of SFBC/SEQC.

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

The rate of NADH consumption is determined photometrycally and is directly proportional to the LDH activity in the sample material.

Procedure

50µl of each sample are mixed with 1.5ml of the working reagent and incubated at 25°C for 1 minute, after which the extintion decrease (ΔE) is measured at λ = 340 nm against distilled water per minute during 3 minutes

Calculation

LDH activity (U/I) = (ΔE/min) x 4925

2.6.6 Determination of Serum GOT-AST

Principle

Using the GOT-AST assay kit (SGMitalia), AST catalyses reaction between alpha ketoglutaric acid and L-aspartate, giving glutamate and oxaloacetate, in presence of malate dehydrogenase (MDH), reacts with NADH giving malate and NAD. The O.D variation is proportional to the AST activity.

Procedure

100µl of each sample are mixed with 1ml of the working reagent and incubated at 37°C for 1 minute, after which the extintion decrease (ΔE) is measured at λ = 340 nm against distilled water per minute during 3 minutes

Calculation

SGOT activity (U/I) = (ΔE/min) x 1746

2.6.7 Determination of Serum GPT-ALT

Principle

Using the GPT-ALT assay kit (SGMitalia), ALT catalyses reaction between alpha ketoglutaric acid and L-alanine, giving L-glutamatic acid and pyruvic acid. Pyruvic acid in presence of lactate dehydrogenase (LDH), reacts with NADH giving lactic acid and NAD. The O.D variation is proportional to the ALT activity.

Procedure

100µl of each sample are mixed with 1ml of the working reagent and incubated at 37°C for 1 minute, after which the extintion decrease

(ΔE) is measured at λ = 340 nm (Helios-γ spectrophotometer, UVG 101103)against distilled water per minute during 3 minutes

Calculation

SGPT activity (U/l) = (ΔE/min) x 1746

2.6.8 Determination of Serum LDL-Cholesterol

Concentration of LDL cholesterol was determined according to the Friedwald equation:

LDL cholesterol = Total cholesterol – (TAG / 5) – HDL cholesterol

2.7 Calculation of Dry Weight of *I.viscosa* Extracts

2.7.1 Dry Weight of *I.viscosa Extracts*

Procedure

Dry weight of the water extracts was determined as described in section 2.1.1, 3g of plant material were soaked in 100ml of pre-boiled distilled water and incubated for 15 min, filterates were collected in an empty beaker. Water was totally evaporated at 65°C, and the weight of the beaker with the dried filtrates was recorded.

Calculation

Dry weight of water extracts of *I.viscosa* (g) = weight of beaker containing dried filtrate – weight of empty beaker

2.8 Anti-Inflammation Effect

2.8.1 Carrageenan Induced Paw Edema in Rats

To study the effect of acute inflammation, five groups of animals were selected in which six animals were assigned to each group. In all groups inflammation was produced by a single subplantar injection of 0.02ml feshly prepared 1% carrageenan in normal saline in the right hind paw of rats. One group with carrageenan alone served as a positive control. Three groups received the water extract of I.viscosa at a concentration of either 250, 500, or 1000mg/Kg body weight intraperitoneally 30 min prior to carrageenan injection. One group received diclofenac (10mg/Kg, i.p), as a standard reference drug. The paw thickness was measured using vernier calipers before and 3 hours after carrageenan injection (Ajith and Janardhanan, 2001).

2.8.2 Formalin Induced Paw Edema in Rats

To study the effect of chronic inflammation, five groups of animals were selected in which six animals were assigned to each group. In all groups chronic inflammation was produced by a single subplantar injection of 0.02ml of 2% formalin in the right hind paw of rats (Ajith and Janardhanan, 2001). Three groups received the water extracts of I.viscosa at a concentration of either 250, 500, or 1000mg/Kg body weight, and one group received the standard reference drug diclofenac (10mg/Kg) intraperitoneally 30 min prior to formalin injection. Control group did not receive any drug. The administration of the extract (250, 500, and 1000mg/Kg body weight) and diclofenac was continued once daily for 6 consecutive days. The paw thickness was measured using vernier calipers before and 6 days after formalin injection (Jose et al., 2004).

2.8.3 Paw Thickness Calculation

The increase in paw thickness in both models was calculated using the formula: $P_t - P_o$

Where P_t is the thickness of paw at time t (i.e. 3 hours after carrageenan injection and 6 days after formalin injection) and P_0 is the paw thickness at 0 time.

The percent inhibition was calculated using the formula:

(C – T/C) x 100.

Where C is the increase in paw thickness of the control and T is that of treatments (Ajith and Janardhanan, 2001).

2.9 Anti-Bacterial Anti-Fungal Effect

2.9.1 Media and Growth Conditions

Methanol and PE extract of inula viscosa were studied for the antibacterial and antifungal effect as follows:

1. To study the anti-bacterial effect, nutrient broth (13g/L) was prepared, autoclaved, and inoculated with Gram negative (*Escherichia coli* O157:H7), or Gram positive bacteria (*Bacillus sp.*) bacteria, and remaining nutrient broth was used for dilutions.
2. To study the anti-fungal effect, potato dextrose broth (24g/L) was prepared, autoclaved, and inoculated with yeast (*Candida albicans* wild type CA112), or with another fungus *(Penicillium sp.)*. Remaining potato dextrose broth was used for dilutions.
3. Inoculated flasks were incubated overnight at 30°C, in a reciprocating shaker (150 strokes/min) through which growth occurred and flasks turned turbid.
4. Under aseptic conditions the cultures were diluted with appropriate broth to a final absorbance of 0.1 at λ=625nm

(0.5ml culture and 5.5ml broth showed to be sufficient as a final concentration).

2.9.2 Petroleum Ether and Methanol Extract of *I.viscosa* Preparation

Petroleum ether and methanol extracts were introduced to the diluted culture broth (10 ml) in two doses so that the final concentrations were 2 and 10 mg/ml. Diluted culture broths were used as controls.
All inoculated sterile tubes were incubated with continuous shaking of (150 strokes/min) at 30°C for overnight, after which absorbance of controls and reference drugs was read at λ= 625nm blanked against nutrient or potato dextrose broths accordingly. Samples (1 ml) containing the methanol and petroleum ether extracts were centrifuged for 10 min at 13000rpm at 10°C. Supernatant containing broth was discarded; bacteria remaining in pellet are washed with 1ml 0.85%NaCl solution several times until the color contributed by drug disappeared. Then, the absorbance of the different samples were determined.

2.10 Insulin determination using Enzyme Linked Immunosorbent Assay (ELISA) technique

Principle of procedure

This assay (Rat/Mouse Insulin ELISA kit, LINCO-USA), is a sandwich ELISA based, sequentially on: 1) capture of insulin molecules from samples to the wells of a microtiter plate coated by pre-titered amount of a monoclonal mouse anti-rat insulin antibodies and the binding of biotinylated polyclonal antibodies to the captured insulin, 2) wash away of unbound materials from samples, 3) binding of horseradish peroxidase to the immobilized biotinylated antibodies, 4) wash away of free enzyme conjugates, and 5) quantification of immobilized antibody-

enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine.

The enzyme activity is measured spectrophotometrically by the increased absorbancy at 450nm, corrected from the absorbancy at 590nm, after acidification of formed products. Since the increase in absorbancy is directly proportional to the amount of captured insulin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of rat insulin.

Calculation:

The dose response curve of this assay fits best to a sigmoidal equation. The results of the unknown samples can be extrapolated from the graph plotted by the absorbance unit of 450nm, less that of 590nm, on the Y-axis against the concentration of rat insulin standards on the X-axis (SOFTMAX Pro 2.6.1).

2.11 Data Handling and Statistical Analysis

Values of the different tested parameters within each group are presented as mean ± SEM. Comparison between each two groups was made by independent t-test. A p value of less then 0.05 ($p<0.05$) was considered significant.

Chapter 3

RESULTS

3.1 Effects of Water Extracts of *I.viscosa* on Inflammation

The present study was undertaken to assess the effect of different doses (250, 500, and 1000mg/kg BW) of *I.viscosa* water extract on acute inflammation induced by carrageenan, and chronic inflammation induced by formalin on rat hind-paw.

3.1.1 Carrageenan

Percent inflammation inhibition induced by diclofenac and the different dosages of *I.viscosa* are shown in Figure 3.1. The 250 mg dose of *I.viscosa* water extract did not show any anti-inflammatory effect on carrageenan induced paw-edema. The 500mg dose had an inhibitory effect on inflammation reaching about (16%) which was significantly less than that observed with diclofenac (39%). However the 1000mg dose showed a (33%) inhibition and was not statistically different from the reference drug (diclofenac).

3.1.2 Formalin

Percent inhibition induced by diclofenac and the different dosages of *I.viscosa* are shown in Figure 3.2. The doses of *I.viscosa* water extract showed a dose dependent anti-inflammatory effect on formalin induced paw-edema. Compared with diclofenac (44%) the 250mg and 500mg doses showed 11% and 20% inhibition of inflammation respectively, and both values were significantly lower than that observed with diclofenac. A maximum inhibition rate (44%) was observed with the 1000mg dose and it is similar to that of the reference drug.

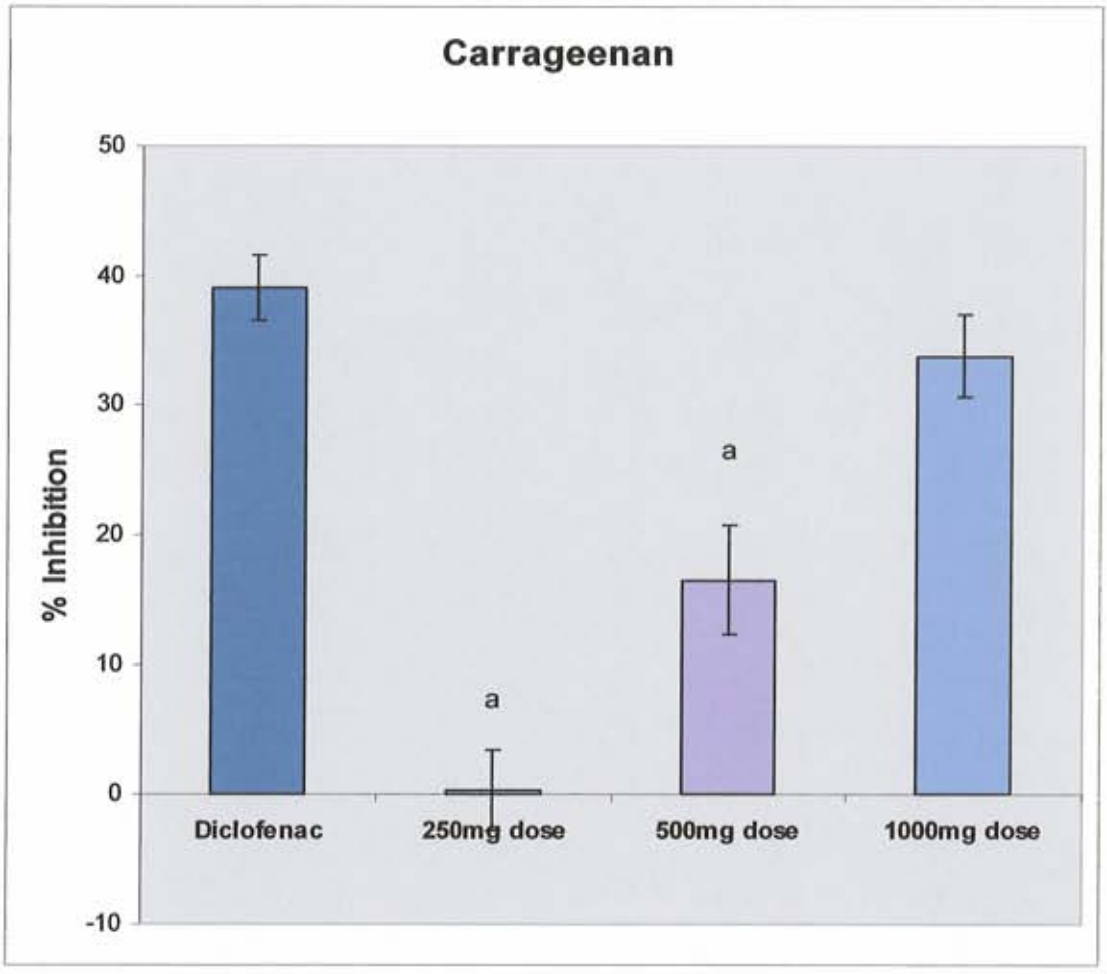

Figure 3.1 Diclofenac (10mg/kg BW), 250mg, 500mg and 1000mg/kg BW doses of *I.viscosa* water extract and their percent inflammatory inhibition rate in Carrageenan induced paw-edema in the rat model.
Bars denote mean ± SEM (n=6)
[a]Significantly different ($p<0.05$) from diclofenac group

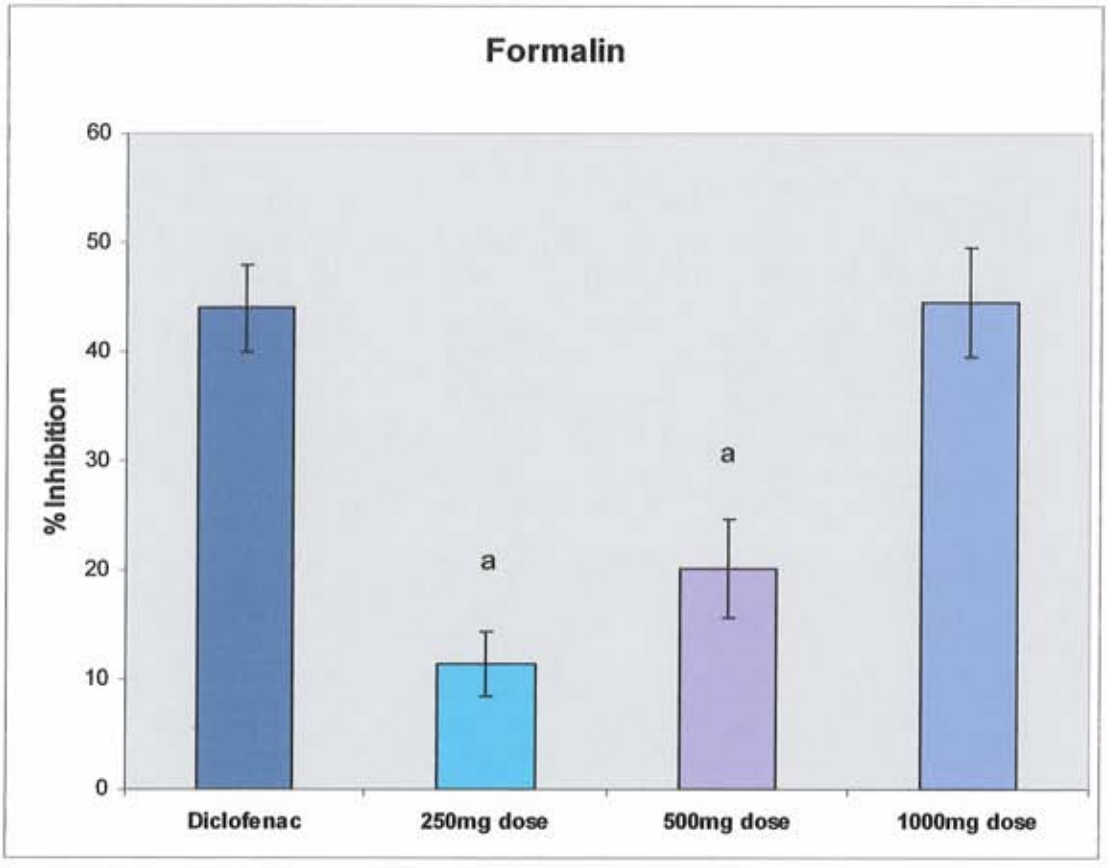

Figure 3.2 Diclofenac (10mg/kg BW), 250mg, 500mg and 1000mg/kg BW doses of *I.viscosa* water extract and their percent inflammatory inhibition rate in formalin induced paw-edema in the rat model.
Bars denote mean ± SEM (n=6)
[a]Significantly different ($p<0.05$) from diclofenac group

3.2 Anti-Microbial Effect

The following results denote the percent inhibition rate in absorbance, upon different microbial cultures of methanol and petroleum ether extracts of *I.viscosa*.

3.2.1 Gram Positive Inhibition

Figure 3.3 showed a mild inhibition rate in OD of 34% reached with the (2mg/ml) dose and 39% reached when (10mg/ml) methanol extract was used. However, petroleum ether extract exhibited almost a similar inhibition ranging between 26% and 36% in a dose dependent manner.

3.2.2 Gram Negative Inhibition

The methanol extract showed a maximum of 56% inhibition rate in OD reached with the (10mg/ml) dose. However, petroleum ether extract exhibited a lower inhibition rate (34%) with the (10mg/ml) dose used. The (2mg/ml) doses of methanol and petroleum ether extracts had mild inhibitory effect. Results are shown in Figure 3.4.

3.2.3 Yeast Inhibition

Mild inhibition in OD is observed in the methanol and petroleum ether extract with the lowest dose of both extract. A dose dependent inhibition is observed in both the methanol and petroleum ether extracts with the highest dose reaching (45%) with the (10mg/ml) methanol dose. Results are shown in Figure 3.5.

3.2.4 Fungal Inhibition

A dose dependent Inhibition in OD is observed in the methanol and petroleum ether extracts. Maximum inhibition (48%) is reached with the (10mg/ml) methanol extract. Results are shown in Figure 3.6.

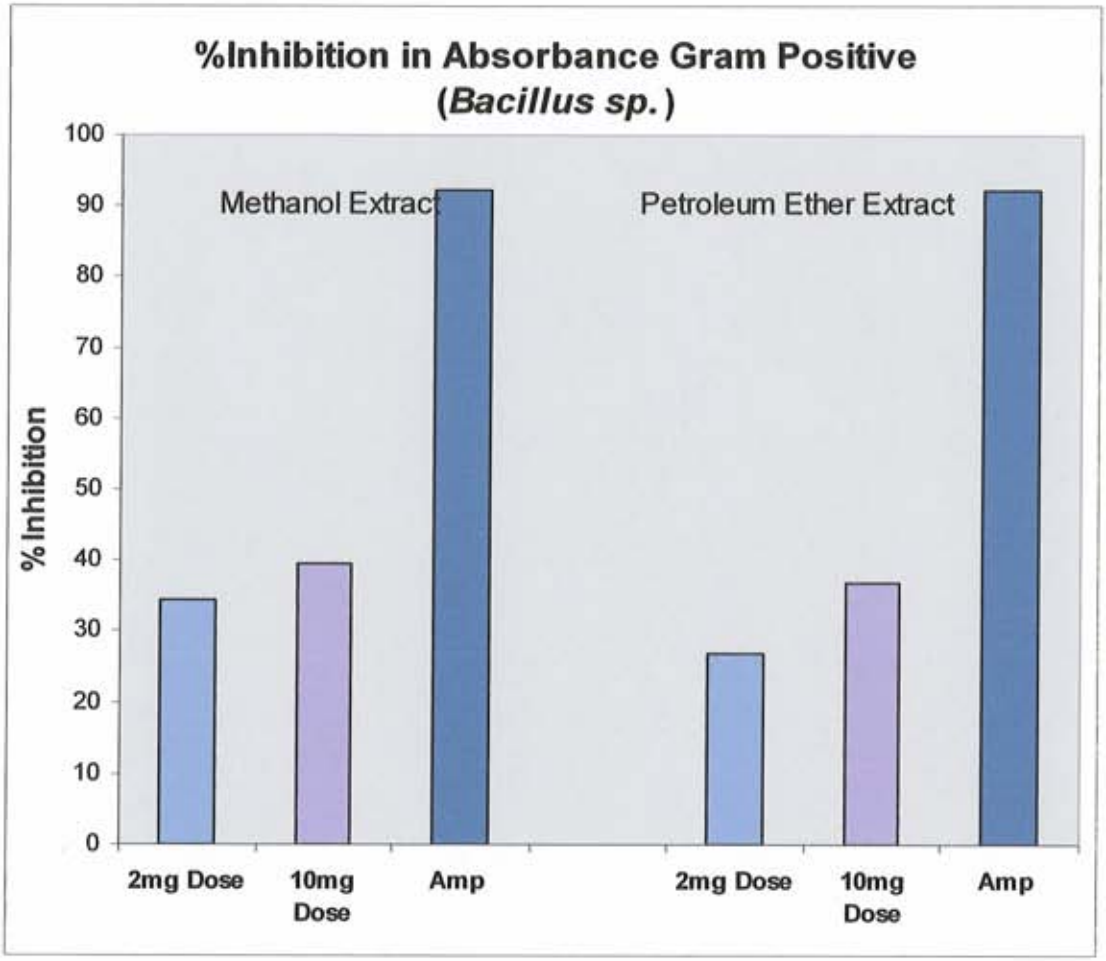

Figure 3.3 Growth inhibition rate in OD of Gram Positive bacterial culture in the presence of Ampicillin (1mg/ml), and *I.viscosa* methanol and petroleum ether extracts (2 mg/ml and 10 mg/ml)

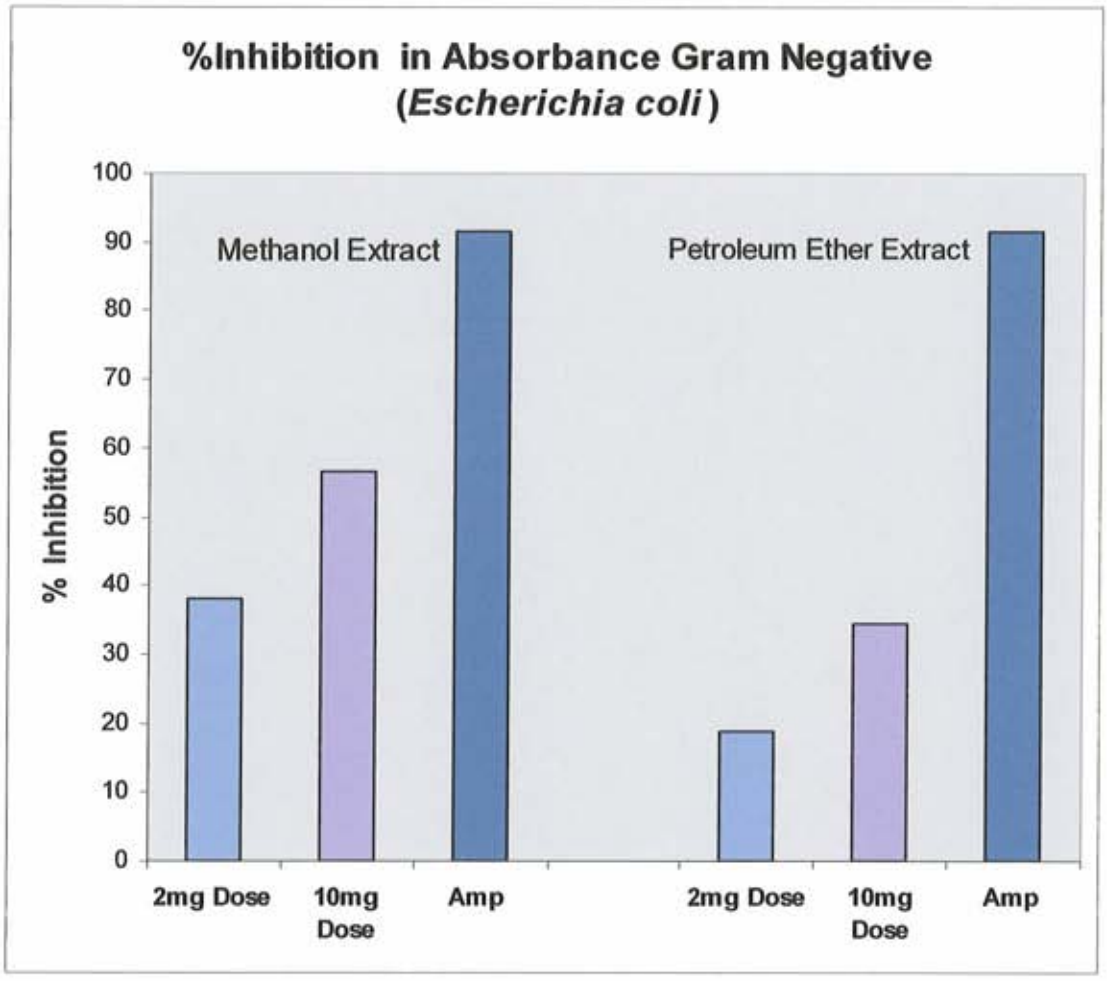

Figure 3.4 Growth inhibition rate in OD of Gram negative bacterial culture in the presence of Ampicillin (1mg/ml), and *I.viscosa* methanol and petroleum ether extracts (2 mg/ml and 10 mg/ml)

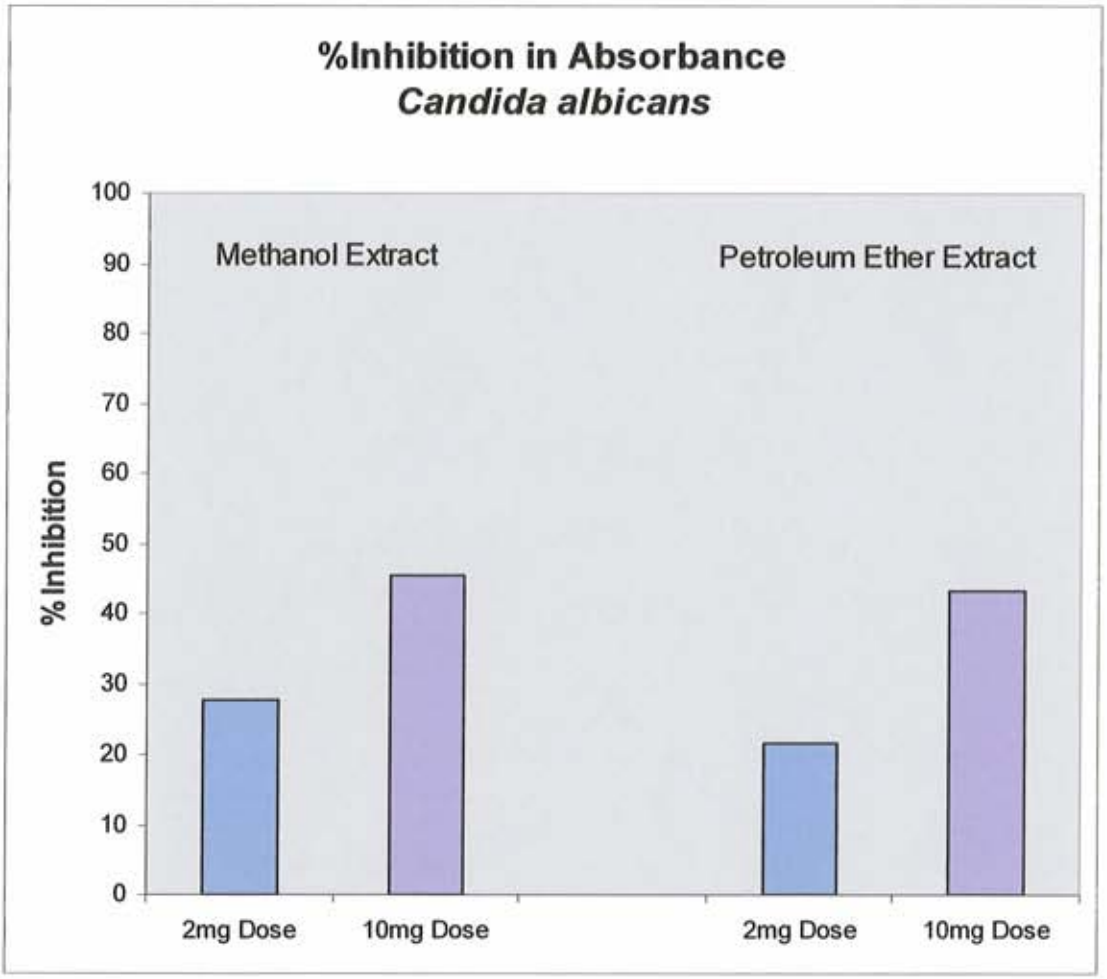

Figure 3.5 Growth inhibition ratein OD of *Candida albican* culture in the presence of *I.viscosa* methanol and petroleum ether extracts (2 mg/ml and 10 mg/ml)

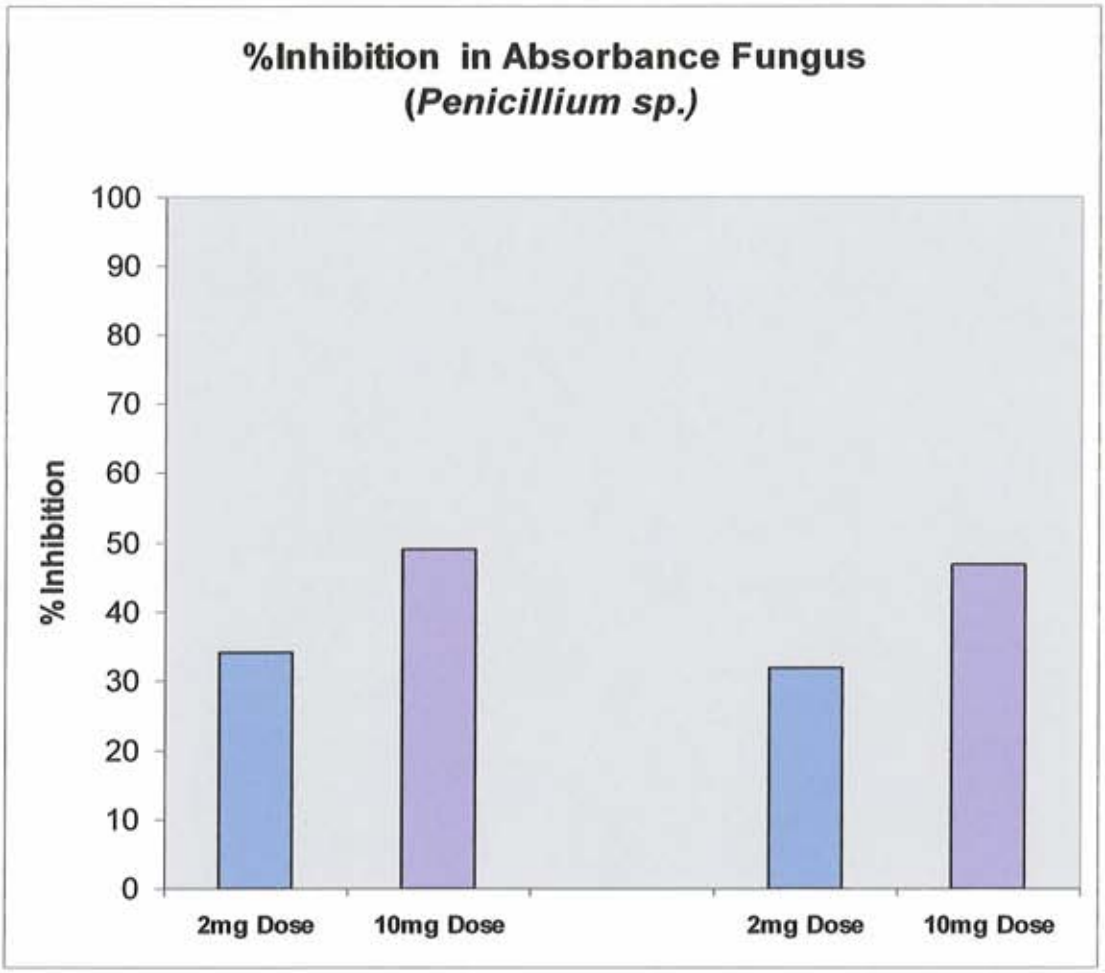

Figure 3.6 Growth inhibition rate in OD of *penicillium sp.* culture in the presence of *I.viscosa* methanol and petroleum ether extracts (2 mg/ml and 10 mg/ml)

3.3 Effects of Water, Methanol and Petroleum Ether Extracts of *I.viscosa* on blood lipid profile

Animals were maintained for a period of one month on water, methanol, or petroleum ether extracts of *I.viscosa* and had a regular diet. At the end of the treatment, all rats were fasted for 18 hours, and rapidly blood was collected. Concentrations of the following are measured in serum: TAG, total cholesterol, HDL cholesterol, LDL cholesterol, LDL/HDL ratio, GOT-AST, GPT-ALT, LDH, Insulin and glucose. Plasma apo B100 concentrations in the VLDL lipoprotein fraction Sf > 20 were also determined.

3.3.1 Serum Lipid Profile

Table 3.1 summarizes the mean serum concentration values in mg/dl of glucose, TAG, cholesterol, HDL-cholesterol, LDL-cholesterol and the ratio LDL/HDL in control and experimental groups.

3.3.1.1 Serum Glucose

The water extract group showed significantly the lowest serum glucose concentration among the different groups ($p<0.05$). All other groups had similar serum glucose concentrations.

3.3.1.2 Serum TAG

No significant changes in the concentrations of circulating TAG is observed among the different groups.

3.3.1.3 Serum Cholesterol

The concentrations of serum cholesterol were significantly lower ($p<0.05$) in all experimental groups with respect to the control. The

serum cholesterol of the methanol and petroleum ether extract groups were also significantly lower ($p<0.05$) than the water extract group.

3.3.1.4 Serum HDL-Cholesterol

There is a significant ($p<0.05$) decrease in the concentrations of HDL-cholesterol in the methanol group with respect to the control and the other two experimental groups.

3.3.1.5 Serum LDL-Cholesterol

All experimental groups showed a significant decrease in their LDL cholesterol levels with respect to the control group. No significant changes has been observed among the experimental groups.

3.3.1.6 LDL/HDL ratio

All experimental groups showed a better LDL/HDL cholesterol ratios with respect to the control group. The lowest ratio was observed with petroleum ether group.

3.3.2. VLDL Apo B 100

The concentrations of VLDL apoB 100 determined in the (Control, water, methanol, and Petroleum Ether Extract) groups are shown in Figure 3.7. No significant differences ($p< 0.05$) have been observed among the different groups.

3.3.3 Serum Enzymes

Table 3.2 summarizes the serum activities of sGOT, sGPT and LDH in the control and experimental groups.

3.3.3.1 Serum GOT-AST

When compared with the other groups there is a significant decrease ($p<0.05$) in the concentration of serum GOT of the experimental

methanol group. No significant changes were observed among the other groups.

3.3.3.2 Serum GPT-ALT

There are no significant differences in sGPT-ALT activities among the different groups.

3.3.3.3 Serum LDH

There is a significant decrease ($p<0.05$) in the concentration of circulating LDH between the control and the other different experimental groups.

3.3.4 Serum Insulin

Figure 3.8 shows the results of serum insulin concentration. Insulin concentrations of the experimental groups were relatively higher than that of the control group. However significant difference was achieved between the control and the water extract group only.

Table 3.1: Glucose (mg/dl), TAG (mg/dl), Cholesterol (mg/dl), HDL-Cholesterol (mg/dl), LDL-Cholesterol (mg/dl) levels in serum after a one month chronic consumption of water, methanol, or petroleum ether extracts of *I.viscosa* in the rat model, and LDL-HDL ratio. Values denote mean ± SEM (n=8)

Parameters	Control	Water Extract	Methanol Extract	Petroleum ether Extract
Glucose	91.93 ± 3.98	80.89 **ab** ± 1.08	93.79 ± 4.0	90.08 ± 3.16
TAG	52.13 ± 2.11	50.71 ± 1.21	55.77 ± 4.27	55.6 ± 3.73
Cholesterol	74.83 ± 2.42	66.85 **ab** ± 1.49	58.62 **a** ± 1.75	56.49 **a** ± 2.67
HDL-Cholesterol	37.23 ± 1.14	36.92 ± 1.21	30.16 **c** ± 1.36	39.77 ± 1.44
LDL-Cholesterol	27.17 ± 2.4	19.80 ± 2.5	17.31 ± 3.1	14.8 ± 3.2
LDL/HDL	0.73	0.54	0.57	0.37

a Significant difference ($p<0.05$) with respect to control

b Significant difference ($p<0.05$) water versus methanol and petroleum ether.

c Significant difference ($p<0.05$) methanol versus control, water and petroleum ether.

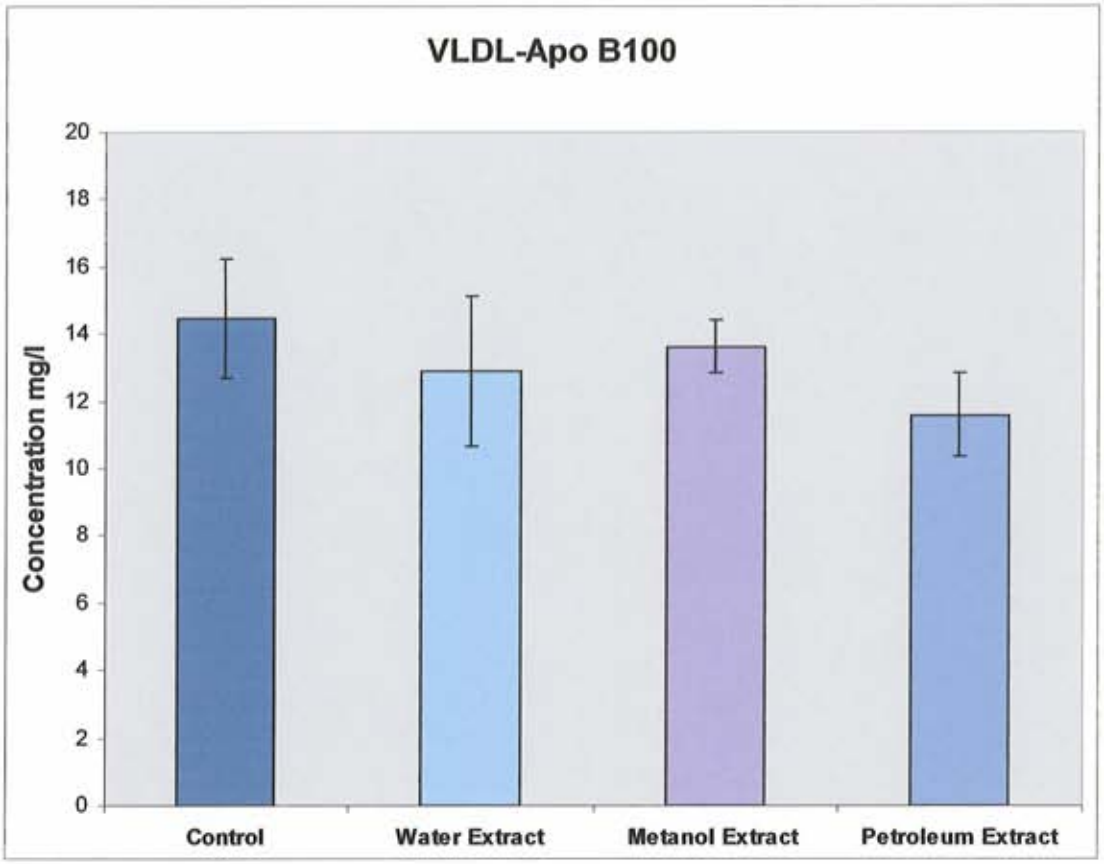

Figure 3.7 VLDL Apo B concentration (mg/L) one month after *I.viscosa* treatment. Values denote mean ± SEM (n=8)

Table 3.2: Serum GPT, GOT and LDH activities after a one month chronic consumption of water, methanol, or petroleum ether extracts of *I.viscosa* in the rat model. Values denote mean ± SEM (n=8)

Parameters	Control	Water Extract	Methanol Extract	Petroleum ether Extract
sGPT	28.16 ± 2.63	22.48 ± 2.43	27.79 ± 2.57	26.96 ± 2.70
sGOT	66.24 ± 3.84	58.16 ± 1.49	40.92 **b** ± 2.63	64.88 ± 4.47
LDH	753.54 ± 30.71	631.95 **a** ± 21.33	631.63 **a** ± 32.20	695.68 ± 34.37

a Significant difference ($p<0.05$) control versus water, methanol and petroleum ether.

b Significant difference ($p<0.05$) methanol versus control, water and petroleum ether.

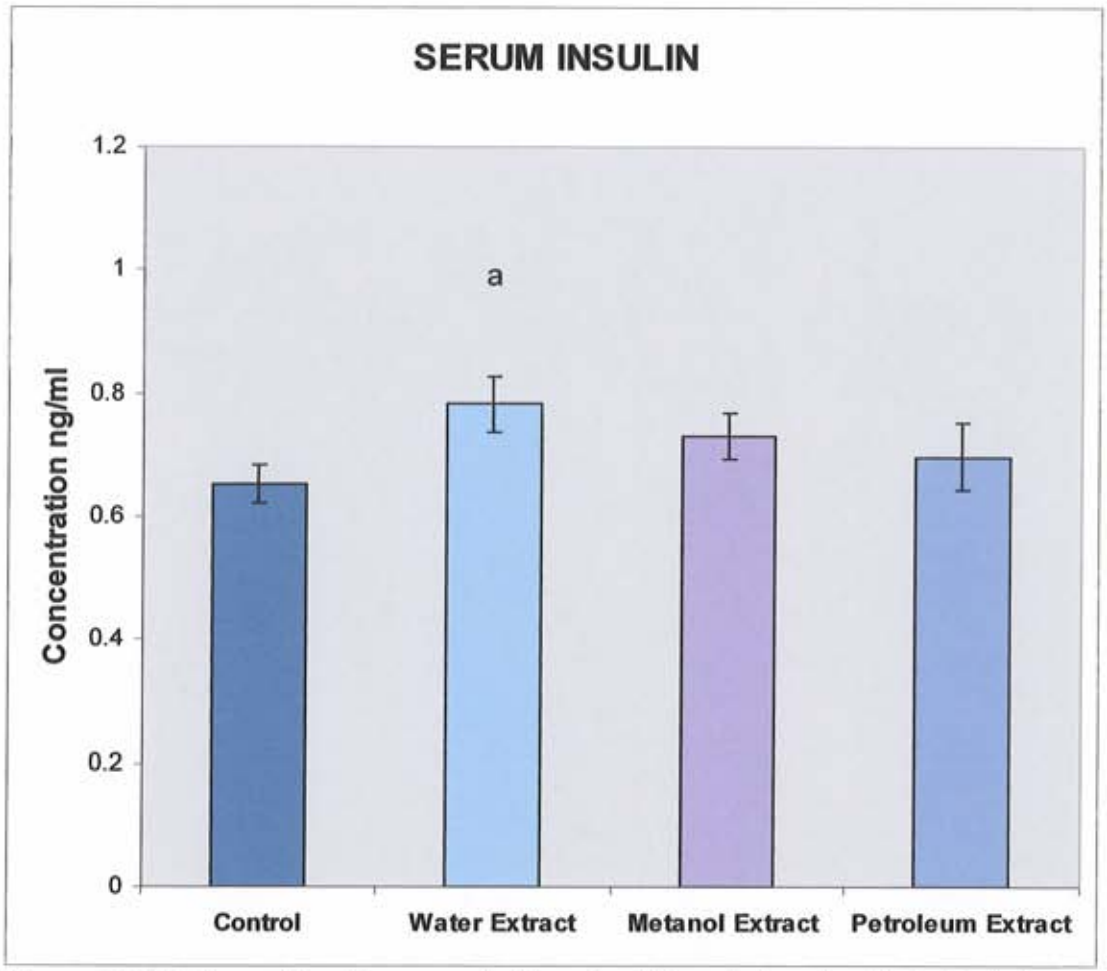

Figure 3.8 Rat Serum Insulin concentrations (ng/ml) as determined in fasted rats subjected to a one month period of *I.viscosa* (water, methanol, and petroleum ether extracts) treatment. Values denote mean ± SEM (n=8).

[a]Significant difference ($p<0.05$) between the water extract group and control.

Chapter 4

DISCUSSION

The present investigation focuses on assessing the effects of a one month period of chronic consumption of water, methanol, and petroleum ether extracts of *Inula viscosa* on blood lipid profile, glycemia and liver enzymes. The anti-inflamarory and antimicrobial effects of the extracts were also investigated. This study was conducted in the rat model fed a regular fat diet. The rat model was chosen in order to avoid any possible toxic effect on human since no human studies have been reported. Also, animal studies are easier to control, since several conflicting differences such as liver function, nutritional factors, medication, smoking, physical activity, and other confounding factors might intervene in chronic human studies.

The antimicrobial effects of extracts derived from *I.viscosa* are studied. Only methanol and petroleum ether extracts were included in the study since it has been shown that water extract exhibited a poor yield of antifungal compounds which are mostly lipophilic Cohen et al, 2002). In the present study, a gram-positive bacteria (*Bacillus sp.*), a gram-negative bacteria (*Escherichia coli*), and two fungi species (*Candida albicans, Penicillium sp.*) were used. Both petroleum ether and methanol extracts showed an inhibitory effect in OD on bacterial growth. The trend in inhibition appeared to be dose dependent and not restricted to a single type of bacteria. However, the inhibition in OD reached a maximum with *Escherichia coli* (57%) when the methanol extract was used. This is consistent with *in vitro* studies conducted by Mehta et al. (1984) where *Inula viscosa oil* was an effective antimicrobial agent against gram-

positive and gram-negative human pathogen. The inhibition observed in the present study, suggest further purification of the crude extract in order to isolate and locate the active ingredient behind the inhibition. Similarly, the extracts used showed to contain antifungal compounds since between 40-50% inhibition of growth was observed with both type of extracts. Several studies (Mehta et al, 1984; Abou-Jawdah, 2002; Cohen, 2002; Cafarchia et al, 2002) confirmed the antifungal effect of *Inula viscosa*. Abou-Jawdah et al. (2002) reported that at least two major compounds or two sets of compounds are responsible for the anti-fungal activity in petroleum ether extract. He also reported that at least one additional inhibitory compound that is not present in the petroleum ether extract is present in methanolic extract. Some reports showed that the anti-microbial activities of inula sp. was associated with the sesquiterpenoid lactones (Bourrel et al,1993; Cafarchia et al, 2001) or with a new sesquiterpene, tayounin (Maoz et al,1999). Maoz et al. (1999) reported that *Inula viscosa* extracts cause a decline in chitin content in dermatophytes and candida albicans. Chitin is a component of the fungi cell wall that does not exist in human cells. Thus, this might have a good beneficial implication on fungi attacking plants of agricultural importance. Sometimes partial purification may prove to be more effective against microbes since crude plant extracts may contain growth factors that will favor the pathogen growth or interfere with the anti-microbial activity. As future work, E-test is recommended to make the results quantitative rather than qualitative.

The anti-inflammatory effect of inula viscosa water extract was investigated by inducing inflammation in the right hind-paw of rats using carrageenan and formalin, hence simulating acute and chronic inflammation. The anti-inflammatory effects were compared to diclofenac, a common reference drug (Ajith and Janardhanan, 2001). In the acute inflammation induced by carrageenan, no anti-inflammatory

effect was observed with the 250mg/kg B.W. dose. However, a substantial anti-inflammatory effect was observed with the 500, and 1000 mg/kg B.W. doses. A dose dependent anti-inflammatory effect was observed, and the 1000 mg dose was as effective as diclofenac in reducing the inflammation. In the chronic study, where inflammation was induced by formalin, all doses used of the water extract were capable of substantially reducing the inflammation in a dose dependent manner. The 1000 mg dose was even slightly more efficient than the reference drug diclofenac. The effect observed with the 250mg dose in the chronic but not acute study may suggest a possible long half-life of the anti-inflammatory agent, which over the days accumulated in the body and became effective in reducing the inflammation. Abu Jawda et al (2002) reported that azulene derivatives are present in *Inula viscosa* extract. Azulene is an extract from the volatile oil of several perennial herbs and possess an anti-inflammatory action (Saki and Miswa, 2005). Possibly azulene is one of the active ingredients in the anti-inflammatory process. On the other hand, the anti-inflammatory effect may be attributed to flavonic content of the plant, as these compounds have been widely studied as inhibitors of some inflammation mediators, for example PGE_2 (Ferrandiz et al., 1991; Della Loggia et al., 1992). Hernandez et al (2001) reported that inuviscolide is the main anti-inflammatory sesquiterpenoid from *Inula viscosa*, and may act by interfering with leukotriene synthesis and phospholipase A(2)- induced mastocyte release of inflammatory mediators. A possible anti-inflammatory activity may be attributed to the anti-oxidant property of the plant extract (schinella et al 2002). Thus, a tea made from the leaves of *Inula viscosa* may be used as an anti-inflammatory remedy in several chronic inflammatory diseases such as in rheumatoid arthritis. In order to assess which of the active ingredient may be behind the anti-inflammatory effect in the water extract used, further purification and separation of the crude extract is recommended. On the other hand, the antioxidant property reported by Hernandez et al.

(2001), is another important issue that must be covered in future studies. LDL oxidation is one of the major causes of atherosclerotic lesion formation. Thus, determination of the antioxidant effect of *Inula viscosa* extract on LDL may also be added to the potential benefits of the plant.

Surprisingly and up to our knowledge, the effect of *Inula viscosa* extract upon blood lipid profile is not reported in the literature. In the present study, water, methanol and petroleum ether extract were used in order to investigate the relationship between chronic ingestion of these extracts and their impact on blood lipid levels in the fasted state. Data have shown that none of the extracts was able to modify serum TAG levels with respect to the control. However, a significant decrease in serum total cholesterol concentration was observed with all three plants extracts,(water extract 66.85mg/dl,methanol extract 58.62mg/dl and petroleum ether extract 56.49mg/dl) when compared with the control group(74.83mg/dl). The decrease was more drastic with the methanol and petroleum ether extracts. The drawback of methanol extract was the significant reduction in serum HDL cholesterol concentration unlike the water and petroleum ether extracts that had HDL cholesterol levels similar to that of the control. In spite of the decrease in HDL cholesterol concentration in the methanol group the LDL/HDL cholesterol ratio was (0.57) with respect to the control (0.73). This may be attributed to the drastic decrease in LDL cholesterol observed with the methanol extract group((17.31mg/dl). Similar drop in LDL cholesterol levels and LDL/HDL cholesterol ratios were observed with both water and petroleum ether extracts(19.80mg/dl,14.8.g/dl) respectively. All these effects are consistent with the cardioprotective effect of the extract, especially water and petroleum ether extracts. In order to understand the mechanism behind the hypocholestrolemic effect observed, the concentration of plasma VLDL particles secreted by the liver were determined. Knowing that each VLDL particle contains one apo B molecule (Elovson et al.,

1988), measuring VLDL apo B reflects exactly the total number of newly secreted VLDL. Since there were no significant changes in VLDL apo B concentrations in the plasma of the different groups (although VLDL apo B were relatively lower in the different extract group) the drug does not appear to affect the number of VLDL particles secreted from the liver into the circulation regardless of the type of extract used. Thus, the drop in serum total cholesterol and LDL cholesterol concentrations may partially be explained through an increase in lipoprotein particle metabolism in the blood rather than through a direct effect of the drug on liver VLDL secretion. To confirm this hypothesis, measurement of plasma LDL apo B concentration is recommended. On the other hand, postprandial lipemia is not covered in the present report. Consequently, the impact of the plant extract on postprandial lipemia, including chylomicron secretion and metabolism, and the impact of the drug on gastric emptying may be important issues to consider in the future. Also it is recommended that a stool lipid content be assessed in future work to determine if the extracts have a direct effect on the gastrointestinal tract absorption.

In order to investigate whether the different extracts have resulted in extract-induced hepatotoxicity and to assert the cardioprotective role of the plant extracts, the activities of SGOT (AST), SGPT (ALT), and LDH were determined. In the present study, it was shown that there were no increases in the plasma activities of these three enzymes, but on the contrary, a significant decrease in the activities of LDH was observed with the different extract groups(631.95 in water extract,631.95 in methanol extract, 695.68 in petroleum ether extract) with respect to the control(753.54). Thus, after a month period of chronic intake of the *Inula viscoa* extracts there has been no liver damage during which the activities of the enzymes would increase rather than decrease. Still, it is too early to recommend *Inula viscosa* extract in the community as a drug that helps in improving blood lipid levels or for any other benefit since more

thorough toxicologic studies are needed to assure the lack of toxic effects of the extract on different body organs.

The effect of *Inula viscosa* extract on fasting blood sugar levels in normolipidemic rats was also investigated. The data have shown that only chronic water extract intake resulted in a significant decrease in serum glucose concentration(80.89mg/dl) with respect to all other groups(control91.93mg/dl,methanol93.79mg/dl,petroleum ether extract90.08mg/dl). This decrease in the glucose level was accompanied with a significant increase in serum insulin concentration of the water extract group. Thus, the rise of serum insulin may be the reason behind the drop in sugar level with chronic water extract intact. The present study, hence suggests that water extract may enhance pancreatic insulin secretion in the blood and thus reduces hyperglycemia. Another possible area for future research studies then may include pancreatic perfusion experiments that reveal the impact of the *Inula viscosa* water extract upon pancreatic insulin secretion.

In conclusion, *Inula viscosa* is a plant having several beneficial potentials. Regarding inflammation, water extract appeared to be very effective as an anti-inflammatory drug that showed comparable benefits to diclofenac in both acute and chronic inflammatory conditions. As an antimicrobial agent, the methanol and petroleum ether extracts showed a substantial trend in inhibition in both bacterial and fungal growth. Thus, plant extracts may have potential usage in agriculture, in order to reduce microbial infection in susceptible plants. At the same time, the use of the plant extract is environment friendly and help in reducing the use of toxic chemicals commonly sprayed on plants against many pathogenic microorganisms. The plant extract also appeared to have a beneficial impact on blood cholesterol level via improving lipoprotein metabolism, and this helps in reducing atherosclerosis occurrence. *Inula viscosa* water extract seems to enhance pancreatic insulin secretion and consequently reduce serum glucose level. At the same time, assessment

of liver enzyme activities showed no toxic effects on liver cell, which somehow reveals preliminary safe use of the extract.

REFERENCES

Ajith, T.A., & Janardhanan, K.K. (2001). Antioxidant and anti-inflammatory activities of methanol extract of phellinus rimosus (Berk) pilat. *Indian Journal of Experimental Biology, 39*, 1166-1169.

Abou-Jawdah, Y.,Sobh, H. & Salameh, A. (2002). Antimycotic activities of selected plant flora, growing wild in lebanon, against phytopathogenic fungi. *Journal of Agriculture and Food Chemistry, 50,* 3208-3213.

Al- Dissi, N.M., Salhab, A.S., & Al-Hajj, H.A. (2001). Effects of Inula viscosa leaf extract on abortion and implantation in rats. *Journal of Ethnopharmacology, 77,* 117-121.

Al- Khalil, S., Al-Eisawi, D., & Fischer, N. (1992). Phytochemical analysis of Jordanian *Inula viscosa* . *Journal of Pharmaceutical Sciences, 6,* 307-309.

Al-Eisawi, D. (1998*). Field guide to wild flowers in Jordan and Neighboring countries* (p.97). Amman: Jordan Foundation Press.

Alkofahi, A. & Atta, A.H. (1999). Pharmacological screening of the anti- ulcerogenic effects of some Jordanian medicinal plants in rats. *Journal of Ethnopharmacology, 67,* 341- 345.

Anderson, T.A., Levitt, D.G. & Banaszak, L.J. (1998). The structural basis of lipid interactions in lipovitellin, a soluble lipoprotein. *Structure, 6,* 895-909.

Aruoma, O.I. (1996). Characterization of drugs as antioxidant prophylactics. *Free Radical Biology and Medicine,20, 675-705.*

Aruoma, O.I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society, 75*, 199-212.

Barbetti, P., Chiappini, I., Fardella, G.& Menghini, A. (1985). A new eudesmane acid from *Dittrichia (*Inula) *viscose. Planta Medica, 51*, 471-473.

Baytop, T. (1984). *Therapy with medicinal plants in turkey.* Istanbul, Turkey: Nobel medicine bookstores. Istanbul University.

Beye, F. (1978). Insecticides from the vegetable kingdom. *Plant Research Development,7,* 13-31.

Blackshear, P.J. (1984). Systems for plyacrylamide gel electrophoresis. *Methods in Enzymology ,104*, 237-255

Bogdanov, M., & Dowhan, W. (1999). Lipid-assisted protein folding. *Journal of Biological Chemistry, 274*, 36827-36830.

Bohlmann, F., Mahanta, P.K., Jakupovic, J., Rastogi, R.C., & Natu, A.A.(1978). New sesquiterpene Lactones from inula species. *Phytochemistry, 17*, 1165-1172.

Bollag, D.M., Rozycki, M.D. & Edelstein, S.J. (1996). *Protein Methods* (2nd ed. pp. 107-154). New York: Wiley.

Bourrel, C., Vilarem, G. & Perineau, F. (1993). Chemical analysis, bacteriostatic and fungistatic properties of the essential oil of Elecampane (*Inula helenium* L.). *Journal Of Essential Oil Research, 4*, 411-417.

Broun, P., Gettner, S. & Somerville, C. (1999). Genetic engineering of plant lipids. *Annual Review of Nutrition,19,* 197-216.

Cafarchia, C., De Laurentis, N., Milillo, M.A., Losacco, V., & Puccini V. (2002). Antifungal activity of essential oils from leaves and flowers of Inula viscosa (Asteraceae) by Apulian region. *Parasitologia*, 41, 587-90.

Cafarchia, C., De Laurentis, N., Milillo,M.A. Losacco, V., Puccini, V. (2001). Fungistatic activity of a sequiterpene lactone (tomentosin) isolated from fresh inula viscose (Asteraceae) flowers from the Pulgia region. *Parasitologia, 43,* 117-121.

Champ, P.C., & Harvey, R.A. (1994). *Lippincott's Illustrated Reviews: Biochemistry* (2nd edition). Philadelphia: Lippincott Company.

Cohen, Y., Baider, A., Ben-daniel, B. & Ben Daniel, Y. (2002). Fungicidal preparations from Inula viscose *Phytopathology*, 152-156

Dafni, A., Yaniv, Z., & Palevitch, D. (1984). Ethanobotanical survey of medicinal plants in Northern Israel. *Journal of Ethnopharmacology ,10,* 295-310.

Deans, S.G., & Svoboda, K. P. (1990). The antimicrobial properties of marjoram (*Origanum origanum* L.) volatile oil. *Flavour Fragrance Journal, 5*, 187-190.

Deans, S.G., & Svoboda, K.P. (1990). Biotechnology and bioactivity of culinary and medicinal plants. *Agbiotechnology News, 2,* 211-216.

Duplus, E., Glorian, M., & Forest, C. (2000). Fatty acid regulation of gene transcription. *Journal of Biological Chemistry,275,* 30749-30752.

Duthie, S.J., Ma, A., Ross, M.A. & Collins, A.R. (1996). Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Research, 56,* 1291-1295.

Elovson, J., Chatterton, J.E., Bell, G.T. (1988). Plasma very low density lipoproteins contain a single molecule of apolipoprotein *Journal of Lipid Research, 29*, 1461-1473.

Farnsworth, N.R., Bingel, A.S., Cordell, G.A., Crane, F.A., & Fong, H.S. (1975). Potential value of plants as sources of new antifertility agents II. *Journal of pharmaceutical sciences, 64*, 717-754.

Fawcett, C.H., & Spencer, D. M. (1970). Plant chemotherapy with natural products. *Annual Review of Phytopathology, 8*, 403-418.

Feher, J., Csomos, G., & Vereckei, A. (1987). *Free radical reactions in medicine.* p 40-43. Berlin-Heidelberg: Springer-Verlag.

Fielding, C.J., & Fielding, P.E. (1985). Metabolism of cholesterol and lipoproteins. In D.E. Vance, & J.E. Vance (Eds.), *Biochemistry of lipids and membranes* (pp.404). [s.l.]: Benjamin/Cummings.

Gotto, A. & Pownall, H. (1999). *Manual of Lipid Disorders.* (2nd ed). Baltimore, MD: Williams and Willkins.

Gurr, M.I., & Harwood, J.L. (1991). *Lipid Biochemistry: An introduction* (4th ed). [s.l.]: Chapman & Hall.

Halliwel, B., & Gutteridge, JMC. (1989). *Free Radicals in Biology and Medicine.* (pp.416-494). Oxford: Clarendon Press.

Hames, B.D. (1990). One-dimensional polyacrylamide gel electrophoresis. In *Gel electrophoresis of proteins: A practical approach.* (pp. 1-147). Oxford: IRL Press.

Hernandez, V., Del Carmen Recio, M., Manez, S., Prieto, J.M., Giner, R.M. & Rios, J.L. (2001) Amechanistic approach to the in vivo anti-inflammatory activity of sesquiterpenoid compounds isolated from Inula viscose. *Planta Medica, 67*(8), 726-731.

Jose, N., Ajith, T.A., & Janardhanan, K.K. (2004). Methanol extract of the Oyster Mushroom, Pleurotus Florida, inhibits inflammation and platelet aggregation. *Phytotherpy Research, 18*, 43-46.

Karim, F., Al-Okleh, A., Suleiman, S., & Quraan, S. (1990). *Poisonous plants in Jordan.* (pp.47). Irbid: Jordan Natural History Museum.

Karim, F., & Quraan, S. (1986). *Medicinal plant of Jordan.* (pp.65). Irbid : Jordan Natural History Museum.

Keli, S.O., Hertog, M.G.L., Feskens E.J.M., & Kromhout, D. (1996) Dietary flavonoids, antioxidant vitamins, and incidence of stroke: The Zutphen study. *Archive of Internal Medicine, 156,* 637-42.

Laemmeli, U.K. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T_4. *Nature, 227,* 680-685.

Lardy, H., & Shargo, E. (1990). *Biochemical aspects of obesity.* [s.l.]:[s.n.].

Lastra, C.,Lopez, A., & Motiva, V. (1993). Gastroprotection and prostaglandin E2 generation in rats by flavenoids of *Dittrichia viscose. Planta Medica, 59,* 497-501.

Lauro, L., & Rolih, C. (1990). Observations and research on an extract of *Inula viscose. Bollettino Societa Italiana Biological Sperimentable, 66,* 829-834.

Lewis, W.H. & Elvin-Lewis, M.P.F. (1977) *Medical Botany.* New York: Wiley.

Liscum, L. & Munn, N.J. (1999). Intracellular cholesterol transport. *Biochemica et Biophysica Acta, 1438,* 19-37.

Maoz, M., & Neeman, I. (1998). Antimicrobial effects of aqueous plant extracts on the fungi *microsporum canis* and *trichophyton rubrum* and on three bacterial species. *Letters in Applicable Microbialogy, 26,* 61-63.

Maoz, M., & Neeman,I. (2000). Effect of Inula viscosa extract on chitin synthesis in dermatophytes and Candida albicans. *Journal of Ethnopharmacology, 71,* 479-482.

Manez, S., Recio, M.C, Gil, I., Gomez, C., Giner, R.M., Wareman, P.G., et al.(1999). A glycosyl analogue of diacylglycerol and other anti-inflammatory constituents from Inula viscosa. *Journal of Natural Products, 62*(4), 601-604.

Mathews, C.K., Van Hold, K.E., & Ahern, K.G. (2000). *Biochemistry* (3rd ed). [s.l.]:Benjamin-cummings Publishing Co.

Mehta, B.k., Batra, A., & Mehta, S.C. (1984) In vitro antimicrobial efficiency of Inula oil against gram-positive, gram negative human pathogen and phytopathogenic fungi. *Indian Perfume, 28,* 123-125.

Murray, R.K., Granner, D.K., Mayes, P.A., & Rodwell, V.W. (1993). *Harper's Biochemistry* (23rd ed.). Connecticut: Prentice Hall.

Saki, H., & Misawa, M. (2005). Effect of sodium azulene sulfonate on capsaicin-induced pharyngitis in rats. *Basic and Clinical Pharmacology and Toxicology ,96,* 54-64.

Schinella, G.R., Tournier, H.A., Prieto, J.M., Mordujovich de Buschiazzo.P., & Rios, J.L. (2002). Antioxidant activity of anti-inflammatory plant extracts. *Life Science, 70,* 1023-1033.

Schinella, G., Troiani, G., Davila, V., Buschiazzo, P.M., & Tournier, H. (2000). Antioxidant effects of an aqueous extract of Ilex paraguariensis. *Biochemical and Biophysical Research Communication,* 269, 357-60.

Sniderman, A.D., Bergeron, J., & Frohlich, J. (2001). Structure of apolipoprotein B-100 in low density lipoproteins. *Journal of Lipid Research, 42,* 1346-1367.

Trease, G.E., & Evans, W.C. (2002). *Pharmacognosy* (12th ed. pp. 1,4,224,225,418). [s.l.]:[s.n.].

Ulubelen, A., Oskuz, S., & Goren, N. (1987). *Phytochemistry, 26,* 1223-1224.

Vance D.E. & Vance, J.E. (1991). *Biochemistry of lipids, lipoproteins and membranes.* (2nd ed.)[s.l]: Elsevier.

Vance, D.E. & Vance, J.E. (2002). *Biochemistry of lipids, lipoproteins and membranes* (4th ed.). [s.l.]: Elsevier.

Wollenweber, E., Mayer, K., & Roitman, J.N. (1991). Exudate flavonoids of Inula viscosa. *Phytochemistry, 30,* 2445-2446.

Yaniv, Z., Dafni, A., Friedman, J., & Palevitch, D. (1987). Plants used for treatments of diabetes in Israel. *Journal of Ethnopharmacology ,19,* 145-151.