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Effect of methanolic extract of *Quercus infectoria* bark
on blood lipid profile, glycemia, inflammation, platelet
aggregation, ulcer and bacterial activity

by

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Lara N. Khouzami

Effect of methanolic extract of *Quercus infectoria* bark on blood lipid profile, glycemia, inflammation, platelet aggregation, ulcer and bacterial activity

ABSTRACT

Nowadays, the surge of consumption of herbal remedies has been stimulated by several factors, including notion that all herbal products are relatively safe and effective. The present investigation was undertaken to explore and shed light on possible medicinal effects of methanolic extract of oak tree (*Quercus infectoria*) bark upon blood lipid profile, glycemia, inflammation, platelet aggregation, ulcer and bacterial activity. After one month of chronic extract (0.5% w/v) intake via drinking water, only HDL-cholesterol levels were significantly increased, thus revealing a possible cardioprotective effect. No significant changes were observed in other lipid parameters studied. An increase in both serum glucose and insulin levels was observed in animals receiving the extract over the study period, which raise the possibility of development of insulin resistance syndrome. Liver enzymes activities: GOT, GPT and LDH, as well as urea and creatinine levels were assessed and the extract appeared not to have a negative impact on these parameters.

The extract also exhibited an anti-inflammatory effect in cases of acute and chronic inflammation induced by carrageenan and formalin respectively. The 250mg/kg body weight dose showed the highest inhibitory activities in the two models used. The inhibition was even much greater than the reference drug diclofenac (10mg/kg body weight). In vitro studies showed a time-dependant stimulation of platelet aggregation when platelets were incubated in presence of 0.5mg/ml of the extract. Pre-treatment of fasted rats with two different doses of the extract (100 and 500 mg/kg body weight) showed a dose-dependant anti-ulcerogenic and gastroprotective effect against ethanol-induced gastric ulcer. The methanolic extract of *Quercus infectoria* bark was tested

for its ability to inhibit 11 bacterial hospital isolates from different patients. The extract showed an antibacterial effect against four of the bacteria tested: *Proteus mirabilis*, *Citrobacter braaki*, and *Staphylococcus aureus* methicillin resistant and sensitive.

These findings suggest that the methanolic extract of oak bark provides an inexpensive and powerful source of herbal supplement used to treat various conditions.

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*"Advance, and never halt, for advancing is perfection. Advance and do not fear
the thorns in the path, for they draw only corrupt blood"*
Jibran Khalil Jibran

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GLOSSARY

ACAT: Acetyl-CoA-cholesterol acetyltransferase

ADP: Adenosine diphosphate

Apo: Apolipoprotein

APS: Ammonium persulfate

AST: Aspartate aminotransferase

BSA: Bovine serum albumin

CHD: Coronary Heart Disease

CE: Cholesteryl esters

CETP: Cholesterol ester transfer protein

CHE: Cholesterol esterase

CHO: Cholesterol oxidase

CM: Chylomicrons

DTT: Dithiothreitol

EDTA: EthylenediamineTetraacetic Acid

ELISA: Enzyme Linked Immuno- Sorbent Assay

FA: Fatty Acids

FFA: Free fatty acids

GK: Glucose kinase

GOD: Glucose oxidase

GPO: Glycerol-3-phosphate oxidase

HDL: High Density Lipoprotein

HL: hepatic lipase

HSDA: Sodium N- (2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline

IDL: Intermediate Density Lipoprotein

LCAT: Lecithine cholesterol acyltransferase

LDH: Lactate Dehydrogenase

LDL: Low Density Lipoprotein

LPL: Lipoprotein lipase

MRSA: Methicillin resistant *Staphylococcus aureus*

MSSA: Methicillin sensitive *Staphylococcus aureus*

PMSF: phenylmethylsulfonylfluoride

POD: Peroxidase

PRP: Platelet rich plasma

SDS: Sodium dodecyl sulfate, Lauryl sulfate

SDS-PAGE: Sodium Dodecyl Sulfate - PolyAcrylamide Gel Electrophoresis

SGOT: Serum-Glutamic Oxaloacetic – Transaminase

SGPT: Serum-GlutamicPyruvic– Transaminase

TAG: Triacylglycerol

TEMED: Tetraethylenediamine

VLDL: Very Low Density Lipoprotein

INTRODUCTION AND LITERATURE REVIEW

Folk medicine is an unofficial health-related practice that has traditionally existed, and is learned informally by word of mouth, and through observation and demonstration. Long before the discovery and development of modern scientific medicine such as the use of pharmaceutical drugs and doctor's surgery, traditional healing methods had been in use, and are still being used today in every culture. Various types of traditional medicine such as herbs, tree or plant roots, fruits, and food items are used for treatments of any illness ranging from AIDS, cancer, malaria, sore throats to arthritis, high blood pressure and infected wounds. Finding healing powers in plants and the use of plant extracts, as well as other alternative forms of medicinal treatments, is enjoying great popularity especially for people in developing countries. Side effects or toxic reactions associated with herbal medicine in any form are rare. This may be due to the fact that herbal medicine products are natural products, generally safe and widely available at low cost, and that adverse reactions following their use are under reported or because the side effects are of such a nature that reporting them is not done, such as minor allergic reactions (Farnsworth, 1993). Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of health problems due to some effective compounds they contain (Logan, 1981).

1.1. Plant distribution and taxonomy

Oak trees make up the genus *Quercus* of the Family Fagaceae, which includes as many as 600 species found mainly in the North Temperate Zone. They grow in a variety of habitats including seacoast, mountain slopes and wet lowlands. Oak is a widely available species varying from small bushes to great trees. Various species can be distinguished by identification of their fruit, the acorn. *Quercus infectoria* (Olivier) (Fagaceae), popularly known in Arabic as "ballout", common oak, oak bark, tanner's bark, dyer's oak, pedunculata oak or nut gall, is a species that is most abundant in Lebanon, Asia minor, Greece, Syria and Iran with a height of 8-10 meters. Acorns are long and narrow, with short cups. This tree is recognized for its galls. Galls are excrescences, which are formed upon the young branches. They are the result of a

puncture made in the bark by an insect (gall wasp *Cynips gallae-tinctoriae*) laying its eggs in the leaf buds (Stone et al., 2002; Cook, 1869).

1.2. Medicinal effects of certain oak species

Certain oak species were screened for their ability to possess some medicinal effects. Mainly, methanol extract of *Quercus ilex* leaves have been reported to have antibacterial activity against a wide range of clinical and plant-associated microorganisms including fungi, yeast and bacteria (Güllüce et al., 2004).

Another study revealed that water, ethanol and methanol in acetyl acetate (MeOH) extracts from *Quercus robur* (L.) bark were tested against Gram positive, Gram negative and yeast and have been shown to possess potent antioxidative and antimicrobial activity (Andrenšek et al., 2004). *Quercus pedunculata* have also been shown to have an inhibitory effect on human immunodeficiency virus (HIV) reverse transcriptase (El- Mekkawy et al., 1995). Inhibition of HIV RT is currently considered as a useful approach in the prophylaxis and intervention of AIDS. Goun et al. (2002) reported that methanol extract of *Quercus robur* (L.) demonstrated a very high activity against both cancer and thrombin.

To find antioxidative compounds present in plants, *Quercus robur* (L.) as well as other plants were tested and *Quercus robur* showed a potent active-oxygen scavenging activity when tested in murine dermal fibroblast culture system (Masaki et al., 1995). *Quercus suber* acorn's skin extracts also showed antioxidant activities (Cantos et al., 2003).

Possible gastroprotective effect against ethanol-induced gastric damage was also investigated by many researchers and the results showed that aqueous extract from *Quercus ilex* root bark was tested for its protective effect against ethanol-induced gastric damage in rats (Gharzouli et al., 1999). Moreover, ethanolic extracts of *Quercus coccifera* and *Quercus aegilops* fruit showed a very high curative rate after ethanol-induced gastric damage in rats suggesting their use in treatment of stomach pain (Alkofahi and Atta, 1999). Khennouf et al. (2003) showed that purified tannins, acetone extracts of *Quercus suber* and *Quercus coccifera* leaves prevented the formation of ethanol-induced gastric lesions in the stomach. Both extracts and tannins strongly inhibited lipid peroxidation in the rabbit brain homogenate. It was also reported that this gastroprotective effect of extracts of *Quercus suber* and *Quercus*

coccifera leaves are related to their anti-lipoperoxidant properties (Khennouf et al., 2003).

Last but not least, the effect of a manufactured soluble fiber made from wood chips of oak (*Quercus mangolica*) on lipid metabolism in rats fed high cholesterol diets showed that HDL-cholesterol concentration was significantly increased in experimental rats supplemented with the soluble dietary fiber. Hepatic Triglycerides (TAG) concentrations were reduced. Furthermore, hepatic microsomal HMG-CoA reductase responsible for cholesterol synthesis was also lower in fiber supplemented groups. The number of lipomas in the dietary soluble fiber fed groups was lower compared with control groups (Chai et al., 2003).

1.3. *Quercus infectoria* uses and constituents

Quercus infectoria is distinguished by its galls that are extensively used in folk medicine for being purely and powerfully astringent. By coagulating the blood, they frequently will arrest hemorrhage from small vessels, and sometimes are used for bleeding surfaces. Tannin is what makes oak bark so valuable for minor wounds and inflammation, for tightening tissue and lessening oozing. Astringent qualities of oak bark account for its use for treating inflammatory skin condition, irritating and itchy patches of eczema, bleeding or infected skin. Oak bark's powerful anti-inflammatory qualities make it very useful (in strong tea form) for sore throat and inflamed gums. Weaker teas, capsules, and liquid extracts are worth for acute diarrhea. It is used in the treatment of kidney stones. Litiex, a commercial product of oak bark, acts as a diuretic (water pill), it reduces pain and inflammation and it is believed to prevent formation of new kidney stones. Researchers are now exploring the value of oak bark for treating vaginal infections (Culberth, 1927).

The galls of *Quercus infectoria* have been pharmacologically documented to possess: antibacterial (Voravuthikunchai et al., 2005; Basri and Fan, 2005; Hwang et al., 2004; Voravuthikunchai et al., 2004; Hussein et al., 2000; Abou-Karam and Shier, 1999) larvicidal (Redwane et al., 2002), anti-inflammatory (Kaur et al., 2004), anti-amoebic effect on caecal amoebiasis (Sawangjaroen, 2004). Litiex, a commercial oak bark preparation, present therapeutic effects in urolithiasis (Rodriguez and Gausa, 1980).

Another study had also focused on the α -Glycosidase inhibitory activity of hexagalloylglucose from the galls of *Quercus infectoria* (Hwang et al., 2000). Briefly, the methanol extract of the galls of *Quercus infectoria*, significantly inhibited α -

glycosidases such as sucrase, maltase and isomaltase. Hexagalloylglucose showed higher activity on sucrase and isomaltase, but lower activity on maltase. This supports the hypoglycemic activity reported by Dar et al. (1976). When compared to acarbose, a hypoglycemic agent, hexagalloylglucose reduce the side effects such as diarrhea and abdominal discomfort in diabetes treatment due to the lower inhibition on α -amylase (Hwang et al., 2000).

Quercus infectoria galls were tested against the in vitro growth of fresh isolates of the intestinal protozoan parasite, *Blastocystis hominis* and showed a high activity either by killing or inhibiting such parasites in samples tested (Sawangjaroen and Sawangjaroen, 2005).

Polyphenols of the aqueous extract from *Quercus infectoria* have been shown to have an inhibitory activity against venom of the snake, *Naja kaouthia* (Monocle Cobra) by selectively blocking acetylcholinesterase activity. Inhibition was due to the condensed tannins and their concentration in the extract (Pithayanukul et al., 2005).

Galls of *Quercus infectoria* were shown to contain ellagic acid, gallotannins, sitosterol, methyl betulate and methyl oleanolate. They were studied pharmacologically. Acetone-treated methanolic extract and chloroform-methanol extracts were investigated. The first extract was active as an analgesic in rats and significantly reduced blood sugar levels in rabbits. The second extract had CNS depressant activity manifested by abolishment of action potential, indicating a complete nerve blockade. In addition, the second extract exhibited a moderate antitremorine activity by causing a delay in the onset and a decrease in the severity of tremorine-induced tremors (Dar and Ikram, 1979; Dar et al., 1976).

Finally, bark of *Quercus infectoria* is used in folk medicine for treating eczema and impetigo (contagious skin infection forming pimples and sores) (Dar et al., 1976).

1.4. Tannins used as medicinal agents: Structural characteristics and origin

“Tannin” is a general descriptive name for a group of polymeric substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Their molecular weights range from 500 to 3,000 (Haslam, 1996). Tannins comprise a large group of natural products widely distributed in the plant kingdom. Thus they are found in every plant part: bark, wood, leaves, fruits, and roots (Scalbert, 1991). Generally, two classes are distinguished, hydrolysable type and the condensed type. Hydrolysable tannins are esters of sugars, mainly glucose, and phenol

carboxylic acids, such as gallic acid, or ellagic acid. As their name suggests, they are readily degraded under hydrolytic conditions into these fundamental components. Hydrolysable tannins are present in many different plant species but are found particularly in high concentrations in nutgalls growing on *Quercus infectoria*, *Rhus semialata* (Chinese and Korean gallotannins) (Health Management Publications, Inc., 2001). The condensed tannins are much more resistant to decomposition and merely yield polymers or amorphous precipitates under the influence of acids.

It was suggested that the consumption of tannin-containing beverages, especially green teas and red wine could cure or prevent a variety of illnesses. Highly purified tannic acid products applied in moderate concentrations could be useful for wound healing and it causes no serious hepatic damage (Health Management Publications, Inc., 2001).

Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins (Texl, 1960). Tannins' mode of action as antimicrobial may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins. Tannins in plants inhibit insect growth and disrupt digestive events in ruminal animals (Cowan, 1999).

1.5. Lipids

1.5.1. Introduction to lipids: general overview

Lipids are a heterogeneous group of molecules that display diversity in both structure and function. They are stored in the body and serve as source of energy and are mainly characterized by their hydrophobic nature. Lipids have physiological importance and serve several major functions: they serve as structural components of biological membranes by formation of a permeability barrier of cells and subcellular organelles in the form of a lipid bilayer, which protect organisms against their environment; they provide energy reserves, predominantly in the form of triacylglycerols (TAG). Both lipids and lipid derivatives serve as vitamins and hormones. Lipophilic bile acids aid in lipid solubilization. Lipids also participate in the transmission of chemical messages in living organisms (Ger, 2004; Vance, 2004). Fatty acids and their derivatives are the major class of important lipids. A second class that play a crucial role in biological systems are cholesterol and its derivatives and metabolic products. Other classes include phospholipids and lipoproteins. Fatty acids

are essential for the formation of phospholipids, building blocks of cellular membranes, and coating of lipoproteins, the lipid transporting particles in blood plasma. Fatty acids are major components of stored fat in the form of TAG. Fatty acids and their derivatives are information carriers interacting with intracellular signaling pathways and nuclear factors involved in the regulation of DNA transcription (Luiken et al., 2000).

Cholesterol is an important constituent of biological membranes, specifically influencing membrane fluidity. Cholesterol is also substrate for formation of bile acids in liver cells. After storage in the gall bladder, bile acids are of paramount importance to facilitate the digestion of dietary lipids and the transport of the digestion products to the epithelial cell of the intestinal wall. Cholesterol is also the precursor of various classes of steroid hormones, including mineralocorticoides and glucocorticoides produced in the adrenal cortex and sex steroid hormones synthesized in the female and male gonads: estrogen and testosterone. Cholesterol is an essential nutrient; it contributes to manufacturing vitamin D in the skin's surface. There is also an evidence for cholesterol helping in cell connections in the brain that are important for learning and memory (Ger, 2004).

Regardless of these benefits, when cholesterol levels rise in the blood, they can have dangerous consequences depending on the type of cholesterol. Although the body acquires some cholesterol through diet, about two-thirds is manufactured in the liver stimulated by saturated fats (Vance, 2004; Gurr and Harwood, 1991).

1.5.2. Blood Lipoproteins

Lipoproteins are protein spheres that transport cholesterol, TAG, and other lipid molecules through the bloodstream. Lipoproteins are categorized into five types according to size and density. Furthermore they can be defined to whether they carry cholesterol or TAG. Low-density lipoproteins (LDL), often called “bad cholesterol”, and high-density lipoproteins (HDL), referred to as “good cholesterol”, which are the smallest and the most dense, are the cholesterol-carrying lipoproteins. Whereas, intermediate density lipoproteins (IDL), very low density lipoproteins (VLDL), and chylomicrons (CM), which are the largest in size and lowest in density, form the class of TAG-carrying molecules (Kingsburg and Bondy, 2003) (Figure 1.1.)

Figure 1.1. Major lipoprotein classes (CM, VLDL, LDL, HDL) based on their density. Lipoproteins diameters range from 6000 Å for CM to 70 Å for HDL. The outer shell (~20 Å) of all lipoproteins consists of apolipoproteins, unesterified cholesterol, and phospholipids; the spherical core contains triacylglycerols and cholesteryl esters. CM and VLDL have the highest content of triacylglycerols, and 1-10% of apolipoproteins by weight; LDL and HDL contain mostly cholesteryl esters in their cores, and 20-50% of apolipoproteins. The major apolipoprotein components of the various classes of lipoproteins are indicated with the solid lines; secondary or minor lipoproteins are indicated with the dashed lines. In this figure, “cholesterol” refers to both esterified and unesterified cholesterol; Triglycerides = triacylglycerols (Vance, 2004).

1.5.3. Transport of lipids in the blood: plasma lipoproteins

Due to their hydrophobic nature, water-immiscible lipids are not able to move in body fluid, so in order to enable their transport in the body, lipids particles are stabilized by a coat of amphiphilic compounds: phospholipids and proteins. The resulting particles are called lipoproteins. Proteins moieties of lipoproteins that form a shell, which surround these particles are called apolipoproteins and are synthesized in the liver. Despite their diversity, the lipoprotein common role is to transport lipids from one tissue to another to supply the lipid needs of different cells. Lipoproteins differ according to the ratio of lipid to protein within the particle as well as having different proportions of lipids: TAG, esterified and non-esterified cholesterol and phospholipids (Berneis et al., 2002; Gurr and Harwood, 1991).

1.5.4. Apolipoproteins functions

Apolipoproteins are amphipathic in nature, in that they have both hydrophobic and hydrophilic regions, and can therefore interact both with lipids of the lipoprotein and with the aqueous environment. Because of the nature of these amphipathic regions, apolipoproteins act as detergents and thus two major functions can be assigned to these particles. First, they provide means of solubilizing the lipid particles and maintain their structural integrity. Second, they help identifying lipoproteins and direct their metabolism in specific ways (Gurr and Harwood, 1991; Schaefer, 1978).

Apolipoproteins can be divided into several sub-classes that are referred to apoAI, apoAII, apoAIV, apoCI, apoB100 and apoB48 (Kingsburg and Bondy, 2003; Gurr and Harwood, 1991). ApoB is an integral protein constituent of three lipoprotein species: chylomicrons, very low density lipoproteins (VLDL), and low density lipoproteins (LDL) (Schaefer, 1978). A major attention was accorded to apoB being an important marker for atherogenicity. ApoB is synthesized both in the liver and intestine in the rat and enters the circulation with either chylomicrons or VLDLs. They appear to be essential for the transport of triglyceride out of the liver and the intestine. ApoB is known to exist in two variants of different molecular masses, usually referred to as apoB-100 and apoB-48. ApoB-100 is the only form of apoB synthesized in the liver and secreted into the plasma as the major protein component of very low-density lipoproteins (VLDL) (Segrest et al., 2001). In contrast, the small intestines of all mammals, and the liver of some species such as rats, synthesize apoB-48, the N-terminal 2152 residues of apoB-100, which is secreted in the intestine in chylomicrons after absorption of dietary lipids (Vance, 2004; Karpe and Hamsten, 1994; Chan, 1992). Interestingly, apoB-100 and apoB-48 generated from a single structural gene (Vance, 2004).

1.5.4.1. Chylomicrons (CM)

Originating from the intestine, CM are the largest and the least dense among lipoproteins particles. They are involved in transport of absorbed dietary fat, thus TAG are their principal components associated with small amounts of phospholipids and proteins that cover the surface. The presence of CM is easily detected in a sample of plasma shortly after the consumption of a fatty meal giving the plasma a cloudy or opalescent appearance by scattering more light. Because of their low density, they can be isolated as a floating layer by a low speed ultracentrifugation. CM are then

transported in the blood to tissues: skeletal muscle, fat, and the liver, where the lipoprotein lipase (LPL) present on the capillary endothelium of adipose and muscle tissues will hydrolyze the triacylglycerol core rendering the molecule smaller and smaller depleting the large CM and giving rise to CM remnants that are relatively rich in cholesteryl ester (CE) and is cleared and removed from the circulation by receptor-mediated endocytosis in liver cells (Cooper, 1997). Each CM has one apoB-48 molecule that remains with the molecule until it is cleared. Thus apoB is a marker of lipoprotein particle number and by assessing the number of apoB-48 molecules we can assess the number of CM in the blood (Karpe and Hamsten, 1994; Chan, 1992). Chylomicrons function to deliver dietary triacylglycerols to adipose tissue and muscle and dietary cholesterol to the liver (Kingsburg and Bondy, 2003).

1.5.4.2. Very low density lipoproteins (VLDLs)

VLDL particles are similar to CM in that they contain predominantly TAG. Their function is to transport TAG of endogenous origin, synthesized mainly in the liver or intestine. The catabolism of VLDL is similar to that of CM. VLDL bind to endothelial LPL and the TAG are hydrolyzed. The resulting particles are called VLDL remnants or intermediate density lipoproteins (IDL). IDL are taken up by the liver via the LDL receptor or is converted to LDL by enzymatic action of hepatic lipase (Gurr and Harwood, 1991).

The dietary intake of both fat and carbohydrate, in excess of the needs of the body, leads to their conversion into TAG in the liver. These TAG are packaged into VLDLs and released into the circulation for delivery to the various tissues (primarily muscle and adipose tissue) for storage or production of energy through oxidation. VLDLs are, therefore, the molecules formed to transport endogenously derived TAG to extra-hepatic tissues. In addition to TAG, VLDLs contain some cholesterol and cholesteryl esters (CE) and the apoproteins, apoB-100, apoC-I, apoC-II, apoC-III and apoE. Like nascent CM, newly released VLDLs acquire apoCs and apoE from circulating HDLs. The fatty acid portion of VLDLs is released to adipose tissue and muscle in the same way as for CM, through the action of LPL. The action of LPL coupled to a loss of certain apoproteins (the apoCs) converts VLDLs to IDLs, also termed VLDL remnants. The apoCs are transferred to HDLs. The predominant remaining proteins are apoB-100 and apoE. Further loss of TAG converts IDLs to LDLs (Vance, 2004).

1.5.4.3. Intermediate Density Lipoproteins (IDLs)

IDLs are formed as TAG are removed from VLDLs. The fate of IDLs is either conversion to LDLs or direct uptake by the liver. Conversion of IDLs to LDLs occurs as more TAG are removed. The liver takes up IDLs after they have interacted with the LDL receptor to form a complex, which is endocytosed by the cell. For LDL receptors in the liver to recognize IDLs require the presence of both apoB-100 and apoE (the LDL receptor is also called the apoB-100/apoE receptor) (Kingsburg and Bondy, 2003).

1.5.4.4. Low-density lipoproteins (LDLs)

LDL particles are the major carrier of plasma cholesterol to tissues where it may serve for membrane structure or conversion into various metabolites such as steroid hormones. LDL particles are derived from VLDL by removing TAG thus producing molecules rich in cholesterol and phospholipids. Cells take up cholesterol via receptor-mediated endocytosis. LDL receptors in peripheral cells or liver cells bind with LDL and remove it from the blood (Rifai et al., 1997).

LDL is normally harmless. However if it is exposed to a process called oxidation, it can penetrate and interact dangerously with the walls of arteries, producing a harmful inflammatory response. It is associated with increased cardiovascular disease risk (Berneis and Krauss, 2002).

LDLs in humans comprise multiple subspecies that differ in their metabolic behavior and pathologic role. Predominance of small dense LDL particles signal atherogenicity. Increased atherogenic potential of small LDL particles is suggested by greater propensity into the subendothelial space, increased binding to arterial proteoglycans, and susceptibility to oxidative modification. Large LDL particles can also be associated with increased coronary heart disease risk, particularly in the setting of low TAG levels (Krauss and Burke, 1982).

The cellular requirement for cholesterol as a membrane component is satisfied in one of two ways: either it is synthesized *de novo* within the cell, or it is supplied from extra-cellular sources, namely, CM and LDLs. In the circulation VLDLs are converted to LDLs through the action of lipoprotein lipase (Gotto and Pownall 1999). The exclusive apolipoprotein of LDLs is apoB-100. LDLs are taken up by cells via LDL receptor-mediated endocytosis. The uptake of LDLs occurs predominantly in liver (75%), adrenals and adipose tissue. As with IDLs, the interaction of LDLs with

LDL receptors requires the presence of apoB-100. The endocytosed membrane vesicles (endosomes) fuse with lysosomes, in which the apoproteins are degraded and the cholesterol esters are hydrolyzed to yield free cholesterol (Gurr and Harwood, 1991).

1.5.4.5. High Density Lipoproteins (HDLs)

HDL particles generally refer to as “Good Cholesterol” due to their major role in carrying cholesterol from peripheral cells to the liver, a process generally known as “reverse cholesterol transport” (Genest, 1990). HDLs are synthesized in the liver and small intestine, as primarily protein-rich particles. They are divided into two subclasses: HDL₂ and HDL₃. Distinction between these two subclasses may have metabolic significance in that HDL₂ appear to have a stronger inverse relationship with cardiovascular disease than HDL₃ (Gotto et al., 2000). ApoI and apoAII are the major apolipoproteins of HDL, although some apoC, apoE and a protein unique to HDL-apoD coat the surface. These particles are disc-shaped rather than spherical and consist of a bilayer of mainly phosphatidylcholine with apoAI, and apoE at the margins of the disc. After secretion in the plasma, HDL acquires additional surface components including phospholipids, cholesterol and apoproteins by transfer from CM and VLDL during their catabolism by LPL (Grundy, 1990).

In plasma, a subfraction of HDL, containing apoAI, and apoD becomes associated with an enzyme: lecithin-cholesterol acyltransferase (LCAT) this results in transfer of fatty acid from phosphatidylcholine to the cholesterol in the surface of the disc-shaped HDL to form CE which transfers in the core of HDL particle. The redistribution of lipid in the HDL particle converts the particles from discoidal to spherical in shape (Krauss and Burke, 1982; Schaefer et al., 1978).

Cholesterol-rich HDLs return to the liver from peripheral cells, where they are endocytosed. Reverse cholesterol transport, or hepatic uptake of HDLs may be mediated through an HDL-specific apoA-I receptor or through lipid-lipid interactions. Macrophages also take up HDLs through apoA-I receptor interaction. HDLs can then acquire cholesterol and apoE from the macrophages; cholesterol-enriched HDLs are then secreted from the macrophages. The added apoE in these HDLs leads to an increase in their uptake and catabolism by the liver (Schaefer et al., 1978).

HDLs also acquire cholesterol by extracting it from cell surface membranes. This process has the effect of lowering the level of intracellular cholesterol, since the

cholesterol stored within the cells as CE will be mobilized to replace the cholesterol removed from the plasma membrane.

The cholesterol esters of HDLs can also be transferred to VLDLs and LDLs through the action of the HDL-associated enzyme, cholesterol ester transfer protein (CETP). This, in addition, allows the excess cellular cholesterol to be returned to the liver through the LDL-receptor pathway as well as the HDL-receptor pathway (Gurr and Harwood, 1991).

Figure 1.2. a. A model of the structure of a low-density lipoprotein (LDL). b. Four classes of lipoproteins visualized under electronic microscope. Chylomicrons ($d > 70\text{ nm}$); VLDL ($30 < d < 80\text{ nm}$); HDL ($5 < d < 12\text{ nm}$) and LDL ($18 < d < 22\text{ nm}$) (Gurr and Harwood, 1991).

1.6. Liver toxicity assessment

Liver absorbs nutrients and oxygen from the blood and regulates blood glucose and protein levels. It is the metabolic hub of the body, which means many things are metabolized, broken down and converted in the liver. It helps to break down drugs and toxins and manufactures proteins and blood clotting factors. Serious harm to the liver caused by drugs, and by the combination of drugs and other substances such as herbal medicine is an important public health problem. Many of these products are chemicals that are processed and detoxified by the liver. These chemical processes may produce substances that injure liver cells in some people and result in impaired liver functioning. Herbal medicines are generally safe but in some cases certain toxic reactions could accompany remedy with herbal medicine, for this reason assessment of liver toxicity should be undergone to test whether liver cells are healthy or damaged. This is done by measuring activity of hepatic enzymes including: serum

lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT). Elevated levels of LDH have been observed in variety of disease states. Highest levels are seen in patients with megaloblastic anemia, disseminated carcinoma and shock. Mild increases in LDH activity have been reported in cases of myocardial or pulmonary infarction, leukemia, hemolytic anemia and non-viral hepatitis. Whereas elevated levels of transaminases AST and /or GPT (ALT) can signal myocardial infarction, hepatic disease, muscular dystrophy, and internal organ damage. However; ALT activity in the serum is rather specific indicator of damage to liver parenchyma, while AST is not necessary a liver-specific parameter (Braunwald et al., 2001).

1.7. Inflammation

The inflammatory response includes three main vascular contributions: vasodilatation, increased vascular permeability, and leucocyte migration to the injured tissues (Matos et al., 2004). An inflammatory response involves a number of mediators secreted by macrophages and neutrophils (ecosanoids, oxidants, cytokines and lytic enzymes). These mediators are responsible for initiation, progression and persistence of chronic or acute state of inflammation (Kaur et al., 2004). Prostaglandins are also important mediators of acute inflammation. Generally, the initial phase of edema is due to release of histamine as opposed to the second phase of swelling, which is related to elevated prostaglandins' production. The swelling phase was also attributed to the induction of inducible cyclooxygenase (COX-2) in the hindpaw (Salvemini et al., 1996). Furthermore, nitric oxide (NO) is another important mediator in acute and chronic inflammation. NO is a potent vasodilator. Its implication during an inflammatory response may be due to increase of vascular permeability and edema through changes in local blood flow at the site of inflammation (Salvemini et al., 1996).

Inflammation has been implicated in the pathophysiology of various clinical disorders including vascular diseases and cancer. Researchers are now investigating potent anti-inflammatory drugs derived from plant extracts and their potential therapeutic use against vascular disorders (Jose et al., 2004).

1.8. Platelet aggregation activity

Platelets antiaggregants are known to be useful for the prevention of thrombus formation, especially in subjects at risk for coronary heart disease and cerebral thrombosis. In addition plants antiaggregants could be used to reduce the risk of stroke and myocardial infarction, leading to a significant decrease in the rate of mortality (Villar et al., 1997; Neiva et al., 1999; Jose et al., 2004). However, some plant extracts such as tannins (Lauque et al., 1991) and ellagitannins (Teng et al., 1997) were shown to be potent stimulators for platelets aggregation *in vivo*. Such active compounds present in different plant parts such as oak bark are used in folk medicine for bleeding wounds when applied directly in the form of powder (Health Management Publications, Inc., 2001).

1.9. Gastric damage

The mucosal surface of the stomach that acts as a barrier limiting the exposure to injurious stimuli is constantly subjected to exogenous and endogenous agents such as acid, pepsin, bile acids, food ingredients, bacterial products, and drugs (Zayachkivska et al., 2005). Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance. Several factors are implicated in the pathogenesis of gastric ulcer. These include increased acid-pepsin secretion, impaired bicarbonate neutralization, impaired mucus secretion and precipitate lesions on the mucosal layer (Cho and Wang, 2002). Pre-treatment or treatment with different substances could have a positive outcome either in prevention or healing of gastric damage. This refers to as cytoprotection which accounts for gastroprotection in response to various irritants and ulcerogens rather than being related to inhibition of gastric acid secretion (Zayachkivska et al., 2005; Gharzouli et al., 1999).

Plant extracts, are some of the most powerful sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers (Toma et al., 2005; Alkofahi and Atta, 1999; Gharzouli et al., 1999).

1.10. Purpose of the project

After extensive literature survey, it appears that no previous studies of any kind were conducted on any extract of *Quercus infectoria* bark. Thus, the present investigation was undertaken on rats in order to evaluate the effect of *Quercus infectoria* bark methanolic extract upon

- Acute and chronic inflammation induced by carrageenan and formalin respectively.
- Blood lipid profile: TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol
- Glycemic profile: glucose and insulin.
- Toxicity by assessing liver enzymes activities: GOT, GPT and LDH, as well as effect on urea and creatinine levels
- Potential antimicrobial activity.
- Platelet aggregation activity.
- Protection against ethanol-induced gastric ulcer.

MATERIALS AND METHODS

2.1. Plant material collection and extraction procedure

Quercus infectoria bark was collected from diverse oak trees widely distributed throughout Lebanon, from young branches ranging in age from 3 to 4 years. The taxonomic identification of the plant material was confirmed by Dr Addel Rahman Barghashoun, a plant taxonomist in the Department of Biology in the Lebanese University- Faculty of Sciences- Tripoli. Collected branches were peeled and chopped into small pieces, dried in shade, and then soaked in methanol for 72 hours. This suspension was filtered. The filtrate was pooled and the solvent was evaporated in a rotavapor at 45° - 50°C under reduced pressure and lyophilized. The lyophilized extract was stored in a well-sealed box in the fridge until use in order to prevent aggregation caused by humidity of the air (Güllüce et al., 2004; Jose et al., 2004).

2.2. Animal treatment

Male Sprague-Dawley rats (n=30) (Lebanese American University stock) were randomly divided into two groups comprising 15 animals each with an average weight of 200 g. The first group served as a control and received 6.5 g of food per 100 g body weight. Food consisted of rat chow to which 5% coconut oil was added. Coconut was used to make the diet atherogenic (Daher et al., 2003). The second group referred to as treatment group received the same food as the control in addition to 1 g of methanol extract of *Quercus infectoria* in 200 ml drinking water for the whole group. Animals were maintained at an ambient temperature of 20 - 22°C. After one month, fasted animals were sacrificed using diethyl ether.

2.2.1. Plasma samples and isolation of lipoproteins fractions

Animals were fasted for 18 hours before being anesthetized with diethyl ether and venous blood was drawn for determination and quantification of apolipoproteins B-48 and B-100 as well as impact of the methanol extract on lipid profile, urea, creatinine, blood glucose levels, serum insulin content, and liver enzymes.

Venous blood (4 ml) was drawn into glass tubes containing disodium ethylenediaminetetraacetic acid (Na₂EDTA, 1mg/ml) using a 10 ml sterile syringe.

Tubes were directly put in ice water. Plasma samples were obtained by low speed centrifugation at 3000 rpm, for 20 minutes at 10°C. Lipoproteins fractions were obtained from plasma samples by density gradient ultracentrifugation. A solution containing 5 µl/ml plasma phenylmethylsulfonylfluoride (PMSF) 10 mM dissolved in isopropanol and 2 mg/L aprotinin were added in order to minimize proteolytic degradation of apoB. Subsequently, 0.21 g of solid NaCl were added to 1.5 ml plasma to increase density to 1.1 g/L. A total volume of 1.5 ml of the density of 1.1 was put in the bottom of an ultracentrifuge tube. A gradient density consisting of 6.5 ml NaCl of a density of 1.065 g/L (10.5 g NaCl/100 ml distilled water; 0.02% sodium azide, NaN₃; and 0.01% EDTA, pH = 7.4) was layered on the top of the plasma using a peristaltic pump. Ultracentrifugation was then performed in an ultracentrifuge at 55,000 rpm and 15°C for 4 hours (Sorval Discovery 90SE, Hitachi, Kendro Laboratory Products)

After centrifugation, the top layer (400µl) was collected for total apoB determination. 100µl of each ultracentrifuged sample was taken and added to 300µl of 2X sample buffer containing 1.51g Tri-sodium Citrate 5.5 hydrate (Tris) in 35 ml water, 20 ml glycerol. pH is adjusted to 6.75 with concentrated HCl then 4 g Sodium Dodecyl Sulfate (SDS), 10ml β- mercaptoethanol, and 2 mg of Bromphenol blue as a front dye were added. The final volume was completed to 100 ml with distilled water. Samples were denatured at 90°C for 4 minutes followed by centrifugation at full speed (13000 rpm) for 1-2 minutes.

The rest of the ultracentrifuged samples, as well as samples mixed with sample buffer were then stored at - 80°C until electrophoresis (Karpe and Hamsten, 1994; Daher et al., 2003).

2.2.2. SDS-PAGE

2.2.2.1. Principle

Electrophoresis is simple, rapid and highly sensitive method that deals with the migration of charged molecules in solution in response to an electric field. The rate of migration depends on the field; on the net charge, size and shape of the molecules and also on the ionic strength, viscosity and temperature of the medium in which the molecules are moving. Sodium Dodecyl Sulfate- PolyAcrylamide Gel Electrophoresis (SDS-PAGE) is an important analytical technique used for quantifying, comparing and characterizing proteins based primarily on their molecular weights or their size,

not on other physical feature. SDS is an anionic detergent, which denatures proteins by binding to their hydrophobic portions, thus disrupting its folded structure and allowing it to exist stably in a solution in an extended conformation. Consequently, the length of the SDS-protein complex is proportional to its molecular weight. A gradient of acrylamide for gel electrophoresis (5-20%) is used because it has two advantages over the use of a linear gel. First, an increased protein sieving effect at higher acrylamide concentrations leads to the formation of sharper bands at lower molecular weight. Furthermore, the gradient allows separation of a wide range of protein molecular weights in a single gel (Bollag, 1996).

2.2.2.2. Stock solutions

Protogel

60 g acrylamide, 1.6 g bisacrylamide dissolved in distilled water to reach a volume of 200 ml

3 M Tris, pH 8.8

36.3g Tris, 90 ml distilled water, pH is adjusted to 8.8 using concentrated HCl, all diluted to achieve a final volume of 100 ml

0.5 M Tris, pH 6.8

6.02 g Tris dissolved in 80 ml distilled water, pH is adjusted to 6.8 with concentrated HCl, dilute to a final volume of 100 ml

2X sample buffer

1.51g Tri-sodium Citrate 5.5 hydrate (Tris) in 35 ml water, 20 ml glycerol. The pH was adjusted to 6.75 with concentrated HCl then 4 g Sodium Dodecyl Sulfate (SDS), 10ml β - mercaptoethanol, and 2 mg of Bromophenol blue as a front dye were added. The final volume was completed to 100 ml with distilled water.

Urea/SDS/DTT

19.2 g urea, 0.0123 g Dithiothreitol (DTT), 0.08 g Lauryl sulfate (SDS) in H₂O. The mixture is dissolved on a magnetic stirrer, then the final volume was adjusted to 40 ml exactly.

APS (Ammonium Persulfate) 1.5 %

0.15 g APS in 10 ml distilled water were added for polymerization.

Water-saturated butanol

90 ml butanol + 10 ml distilled water

NB: Protogel, 3 M Tris, 5 M Tris, 2X sample buffer, and water-saturated butanol could be prepared and stocked whereas Urea/SDS/DDT and APS Solutions should be freshly prepared prior to utilization.

2.2.2.3. Resolving and stacking gels preparation (2 gels)

A linear gradient (5-20%) polyacrylamide gels as well as the resolving gels were prepared as described in table 2.1.

Table 2.1. Gel mixture for 5-20 % gradient gels

	RESOLVING GELS		STACKING GEL
SOLUTIONS	5 %	20 %	
0.5 M Tris	—	—	3.75 ml
3 M Tris	3.7 ml	3.7 ml	—
Protogel	5 ml	20 ml	1.85 ml
Urea/SDS/DDT	3 ml	300 µl	0.3 ml
APS	0.7 ml	0.7 ml	1.15 ml
Distilled water	20.2 ml	2.7 ml	7.5 ml
Sucrose	—	4.5 g	—
TEMED (added last)	13 µl	13 µl	11.25 µl

2.2.2.4. Pouring gels

Electrophoresis apparatus (Hoefer SE 600, Amersham Pharmacia Biotech) was assembled according to the manufacturer. The linear density gradient was prepared using a gradient maker. Once the gels were poured they are overlaid with 0.75 ml water-saturated butanol using a glass pipette to ensure a flat surface and to exclude air. The gels were allowed to set and the polymerization time was adjusted to 45

minutes. Water-saturated butanol was then removed and the gels were rinsed 3 times with distilled water then blotted using a Whatman filter paper. Combs were then added and the stacking gel was poured on polymerized resolving gel using a pipette. Combs served to create wells in the gels where the samples should be loaded. The gels were let to stand for an additional 45 minutes, after which combs were removed.

2.2.2.5. Loading of samples

Samples frozen at -80°C were thawed immediately before use. Molecular weight marker was prepared using a High Molecular weight SDS Calibration Kit for SDS electrophoresis (High Molecular Weight Calibration Kit, Amersham Pharmacia Biotech, USA).

Human apoB was used as a standard for apoB48 quantification since rat apoB48 standards are difficult to prepare (Kotite, 1995). ApoB100 and rat apoB48 have similar chromogenecity when stained with Coomassie Blue R-250, thus proving the use of human apoB100 as a standard (Van Beek et al., 1998). Human LDL ($1.030 < d < 1.040 \text{ g/ml}$), isolated from fasting human plasma samples by density gradient ultracentrifugation, served to prepare apoB100 standards (Karpe and Hamsten, 1994). The LDL subfraction was recovered from a 1 ml portion located between 5.5 and 6.5 below the top if the tube after 16 hours of ultracentrifugation. Desalting was achieved using by passing the LDL through a PD-10 column (Pharmacia). The total protein content was determined with bovine serum albumin (BSA) as standard with addition of SDS (final concentration 1%) to the reagent mixture in order to reduce turbidity. Several determinations were taken, and the mean was taken as the final protein value. Following delipidation of LDL, the dissolved protein mixture was denatured at 95°C for 5 minutes. Delipidated and denatured apo B100 protein standard were stored at -80°C in small vials and thawed immediately before use (Karpe and Hamsten, 1994). Samples (100 μl), molecular weight marker (8 μl), as well as standard apoB samples (5, 15 and 30 μl) were loaded in the wells. Gels are now ready for electrophoresis.

2.2.2.6. Electrophoresis

Electrophoresis was performed using a vertical electrophoresis apparatus connected to a power supply. The upper and lower electrophoresis buffers contained 99.4 g glycine, 21.4 g Tris and 7 g SDS in 7 L distilled water, all dissolved on a magnetic stirrer. Electrode plugs were then attached to proper electrodes. Current

should flow towards the anode. Electrophoresis was run at 220 V, 20 W, and 120 mA for 4 hrs.

2.2.2.7. Staining and quantification of Total apoB

Gels were stained with Coomassie Blue (Coomassie Blue R-250), a method that can detect as little as 0.1 µg of protein in a single band. After electrophoresis, gels were gently removed, stacking gel is discarded; gels are now ready for staining/ destaining protocol performed in an automated gel stainer- destainer (Hoefer Processor Plus, Amersham Pharmacia Biotech, USA) for an overnight. The coomassie Gel stain consists of 1g coomassie blue, 450 ml methanol, 100 ml glacial acetic acid and 400 ml distilled water. Whereas the coomasie gel destain contains: 300 ml methanol, 100 ml glacial acetic acid and the volume is completed to 2 liters with 1600 ml distilled water.

After destaining, gels images were taken and the saved images were used for quantification of apoB-48 and apoB-100 bands using 1-D Advanced software (advanced software Biotechnology, Fullerton, CA, USA). A calibration curve was constructed and the masses of apoB48 (unknown samples) were extrapolated from the curve (Daher et al., 2003; Karpe and Hamsten, 1994).

2.3. Serum assays

2.3.1. Samples preparation:

Venous blood (4 ml) from the same fasting rats was drawn as described above. 4 ml blood from each rat were put into glass tubes and allowed to clot for 30 to 45 minutes at room temperature. Blood was then centrifuged for 20 minutes at 3000 rpm at 10°C. supernatant, serum, was isolated from the pellet and transferred into eppendorf tubes, which are put directly into ice water. Serum fractions serve to assess liver toxicity by measuring activity of liver enzymes: SGOT, SGPT and LDH, lipid profile by measuring levels of HDL cholesterol, LDL cholesterol, TAG, total cholesterol, as well as creatinine, urea and glucose levels and insulin content for both control and treatment groups.

2.3.2. Glucose determination

Principle: Glucose is the most important monosaccharide in the blood and glucose substrate is an indispensable energy supplier, which supports cellular function.

Glucose is oxidized by glucose oxidase (GOD) to gluconolactone in the presence of atmospheric oxygen. The resultant hydrogen peroxide oxidizes 4-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 505 nm. The enzymatic reaction used in the assay is as follows:

Procedure: 10 µl of each sample and standard, run in duplicates, are mixed with 1 ml of the buffer solution and the enzymes and incubated 10 minutes at 37°C. The absorbances of the unknown and the standard samples are measured against the reagent blank at $\lambda = 505$ nm immediately (Jenway 6105 spectrophotometer, U.K.).

Calculation:

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

Whereby the concentration of the standard is 100 mg/dl according to the manufacturer's (SPINREACT) leaflet.

2.3.3. Determination of TAG

Principle: Determination of TAG is based on a method using a lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of TAG to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff. The color intensity is directly proportional to the concentration of TAG and can be determined spectrophotometrically at a wavelength of $\lambda = 505$ nm

Procedure: 10 µl of each sample and standard, run in duplicates, are mixed with 1 ml of the buffer solution and the enzymes and incubated 5 minutes at 37°C. the absorbances of the unknown and the standard samples are measured against the reagent blank at $\lambda = 505$ nm immediately (Jenway 6105 spectrophotometer, U.K.).

Calculation:

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

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

2.3.4. Determination of total cholesterol

Principle: Cholesterol is determined enzymatically using cholesterol esterase (CE) and cholesterol oxidase (CHO)

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids.

Cholesterol is converted by oxygen with the aid of cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.

Hydrogen peroxide created forms a red dye by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The color intensity is directly proportional to the concentration of cholesterol and can be determined spectrophotometrically at a wavelength of $\lambda = 505 \text{ nm}$

Procedure: 10 μl of each sample and standard, run in duplicates, are mixed with 1 ml of the buffer solution and the enzymes and incubated 5 minutes at 37°C . the absorbances of the unknown and the standard samples are measured against the reagent blank at $\lambda = 505 \text{ nm}$ immediately (Jenway 6105 spectrophotometer, U.K.).

Calculation:

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

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

2.3.5. Determination of HDL-Cholesterol

Principle: Low density lipoproteins (LDL and VLDL) are specifically precipitated by phosphotungstic acid and magnesium ions and can then be removed by centrifugation. High density lipoproteins (HDL) remains in the supernatant. Determination of HDL cholesterol is performed using the clear supernatant.

Procedure: 0.5 ml of each sample and standard, run in duplicates, are mixed with 50 μl of 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 is used for cholesterol determination using the cholesterol assay kit as described previously.

Calculation:

$$\text{HDL- Cholesterol (mg/dL)} = \text{Absorbance of sample} \times 320$$

2.3.6. Determination of LDL – Cholesterol

Principle: Direct determination of LDL-cholesterol takes advantage of the selective micellary solubilization of LDL-cholesterol by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fraction increase in this order: HDL < chylomicrons < VLDL < LDL. In the presence of Mg^{2+} , a sugar compound markedly reduces the enzymatic reaction for the cholesterol measurement in VLDL and CM. The combination of a sugar compound with detergent enables the selective determination of LDL-cholesterol in serum.

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4 – cholestenone and hydrogen peroxide.

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-amino-antipyrine and HSDA to form a purple blue dye. The color intensity is directly proportional to the cholesterol concentration and is measured spectrophotometrically at a wavelength of 505 nm.

Procedure: 10 μl of each sample or calibrator is mixed and incubated 5 minutes at 37°C with 900 μl of 25 mM Good's buffer pH= 6.8, 5000 U/L CHE, 5000 U/L CHO, 0.64 mM H- HSDA and 1000 KU/L catalase. The absorbance A1 is read at 650 nm against reagent blank. Then 300 μl of 25 mM Good's buffer pH= 7, 3.4 mM 4-

aminoantipyrine, 20 KU/L POD and sodium azide is mixed with the previous mixture and again incubated for 5 minutes at 37°C. the second absorbance A2 is read.

* *HSDA: Sodium N- (2-hydroxy-3-sulfopropyl)- 3,5-dimethoxyaniline*

Calculation:

Concentration of unknown (mg/dL) = $\frac{[(A2-A1) \text{ sample} - (A2-A1) \text{ blank}] \times \text{Conc. of calibrator}}{[(A2-A1) \text{ calibrator} - (A2-A1) \text{ blank}]}$

Whereby the concentration of the calibrator is 108 mg/dL according to the manufacturer's (DIALAB) leaflet.

2.3.7. Determination of Serum Glutamate Oxaloacetate Transaminase (SGOT)

Principle: Aspartate aminotransaminase (AST) (glutamate oxaloacetate transaminase) is a hepatic enzymes, which catalyses the interconversion of amino acids and α -ketoacids by transfer of amino groups. The enzymatic reactions for AST determination is as follows:

The enzyme AST catalyses this equilibrium reaction. The increase in oxaloacetate is determined in an indicator reaction catalyzed by malate dehydrogenase.

NADH is oxidized to NAD. The rate of photometrically determined NADH decrease is directly proportional to the rate of formation of oxaloacetate and thus the AST activity.

Procedure: 100 μ l of each sample are mixed with 1 mL of the working reagent containing 100 mM Tris buffer pH = 7.8, aspartate 500 mM, NADH 0.18 mM, malate dehydrogenase 1200 U/L and α -ketoglutarate 15 mM. After 1 minute at a temperature of 25-30 °C, the extinction decrease (ΔE) is measured against distilled

water every 1 to 3 minutes using Jenway 6501 spectrophotometer, U.K. at a wavelength of 340 nm.

Calculation:

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

2.3.8. Determination of Serum Glutamate Pyruvate Transaminase (SGPT)

Principle: Alanine aminotransferase (glutamate pyruvate transaminase), is mainly present with higher activity in the liver, although minor activity can be detected in the kidneys, heart, skeletal muscle, pancreas, spleen, and lungs. It catalyses the conversion of amino acids to the corresponding α -keto acids via the transfer of amino groups. Enzymatic reactions underlying direct determination of this enzyme activity are the following:

ALT is the enzyme which catalyses this equilibrium reaction. The pyruvate increase is measured in a subsequent indicator reaction, which is catalyzed by lactate dehydrogenase.

In the second reaction, NADH is oxidized to NAD. The rate decrease in NADH (measured photometrically) is directly proportional to the rate of formation of pyruvate, and thus the ALT activity.

Procedure: 100 μL of each sample are mixed with 1 mL of the working reagent containing 100 mM Tris buffer pH = 7.8, L- alanine 500 mM, NADH 0.18 mM, lactate dehydrogenase 1200 U/L and α - oxoglutarate 15 mM. After 1 minute at a temperature of 25-30 $^{\circ}\text{C}$, the extinction decrease (ΔE) is measured against distilled water every 1 to 3 minutes using Jenway 6501 spectrophotometer, U.K. at a wavelength of 340 nm.

Calculation:

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

2.3.9. Determination of Lactate Dehydrogenase

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

Procedure: 20 µl of each sample are mixed with 1 mL of the working reagent containing 50 mM Tris buffer pH = 7.3, L- alanine 500 mM, NADH 0.18 mM, pyruvate 0.6 mM. After 1 minute at a temperature of 25-30 °C, the extinction decrease (ΔE) is measured against distilled water every 1 to 3 minutes using Jenway 6501 spectrophotometer, U.K. at a wavelength of 340 nm.

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

2.3.10. Urea determination

Principle: The determination of urea is the most widely used test for the evaluation of kidney function. It is the final degradation product of protein and amino acid metabolism. In protein catabolism the proteins are broken down to amino acids and deaminated. The ammonia formed in this process is synthesized to urea in the liver. This is the most important catabolic pathway for eliminating excess nitrogen in the human body.

Urea is hydrolyzed by urease to form CO₂ and ammonia.

The ammonia formed then reacts with α-ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD⁺.

Procedure: 10 µl of each sample and standard are mixed with 1 ml of working reagent and incubated for at least 5 minutes at 37 °C. The decrease in absorbance of the unknown and the standard samples are measured against blank reagent at $\lambda = 580$ nm (Jenway 6105 spectrophotometer, U.K.).

Calculation:

$$\text{Concentration of the unknown (mg/dl)} = \frac{\Delta \text{ OD of unknown sample} \times n}{\Delta \text{ OD of standard}}$$

Where n is the concentration of the standard, which is 0.5 mg/dl according to the manufacturer's (bioMérieux) leaflet.

2.3.11. Determination of creatinine

Principle: In muscle metabolism, creatinine is synthesized endogenously from creatine and creatine phosphate. Under conditions of normal renal function, creatinine is excreted by glomerular filtration. Creatinine determinations are performed for the diagnosis and monitoring of acute and chronic renal disease as well as monitoring of renal dialysis.

The reaction is as follows:

In alkaline solution, creatinine forms a yellow-orange complex with picrate. The color intensity is directly proportional to the creatinine concentration and can be measured photometrically.

Procedure: 100 µl of each sample is mixed with 1 ml of working reagent at a temperature of 25-30 °C. the decrease in absorbance is read immediately within 20-80 seconds at a wavelength of 492 nm.

Calculation:

$$\text{Concentration of the unknown (mg/dl)} = \frac{\Delta \text{ OD of unknown sample} \times n}{\Delta \text{ OD of standard}}$$

Where n is the concentration of the standard, which is 1.5 mg/dl according to the manufacturer's (bioMérieux) leaflet.

2.4. Determination of serum insulin

Non-radioactive quantification of insulin in rat serum was determined using the Rat/mouse Insulin ELISA (Enzyme Linked Immuno-Sorbent Assay) kit.

The Rat insulin ELISA is a solid phase two-site enzyme immunoassay based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation, insulin in the samples reacts with horseradish peroxidase conjugated to anti-insulin biotinylated antibodies and anti-insulin antibodies bound to the microtiter wells. 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 a colorimetric endpoint results, that is read spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm. The increase in the absorbency is directly proportional to the amount of captured insulin in the unknown sample; subsequently this latter can be derived by interpolation from a reference curve generated with reference standards of known concentrations of rat insulin.

2.4.1. Sample preparation and assay procedure

2.4.1.1. Procedure:

Serum samples of control and experimental groups as well as the standards were dispensed in the appropriate wells of the microtiter assay plate. The anti-insulin –HPR conjugate is added into the wells. The plate was then incubated at room temperature for 2 hours on an orbital microtiter shaker at about 500 rpm.

Following incubation, solutions were decanted from the plates, which were tapped to remove residual fluid. Wells were then washed 6 times with diluted TBS wash buffer, 300 μ l per well per wash. Again the fluid is removed by decantation and tapping firmly against absorbent paper. Finally, 100 μ l of substrate solution were added to each well, plates were shaken for additional 15 minutes. A blue color should develop in the wells of insulin standards with intensity proportional to increasing concentrations of insulin. Sealer is removed and 100 μ l of stop solution were added after which plates were shaken to ensure complete mixing of solution in all the wells.

The blue color should turn into yellow after acidification obtained by addition of the stopping reagent and absorbance was read at 450 nm and 590 nm using the Spectra Max Plus ELISA reader, (Molecular Devices, USA).

2.4.1.2. Calculation:

A standard curve is constructed using all standard points for which absorbances are less than 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 the E-LizaMat 300 reader and the DGR ELISA regression program.

2.5. Antiinflammatory activity

Acute antiinflammation activity was assessed using carrageenan-induced inflammation (Jose et al., 2004) and chronic antiinflammatory activity using formalin induced edema (Jose et al., 2004) in mice hind paw. For this purpose male Dawely Sprague rats (n=30), weighing between 280 and 320g, were used. All animals were fed the regular rat chow diet (6.5 g per 100 g body weight) and had free access to water.

2.5.1. Carrageenan induced paw edema

Animals (n=30) were divided into 5 groups comprising 6 animals each. In all groups edema was induced by subplantar injection of 0.02 ml of 1% freshly prepared carrageenan in normal saline in the right hind paw of rats (Jose et al., 2004). One group was administered with carrageenan alone and served as a positive control. Group II was administered with the reference drug diclofenac at a dose of 10mg/kg. The three remaining groups III, IV, and V received the methanol extract of *Quercus infectoria* bark at a concentration of 250, 500 and 1000 mg/kg body weight respectively. In all groups, the drugs were injected intraperitoneally 30 minutes prior to carrageenan injection. Paw thickness was measured before and after 3 hours of carrageenan injection using a vernier caliper (Jose et al., 2004). All extracts were sterilized using syringe filter sterilization.

2.5.2. Formalin induced paw edema

Animals were divided as described for carrageenan induced paw edema. Chronic inflammation was induced by a single subplantar injection of 0.02 ml of 2% formalin in the right hind paw (Jose et al., 2004). Group I served as a positive control and was injected with formalin alone. Group II was injected with the standard reference drug diclofenac (10mg/kg body weight) while all animals received the drugs intraperitoneally 30 minutes prior to formalin injection intraperitoneally and daily for 6 consecutive days. A vernier caliper served to measure the paw thickness before and 6 days after formalin injection (Jose et al., 2004).

Increases in paw thickness in both models were calculated using the formula $P_t - P_0$. P_t represents the paw thickness at time t (ex 3 hrs after carrageenan injection and 6 days after formalin injection), whereas P_0 represents the paw thickness at 0 time. The formula $(C-T/C) \times 100$ serves to calculate the percentage inhibition, where C is the increase in paw thickness of the control and T that of the treatment (Jose et al., 2004).

2.6. Platelet aggregation activity

In order to perform this experiment, fresh blood from healthy rats was collected in anticoagulant solution (2.4% sodium citrate, 1.5% citric acid and 1.8% D-glucose). The ratio of blood to anticoagulant solution was 5:1. Blood was then centrifuged at 1900 rpm for 7 minutes to obtain the platelet rich plasma (PRP), which was then centrifuged at 4500 rpm for 18 minutes. The supernatant was discarded and the sediment was dissolved in washing buffer I containing of (mM) 113 NaCl; 4.3 K_2HPO_4 ; 4.3 Na_2HPO_4 ; 24.44 NaH_2PO_4 and 5.5 D-glucose (pH 6.5). Platelets were collected after centrifugation at 4000 rpm for 10 minutes then they were suspended in buffer II composed of (mM): 113 NaCl; 4.3 K_2HPO_4 ; 16 Na_2HPO_4 ; 8.3 NaH_2PO_4 and 5.5 D-glucose, pH 7.5. The suspension was adjusted to a final optical density of 0.5/ml at 600 nm (Jose et al., 2004).

The methanol extract of *Quercus infectoria* bark (500 μ g) was incubated with a volume of 1 ml washed platelets in siliconized tubes for 5, 10 and 20 minutes in a waterbath at 37°C. 20 μ l of 1 mM freshly prepared ADP was added at the end of the incubation period and mixed thoroughly with the washed platelets then the optical density at 600 nm was measured at 1 min intervals up to 5 minutes. A solution of washed platelets incubated with buffer II served as a control. A normal solution with

the same composition of the control one was also done but no ADP was added to the tubes while measuring the OD (Jose et al., 2004).

2.7. Effect of methanol extract of *Quercus infectoria* bark on ethanol induced gastric damage in rats

The effect of methanolic extract of *Quercus infectoria* bark on ethanol-induced gastric ulcer was performed in male Sprague-Dawley rats according to the protocol described by Alkofahi and Atta (1999). Briefly, male Sprague-Dawley rats (Lebanese American University stock), 250-300g, were randomly assigned to 6 groups of 6 rats each. Animals were housed in an ambient temperature of $23 \pm 1^{\circ}\text{C}$ and 65-70% relative humidity. Forty-eight hours before use, animals were starved to ensure an empty stomach. Furthermore, they were kept in cages with raised floors of wide wire mesh to prevent coprophagy (eating of excrement). To prevent excessive dehydration during starvation, all groups were supplied with sucrose 8% (w/v) in NaCl 0.2% (w/v) during the fasting period, which was removed 1 hour before experimentation (Alkofahi and Atta, 1999; Gharzouli, 1999). Group II, which served as a control group was given water (10ml/kg) while the treatment groups III, IV, V received respectively 100 mg/kg, 500 mg/kg of methanol extract of *Quercus infectoria* bark and 11.5 mg/kg of the reference drug Cimetidine (commercially known as Tagamet) (Xu, 1998). Doses were administrated orally via a stainless steel intubation needle. Two doses were given on the first day at 9:00 h and 17:00 h; a third dose was given on the second day 1.5 h before induction of gastric ulceration. To induce gastric ulcer, the controls as well as the treatment groups received by gastric gavage 10ml/kg body weight ethanol 50% (v/v) in distilled water. Group I served as the reference group (no ulcer induction by ethanol) and received equivolumes of water instead. One hour after ethanol administration, all animals were sacrificed by an overdose of chloroform; stomachs were rapidly removed, opened along their greater curvature and rinsed under running tap water. Using an illuminated microscope (10 x) long lesions were counted and measured along their greater length. Petechial lesions (very small lesions) were also counted and each five were taken as a 1 mm of ulcer. The sum of the total length of long ulcers and petechial lesions in each group of rats was divided by its number to calculate the ulcer index (mm). The curative ration was determined by the formula:

$$\text{Curative ratio} = \frac{(\text{Control ulcer index}) - (\text{test ulcer index}) \times 100}{(\text{Control ulcer index})}$$

2.8. Screening of antibacterial activity of methanolic extract of *Quercus infectoria* bark

Methanolic extract of *Quercus infectoria* bark was tested for its ability to inhibit the activity of 11 hospital isolates from different patients by disc diffusion method. The bacterial strains used were *Enterobacter agglomerans*, *Proteus mirabilis*, *Salmonella* spp., *Citrobacter braaki*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Haemophilus parainfluenza*, *Streptococcus pyogenes* (*S. pyogenes*), *Staphylococcus aureus* methicillin-resistant (MRSA), and *Staphylococcus aureus* methicillin-sensitive (MSSA). All the bacterial species tested were identified by API 20E except for *S. pyogenes* (API Streptococcus) and MRSA and MSSA, which were identified by *Staphylococcus* coagulase test at the “Centre Hospitalier du Nord, Zgharta- North Lebanon”.

2.8.1. Disc diffusion assay

The methanolic extract of *Quercus infectoria* bark was dissolved in sterile NaCl solution (0.85%) to a final concentration of 20 mg/ml for disc diffusion assay (Basri and Fan, 2005). Sterile 5-mm diameter filter paper disks were soaked with 20 µl of extract; thus each disc contained 400 µg of extract. Disks were then placed on the surface of Muller-Hinton agar inoculated with the appropriate bacteria tested except for *S. pyogenes* and *Haemophilus parainfluenza* that were grown on blood agar and chocolate agar respectively. All the bacteria were grown and maintained on nutrient agar. The inoculum size of each test strain was standardized by adjusting the optical density of the bacterial suspension to 0.08 (OD₆₂₀=0.08) at 620 nm that corresponds to 10⁸ bacteria / ml of suspension. All the plates were then incubated at 37°C for 24 h. the antibacterial activity was evaluated by measuring the diameter of the inhibition zone. Each experiment was performed in duplicate and the mean of the diameters of the inhibition zones was calculated. A specific reference drug was used for each type of bacteria (Table 2.2).

2.8.2. Microdilution assay

The minimal inhibitory concentration (MIC) values were studied for the microorganisms that were determined to be sensitive to the *Quercus infectoria* extract in the disc diffusion assay. The MIC was determined using the two-fold serial microdilution method with normal saline. The final concentration ranged from 20 mg/ml to 0.00976 mg/ml. The tested extracts were added to sterile NaCl into microtiter plates before the diluted bacterial suspension was added (OD₆₂₀=0.08). The bacterial suspension served as the positive control whereas the extract served as negative controls. Maxipime (500 µg/ml) was used as a reference drug for the bacteria tested. The MIC values were taken as the lowest concentrations of the extracts that showed after 24 h at 37°C. The absorbance of the microtiter plates' wells was read at 620 nm (Basri and Fan, 2005) using an E-LizaMat 300 reader and the DGR ELISA regression program.

Table 2.2. List of bacterial strains tested and reference drugs used to screen the antimicrobial activity of methanolic extract of *Quercus infectoria* bark

Bacteria tested	Reference drug
1. <i>Citrobacter braaki</i>	Gentamycin (10µg/disc)
2. <i>Enterobacter agglomerans</i>	Ceftriaxon (30 µg/disc)
3. <i>Escherichia coli</i>	Ofloxacin (5µg/disc)
4. <i>Heamophilus parainfluenza</i>	Levofloxacin (5µg/disc)
5. <i>Klebsiella pneumoniae</i>	Bactrim* (25µg/disc)
6. <i>Proteus mirabilis</i>	Ciprofloxacin (5 µg/disc)
7. <i>Pseudomonas aeruginosa</i>	Astreonam (30 µg/disc)
8. <i>Salmonella spp</i>	Bactrim* (25µg/disc)
9. <i>Staphylococcus aureus methicillin resitant (MRSA)</i>	Vancomycin (30 µg/disc)
10. <i>Staphylococcus aureus methicillin sensitive (MSSA)</i>	Vancomycin (30 µg/disc)
11. <i>Streptococcus pyogens</i>	Cefrtiaxon (30µg/disc)

* Bactrim consisted of sulfamethoxazol (23.75) + Trimethoprim (1.25)

2.9. Statistical analysis:

Values are presented as means ± S.E.M. The effect of lipid loading in each group was analyzed statistically by analysis of variance. The comparison between two groups was made by independent *t* test. An α level of 0.05 was considered significant for both the *t* test and analysis of variance.

RESULTS

After one month of administration *Quercus infectoria* bark methanolic extract, in drinking water; animals were sacrificed, blood collected and serum was used for screening tests including: glucose, TAG, total cholesterol, HDL-cholesterol, LDL-cholesterol, SGOT, SGPT, LDH, urea, creatinine, and insulin. Total apoB was assayed and obtained by density gradient ultracentrifugation of plasma samples and total apoB quantification was done by SDS-PAGE. Another group of animals (n=30) served to determine the effect of different doses of methanolic extract of *Quercus infectoria* bark on acute and chronic induced inflammation. Effect of the extract protection against ethanol-induced gastric ulcer, platelet aggregation and antibacterial activity were also assayed.

3.1. Effects of methanolic extract of *Quercus infectoria* bark on serum lipid, glycemia, and liver enzymes

3.1.1. Serum lipid profile

Table 3.1. summarizes the mean serum concentration of total cholesterol, TAG, LDL-cholesterol, and HDL-cholesterol in control and treatment group respectively in mg/dL.

3.1.1.1. Total cholesterol

Circulating cholesterol levels didn't show any significant difference between control and treatment groups (Table 3.1.)

3.1.1.2. TAG

As it can be inferred from the results, levels of circulating TAG didn't show any significant difference between control and treatment groups (Table 3.1.)

3.1.1.3. LDL- cholesterol

Results also revealed no significant difference between control and treatment groups concerning LDL-cholesterol (Table 3.1.)

3.1.1.4. HDL-cholesterol

HDL-cholesterol levels reveal a slight significant increase in the treatment group administered with the methanolic extract of *Quercus infectoria* bark over the period of one month by 12% ($p=0.0063$) as compared to the control group (Table 3.1.)

3.1.1.5. Cholesterol/HDL and LDL/HDL ratios

Fig 3.1. shows ratios of total and LDL cholesterol to HDL-cholesterol respectively. There is a very slight decrease in LDL/HDL ratio in the experimental group with respect to the control. Whereas, Cholesterol/HDL ratios were almost the same in both groups.

3.1.1.6. Total apoB concentrations

There was no significant difference in total apoB concentrations among the control and the treatment groups (Table 3.1.).

Figure 3.1. Total cholesterol/HDL cholesterol, and LDL/HDL cholesterol ratios after one month administration of *Quercus infectoria* bark methanolic extract in rats fed a rat chow diet supplemented with 5% coconut oil. Bars denote mean $\pm$ SEM ($n=15$).

Table 3.1. Cholesterol (mg/dl), Triglycerides (mg/dl), LDL-Cholesterol (mg/dl), HDL-Cholesterol (mg/dl), and total apoB obtained after density gradient ultracentrifugation after one month of supplementation with methanolic extract of *Quercus infectoria* bark in control and treatment groups. Values are expressed as mean $\pm$ SEM (n=15).

Parameter	Control (n=15)	Treatment (n=15)
Total cholesterol	57 $\pm$ 1.68	62.7 $\pm$ 1.68
TAG	81.4 $\pm$ 6.27	93.8 $\pm$ 8.59
LDL- Cholesterol	17.5 $\pm$ 1.44	17.6 $\pm$ 1.6
HDL- Cholesterol	23.5 $\pm$ 0.6*	26.4 $\pm$ 0.77*
Total ApoB (n=6)	7.262 $\pm$ 0.75	6.772 $\pm$ 0.78

* Significant difference (< 0.05) with respect to the control

3.1.2. Glycemic profile

3.1.2.1. Glucose

Blood sugar levels in the control and test animals were subjected to statistical treatment (Student *t* test) to determine any significant difference in blood glucose levels between the two groups.

The mean fasting serum concentrations of glucose as determined following one month administration of *Quercus infectoria* bark methanolic extract in rats fed a rat chow diet supplemented with 5% coconut oil showed a significant increase by 25.4% ($p=0.000293$) in the treatment group compared to the control one. (Figure 3.2.a.)

3.1.2.2. Serum insulin levels

The mean fasting serum concentrations of insulin as determined following one month administration of *Quercus infectoria* bark methanolic extract in rats fed a rat chow diet supplemented with 5% coconut oil showed a significant change ($p=0.014738$) between controls and experimental groups (Figure 3.2.b)

Figure 3.2. a. Serum glucose concentrations (mg/dl) in control and experimental groups following one month administration of *Quercus infectoria* bark methanolic extract in rats fed a rat chow diet supplemented with 5% coconut oil. **b.** Serum insulin concentrations (ng/ml) in control and experimental groups following one month administration of *Quercus infectoria* bark methanolic extract in rats fed a rat chow diet supplemented with 5% coconut oil. Bars denote mean $\pm$ SEM (n=15).

* Significant difference with respect to the control

3.1.3. Liver enzymes, urea and creatinine

Table 3.2. summarizes serum activities of SGOT, SGPT, and LDH in both control and treatment groups as well as urea and creatinine levels in both groups. SGOT and SGPT levels do not show any difference between experimental groups. Whereas, a significant decrease in LDH activity by 50% is shown in the treatment group compared to the control one. As it is shown in table 3.2., levels of both urea and creatinine were the same in both control and treatment groups.

Table 3.2. SGOT, SGPT and LDH activities, urea (mg/dl) and creatinine (mg/dl) in serum obtained after density gradient ultracentrifugation after one month of supplementation with methanolic extract of *Quercus infectoria* bark in control and treatment groups. Values are expressed as mean $\pm$ SEM (n=15).

Parameter	Control (n=15)	Treatment (n=15)
SGOT	60.237 $\pm$ 2.68	66.408 $\pm$ 4.64
SGPT	21.933 $\pm$ 1.27	20.200 $\pm$ 1.8
LDH	286.117 $\pm$ 58.08*	141.429 $\pm$ 20.61*
Urea	4.3 $\pm$ 1.5	33.1 $\pm$ 1.9
Creatinine	0.47 $\pm$ 0.01	0.48 $\pm$ 0.02

* Significant difference (< 0.05) with respect to the control

3.2. Antiinflammatory activity

The methanol extract of *Quercus infectoria* bark significantly inhibited the acute inflammation induced by carrageenan and chronic inflammation induced by formalin at concentrations of 250, 500 and 1000 mg/kg body weight in experimental animals (Table 3.3.). In Group III, one animal died and was excluded from data statistical analysis (Figure 3.3. and Figure 3.4.)

Table 3.3. Effect of methanol extract of *Q. infectoria* bark on carrageenan induced acute and formalin induced chronic inflammation

Treatment	Dose (mg/kg)	Carrageenan		Formalin	
		Increase in paw thickness after 3 h	Inhibition (%)	Increase in paw thickness after 6 days	Inhibition (%)
Control		1.916 ± 0.585	—	1.695 ± 0.722	—
<i>Q. infectoria</i> extract	250	0.533 ± 0.408 ^a	72	0.576 ± 0.425 ^a	66
	500	0.716 ± 0.248 ^a	62	0.895 ± 0.607 ^a	47
	1000	0.667 ± 0.408 ^a	65	0.9 ± 0.271 ^a	47
Diclofenac	10	0.95 ± 0.235 ^a	50	1.096 ± 0.498 ^a	35

Values are represented as mean ± SD *n*=6,^a *p* <0. 001 significant with respect to the control.

Figure 3.3. Diclofenac (10 mg/kg BW), 250, 500 and 1000 mg/kg BW of methanolic extract of *Quercus infectoria* bark and their percent inflammatory inhibition in carrageenan induced paw-edema in the rat model. Bars denote mean $\pm$ SEM (n=6).
a: significant difference with respect to diclofenac.

Figure 3.4. Diclofenac (10 mg/kg BW), 250, 500 and 1000 mg/kg BW of methanolic extract of *Quercus infectoria* bark and their percent inflammatory inhibition in formalin induced paw-edema in the rat model. Bars denote mean $\pm$ SEM (n=6).

a: significant difference with respect to diclofenac.

3.3. Platelet aggregation activity

Addition of adenosine diphosphate (ADP) to washed human platelet is manifested by a marked decrease in OD at 600 nm as ADP is an inducer of aggregation.

The methanol extract of *Quercus infectoria* bark (500 µg/ml), preincubated with washed platelets, showed a marked stimulation of platelet aggregation mediated by ADP as shown in figure 3.6. This stimulation increased with increase in incubation time.

Figure 3.5. Platelet aggregation inducing activity of methanol extract of *Quercus infectoria* bark when incubated with washed rat platelets. ADP (1mM) was used as the inducer of aggregation.

Extract 5: absorbance of the extract after 5 minutes incubation with washed rat platelets.

Extract 10: absorbance of the extract after 10 minutes incubation with washed rat platelets.

Extract 20: absorbance of the extract after 20 minutes incubation with washed rat platelets.

3.4. Gastroprotective effect of *Quercus infectoria* against ethanol-induced gastric ulcer:

Ethanol-induced gastric damage was characterized by the presence of elongated and petechial lesions confined in most cases to the glandular region. The number of long ulcers and the ulcer index were highly significant ($P < 0.001$) when compared to normal group (Table 3.4.). Repeated oral administration of 100 mg/kg and 500 mg/kg

of methanol extract of *Quercus infectoria* bark significantly lowered the severity of gastric damage.

Table 3.4. Effect of methanolic extract of *Quercus infectoria* bark on ethanol-induced gastric damage in rats

Treatment groups	Nb of animals	Ulcer index (mm)	Curative ratio (%)
Refrence (Group I)	6	0	0
Control (Group II)	5	43.7	0
Extract (100 mg/kg) (Group III)	5	19.1	56
Extract (500 mg/kg) (Group IV)	6	14.16	67.6
Cimetidine (11.5 mg/kg) (Group V)	5	27.21	37.7

Nb: number of animals

3.5. Antibacterial activity

The methanolic extract of *Quercus infectoria* bark was tested against 11 bacterial hospital isolates and showed antibacterial activities against 4 bacterial strains: *Proteus mirabilis*, *Citrobacter braaki*, MRSA and MSSA. The diameters of the inhibition zones produced by the extract on the 4 strains are listed in table 3.5.

The MIC values were determined for the 3 strains (*Proteus mirabilis*, MRSA and MSSA) shown to be sensitive to the methanolic extract of *Q. infectoria* bark. Based on the results obtained, the MIC value for *Proteus mirabilis* was 5 mg/ml. MIC values for MSSR and MSSA were 1.25 mg/ml.

Table 3.5. Antimicrobial activity of methanolic extract of *Quercus infectoria* bark against the bacterial strains based on disc diffusion method.

Bacteria tested	Zone of inhibition (mm)	Reference drug	Zone of inhibition (mm)
<i>Citrobacter braaki</i>	10	Gentamycin (10 µg/disc)	20
<i>Enterobacter agglomerans</i>	0	Ceftriaxon (30 µg/disc)	28
<i>Escherichia coli</i>	0	Ofloxacin (5µg/disc)	32
<i>Heamophilus parainfluenza</i>	0	Levofloxacin (5µg/disc)	24
<i>Klebsiella pneumoniae</i>	0	Bactrim (25 µg/disc)	21
<i>Proteus mirabilis</i>	10	Ciprofloxacin (5 µg/disc)	32
<i>Pseudomonas aeruginosa</i>	0	Astreonam (30 µg/disc)	24
<i>Salmonella spp.</i>	0	Bactrim (25 µg/disc)	29
MRSA	12	Vancomycin (30 µg/disc)	17
MSSA	12	Vancomycin (30 µg/disc)	17
<i>Streptococcus pyogens</i>	0	Ceftriaxon (30µg/disc)	31

NB: each sterile disc contains 400 µg of *Quercus infectoria* bark methanolic extract.

a

b

Figure 3.6. a. Diameter of the inhibition zone produced by methanolic extract of *Quercus infectoria* bark (OE) on MRSA. b. Diameter of the inhibition zone produced by methanolic extract of *Quercus infectoria* bark (OE) on MSSA. Each disc contains 400µg of the extract.

DISCUSSION AND CONCLUSIONS

Quercus infectoria has been extensively studied pharmacologically, but a great importance was accorded to its galls, which exhibit astringent, anti-inflammatory (Kaur et al., 2004), antiviral (Dar et al., 1976), antidiabetic (Hwang et al., 2000), larvicidal (Redwane et al., 2002), antibacterial (Voravuthikunchai et al., 2005; Basri and Fan, 2005; Pithayanukul et al., 2005; Voravuthikunchai et al., 2004; Hwang et al., 2004; Hussein et al., 2000), antiulcerogenic and gastroprotective activities (Sawangjaroen et al., 2004). A search of the literature on *Quercus infectoria* indicates that the bark of this species has never been subjected to investigation. This study was undertaken to evaluate potent medicinal effects of the methanolic extract of *Q. infectoria* bark and changes it produces on blood lipid profile, liver enzymes and glucose and serum insulin after one month administration of the extract via drinking water, and to monitor its anti-inflammatory, gastroprotective and antiulcerogenic activities using *in vivo* models in male Sprague-Dawley rats, as well as its potential antibacterial effect on 11 isolates of different bacterial species and its platelet aggregation activity.

Lipid and lipoprotein abnormalities are associated with development and progression of coronary heart disease (CHD) (Skoumas et al., 2003). To better understand the effect of methanolic extract of *Quercus infectoria* bark upon blood lipid profile five parameters- total cholesterol, plasma triglycerides, HDL-cholesterol, LDL-cholesterol and cholesterol/HDL and LDL/HDL ratios- were reported.

Based on the results obtained, methanolic extract of *Quercus infectoria* bark appeared not to have a significant effect on blood lipid profile with the exception of HDL-cholesterol. TAG, total cholesterol, LDL-cholesterol and total apoB concentrations were relatively unchanged by the chronic intake of the extract. Plasma apolipoprotein B (apoB) is a better marker of the risk of CHD due to the atherogenic lipoproteins, very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) (Sniderman, 2001). However, the extract interestingly increased significantly HDL-cholesterol level, thereby conferring some cardioprotective effect with chronic extract intake.

This result is of importance as HDL-cholesterol or “Good cholesterol” is the type of lipoprotein that removes cholesterol from the peripheral tissues. This increase in HDL-cholesterol concentration could be probably related to the presence of fibers in the bark of *Quercus infectoria*. This result is consistent with that reported by Chai et al. (2003), who monitored the effects of manufactured soluble dietary fiber from *Quercus mongolica* on blood lipid profile in rats fed high cholesterol diet. They showed that HDL-cholesterol levels were significantly higher in rats fed fiber from bark of *Quercus mongolica*. The improvement in HDL-cholesterol suggests that the extraction of soluble dietary fiber from bark or wood of *Quercus infectoria* could provide an inexpensive source of natural dietary fiber that could be used for treating CHD through increasing HDL- cholesterol.

In folk medicine, the galls of *Quercus infectoria* have been largely used for treating inflammatory diseases. In order to shed light on potential effects of our extract, the anti-inflammatory activity of methanol extract of *Quercus infectoria* bark was investigated using in vivo models of acute and chronic inflammation induced by carrageenan and formalin respectively.

The extract showed a significant reduction in inflammation induced in hind-paw of male Sprague-Dawely rats. It is interesting to note that the lowest dose used (250 mg/kg) produced the highest percentage of inhibition, 66% compared with doses of 500, and 1000mg/kg that produced about 47% inhibition, and diclofenac (reference drug) which produced 35% inhibition of chronic inflammation induced by formalin. The 250 mg/kg dose appeared to be the optimum dose used and increasing the dose beyond 250 mg/kg is no more useful and may reduce the anti-inflammatory effect of the extract. Further studies using doses of the extract smaller than 250 mg must be performed in order to locate better the appropriate dose, which may be even much less than 250 mg/kg body weight. It is important to note that the extract effect was substantially more potent than the reference drug diclofenac, which raise promising effect of the extract on inflammation. The absence of a dose response effect may be due to the fact that no further significant effect could be observed. It could also be due to the complex composition of the extracts. As suggested by Khennouf et al. (2003), it is possible that some compounds present in the extract may antagonize the effect of some other compounds (e.g., tannins). Furthermore, an assessment of percentage inhibition was monitored using the acute inflammation induced by carrageenan.

Carrageenan-induced paw edema is a useful model to assess the contribution of mediators involved in vascular changes associated with acute inflammation (Kaur et al., 2004). Again the lowest dose of the extract used (250 mg/kg) produced 72 % inhibition compared with doses of 500 and 1000 mg/kg of the extract and 10 mg/kg of diclofenac which produced 62, 65 and 50 % inhibition respectively. The significant inhibition by methanol extract of hind paw inflammation suggests that the extract contains compounds which possess analgesic and/or anti-inflammatory activity.

It is known that all *Quercus* species contain tannins in variable percentage according to the part of plant considered: leaves, bark and galls (Mammela et al., 2000; Ikram and Nowshad, 1977; Dar, et al., 1976; Culberth et al., 1927). Texl (1960) has previously reported that tannins possess an anti-inflammatory effect. A very recent study done by Erdelyi et al. (2005) also demonstrated that tannins, which are plant-derived, water-soluble polyphenols, have a wide range of biological activities among which anti-inflammatory activity manifested by the inhibition of transcription of nuclear factor kappa B (NFKappaB). This latter induces the transcriptional up-regulation of various inflammatory response such as interleukins and tumor necrosis factor alpha (TNF α) (Choi et al. 2003). In addition, the mechanism underlying the anti-inflammatory effect of tannins include the scavenging of radicals (antioxidant effect) and inhibition of expression of the mediators of the inflammatory response including cytokines, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (Erdelyi et al., 2005).

Moreover, Oliveira et al. (2004), have shown that resin possess anti-inflammatory activity. However, resin did not modify the hind-paw edema induced by carrageenan, but inhibits effect on collagen formation but no acute edema (Oliveira et al., 2004). In correlation with this study, resin present in the bark of *Quercus infectoria* besides tannins (Culberth et al., 1927), may have contributed to the anti-inflammatory activity of the extract.

The effect of methanolic extract of *Quercus infectoria* bark on platelets aggregation was studied. According to the results obtained, the extract induced a time- dependant aggregation of washed rat platelets in the presence of ADP, a potent activator of platelets aggregation (Jose et al., 2004). This activity could be probably related to the presence of tannins and ellagitannins in the extract. Previous reports have shown that these two compounds stimulate platelet aggregation (Teng et al., 1997; Lauque et al.,

1991). Lauque et al. (1991) showed that tannin induced dose-dependant synthesis of phosphatidic acid, early and transient hydrolysis of phosphatidylinositol monophosphate and bisphosphate, transient synthesis of diacylglycerol, and release of arachidonic acid metabolites. All these metabolites are known to be involved in the process of aggregation. Tannin also induced a reversible rise of intracellular Ca^{2+} due to a mobilization of the internal stores and an influx of extracellular Ca^{2+} (Teng et al., 1997). These results suggest that tannin, as thrombin, activates human platelets by phospholipase C and A2 activations, diacylglycerol release, and intracellular free Ca^{2+} mobilization. Thus, tannins can act as ADP receptor agonist in rat platelets (Teng et al., 1997). The results obtained supported the use of tannins in folk medicine in the healing of bleeding wounds. This finding suggested that further work is needed in order to extract the active compounds responsible for this the platelet aggregation activity and for the use of this extract as a topical cream for stopping wound bleeding.

Plant extracts are some of the most attractive sources of new drugs, and have been shown to produce promising results for the treatment of gastric ulcer (Toma et al., 2005; Alkofahi and Atta, 1999). In an attempt to investigate the possible anti-ulcerogenic and gastroprotective effect methanolic extract of *Quercus infectoria* bark, an ethanol-induced gastric damage was carried out. Ethanol (50%) induced long and petechial lesions in a relatively short time, which makes this technique useful to evaluate anti-ulcer drugs.

In this study, pre-treatment over a 2 days period via oral administration of methanolic extract of *Quercus infectoria* bark resulted in a dose- dependant protection against gastric lesions produced by 50% ethanol. Ethanol is known to be a corrosive substance for the gastric mucosa. The protection was by 56% (dose of 100 mg/kg) and 67.6% (dose of 500 mg/kg) respectively compared with the group that have received cimetidine as a reference drug and which produced 37.7% protection. Cimetidine was tested for its protection of gastric mucosa from ethanol-induced injury and the rate of protection was close to 33%, a value consistent with the data concerning cimetidine (Xu et al., 1998). Alkofahi et al. (1999) tested the ability of *Q. aegilops* and *Q. coccifera* as well as other plants to prevent ethanol-induced gastric damage and pointed out that all of the effective plants extract contain tannins and /or flavonoids to which the anti-ulcerogenic effect could be attributed (Zayachkivska et al., 2005; Alkofahi and Atta, 1999). These results are consistent with those obtained by

Khennouf et al. (2003), which reveal that tannins from leaves of *Quercus suber* and *Quercus coccifera* given orally prevented the formation of ethanol-induced gastric lesions (the percent protection varied from 68 to 91 %). Khennouf et al. (2003) showed that the gastroprotective effects of methanolic extract of *Quercus infectoria* bark are related to their anti-lipoperoxidant properties because tannins from both extracts strongly inhibited the lipid peroxidation of rabbit brain homogenate.

In a correlation with another study that tested the gastroprotective effect of other species of the genus *Quercus* (Gharzouli et al., 1999; Khennouf et al., 1999), it could be inferred that protection of the gastric mucosa against hemorrhagic lesions produced by 50% ethanol was mainly due to the presence of tannic acid in these species. Moreover it is generally known that polyphenols such as tannic acid, quercetin and ellagic acid present in *Quercus infectoria* bark, are able to inhibit the proton pump present in the parietal cells (Gharzouli et al., 1999; Khennouf et al., 1999). Khennouf et al. (2003) showed later on that this inhibition might be related to the inhibition of the acid secretion before administration of ethanol, thus tannins participate by this means in protecting the stomach against damaging agents. This property was also proved by different authors whose findings suggested that tannins extracted from *S. cumini* exhibited gastroprotective and anti-ulcerogenic effects (Ramirez, 2003).

Furthermore, Tannins are used in medicine primarily because of their astringent properties (Mammela et al., 2000; Ikram, 1977; Dar et al., 1976), which are due to the fact that they react with the proteins of the layers of tissue with which they come into contact. Tannins are known to 'tan' the outermost layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation (Borrelli and Izzo, 2000).

Bark of *Quercus infectoria* is also known to contain resin, generally used in folk medicine to treat inflammatory conditions and to hasten wound repair (Culberth, 1927), and reported to have a protective effect in gastrointestinal disorders (Oliveira et al., 2004). So resin in *Q. infectoria* wood could be somehow related to the gastroprotective effect even it is present in small concentrations.

Toma et al. (2005), who tested the preventive effect of 3 extracts from *Mammea Americana* (L.) bark, showed that the ethanol-induced ulcers are not inhibited by antisecretory agents such as cimetidine, but by agents that enhance mucosal defensive factors such as endogenous prostaglandins and mucus synthesis. This refers to as the cytoprotective effect.

Further experiments are required to isolate the active ingredients causing the gastroprotection observed in rats. This can be done by mainly separating different tannins and resin present in the oak bark and then identifying the potential benefit of each compound separately and elucidating the underlying mechanisms involved.

The use of plants extracts is enjoying great popularity especially in developing countries due to the fact that herbal products are relatively safe and effective. Although herbal remedies are generally perceived as harmless, it is not possible to establish absolute safety for medicinal plants preparations based solely on epidemiological studies (Farnsworth, 1993). The liver is essential for metabolism of drugs and exogenous toxins. Therefore, reports of hepatotoxicity must be included in studies conducted on medicinal herbs (Grunhage et al., 2003). Drug-induced hepatitis is the inflammation and hepatocellular damage of the liver caused by a drug or combinations of drugs or possibly by some food supplements or herbal therapies (Pak et al., 2004; Janbaz et al., 2004). To test the effect the methanol extract of *Quercus infectoria* bark, the assessment of hepatoprotective activity was evaluated by measuring the activities of transaminases (GOT and GPT), and lactate dehydrogenase (LDH) in serum of rats after administration of methanol extract of *Q. infectoria* in drinking water over a one month period.

Methanol extract of *Quercus infectoria* bark has no significant effect on transaminases GOT and GPT, indicating that this extract shows no toxicity on liver cells. On the other hand, the level of LDH was significantly decreased in the extract group with respect to the control. Since LDH is an enzyme present in almost all body cells and released in the bloodstream whenever cells are damaged, indicates that extract didn't affect adversely most body cells, but it may possibly resulted in a protective effect (Braunwald et al., 2001).

Medicinal plants from various parts in the world can provide a rich source of antibacterial activities and many plant species have been used widely to cure infectious diseases. Such plants are available locally, are inexpensive and have become increasingly popular (Basri and Fan, 2005; Güllüce et al., 2004). In the present study, methanolic extract of *Quercus infectoria* bark was screened against 11 hospital isolates of different bacterial strains. The extract was shown to have a potential antibacterial effect against 4 bacterial species including *Proteus mirabilis*,

Citrobacter braaki, MRSA and MSSA. The diameters of the inhibition zones ranged from 10 to 12 mm respectively. The MIC value, defined as the lowest concentration of the compounds to inhibit the growth of microorganisms, was also determined for *Proteus mirabilis*, MRSA and MSSA and ranged from 5 mg/ml for *Proteus mirabilis* to 1.25 mg/ml for MRSA and MSSA.

The results obtained are consistent with those of Basri and Fan (2005), who showed that water and acetone extracts from galls of *Quercus infectoria* have a high potential antibacterial activity. The antimicrobial activity seems to depend on the presence of tannins in the galls of *Quercus infectoria*. This implies that tannins might be the active compounds that may be responsible for the antibacterial activity (Basri and Fan, 2005). A very recent study conducted by Voravuthikunchai et al. (2005) also showed that ethanolic extract of *Quercus infectoria* was active against hospital isolates of methicillin-resistant *Staphylococcus aureus*. The antimicrobial action of tannins may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins (Cowan, 1999).

In conclusion, the potential antimicrobial effect of methanolic extract of *Quercus infectoria* bark is due the fact that bark of this plant is known to possess high amounts of tannins, and the antimicrobial properties are well established (Voravuthikunchai et al., 2005). This finding provides an insight into the usage of this extract in treatment of wounds or burns associated with bacterial infections. Furthermore, as suggested by Voravuthikunchai et al. (2005) the extract can be effective in the form of a nasal ointment or body wash, but much more experimental work is needed. However, further studies are needed to purify the active compounds responsible for the antibacterial effect.

The effect of methanolic extract of *Quercus infectoria* bark upon glycemic profile was studied. In the present study, the extract appeared to increase significantly blood glucose level at the end of the study period. This increase in blood glucose level was accompanied with an increase in serum insulin concentrations. The concomitant increase of serum insulin and glucose levels may raise the possibility of insulin resistance syndrome. Such finding raises several questions about the feasibility of using this extract as a herbal supplement. However, further purification of the extract into its simple components may allow the removal of the compounds responsible for

hyperglycemia and hyperinsulinemia. Then, it would be safer to use the purified extract in treatment of inflammation, ulcer, and other possible conditions.

Previous studies by Hwang et al. (2000) have shown that the methanol extract of galls of *Quercus infectoria* significantly inhibited α -glycosidases such as sucrase, maltase and isomaltase, thus exhibiting a hypoglycemic activity. However, such a finding is not in contradiction with the results obtained since the present study was conducted on bark rather than gall extract and it reports the glucose and insulin values in the fasted state and not the postprandial state. For instance, it may be possible that the extract inhibits sugar digestion thereby delaying absorption and consequently lowering postprandial glycemia. At the same time the extract may also have an inhibitory effect on insulin sensitivity when taken chronically leading to hyperglycemia and hypereinsulinemia. Anderson and Polansky (2002) reported that some polyphenols present in tea can increase insulin activity in epididymal fat cells *in vitro*. High performance liquid chromatography showed that some of the insulin-potentiating activity was due to the presence of tannins in tea as well as other active compounds. Other *in vitro* studies on rat adipocytes (Hattori et al., 2003) showed insulin-potentiating activity with lagerstroemin that belongs to ellagitannins, a class of plant polyphenols, which have been reported to bind several polypeptides such as the regulatory subunit of protein kinase A. A possible explanation for the insulin-like actions of lagerstroemin is that the tannin binds the extracellular part of the insulin receptors in a manner causing their activation (Hattori et al., 2003). Although these data may contradict with the hyperinsulinemia and hyperglycemia observed in the present study, one should not forget that the discrepancies in the results may be attributed to the fact that the extract used in the present study was crude, chronically given to rats, and most importantly *in vivo* studies may completely give different results from *in vitro* studies because of the complexity of the organism studied. In addition, the concentration of the active ingredient used in the *in vitro* studies may also be very much different from their concentration in the crude extract used and last but not least the interaction between the different compounds present in the crude methanolic extract may modify the action of certain active ingredients.

Further experiments are needed in order to evaluate the effect of the extract upon insulin secretion and insulin resistance. For instance, pancreatic perfusion experiments with and without the extract may clarify the reason behind the high serum insulin concentration observed. Also, other studies on insulin sensitivity may also be

necessary to clarify the mechanism of action of the extract on serum blood glucose level. However, it is more important to purify the extract into its active components and assess the effect of each compound separately *in vivo*.

Briefly, the crude methanolic extract of *Quercus infectoria* displayed various important and promising biological activities in the rat model. Although the different activities observed may be the result of action of different active ingredients, however the present study constitutes “essential database” for future studies where further purification and assessment of the activity of each component may be done taking into consideration the preliminary results obtained in the present investigation.

The importance of the extract with respect to cardiovascular diseases was manifested by a significant increase in HDL-cholesterol levels after one month of chronic intake of the extract. Furthermore the extract showed both anti-inflammatory and anti-ulcerogenic activities that appeared to be greater than current reference drugs used in the market. Substances responsible for the anti-inflammatory activities, such as tannins, appeared to have an antiulcerogenic effect at the same time. Such compounds are of particular therapeutic importance as most of the anti-inflammatory drugs used in modern medicine are ulcerogenic. In addition, the extract was shown to stimulate the aggregation of platelets *in vitro*, which make it useful for use as a topical agent for curbing of wounds bleeding. Moreover, the extract was screened for its ability to inhibit certain bacterial species and was shown to inhibit four bacterial strains from hospital patients isolates. This result sheds light on the use of the methanolic extract of *Quercus infectoria* in the treatment of certain infectious diseases. Finally, chronic intake of the extract resulted in concomitant increase in both plasma glucose and insulin levels, thus raising questions about the use of the crude extract as herbal supplement. Further studies are needed to fractionate the extract into its active components, where removal of ingredients responsible for hyperglycemia and hyperinsulinemia seems to be important in order to avoid undesirable secondary effects of the extract.

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