THE EFFECTS OF SALVIA OFFICINALIS
LEAVES ON HYPERLIPIDEMIA, GLYCEMIA,
ULCER, INFLAMMATION AND
BACTERICIDAL ACTIVITY

by

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Iman H. Alayan
Abstract

*Salvia Officinalis* plants, commonly known as Sage, possess a wide range of biological activities. Although many studies were conducted on *S. Officinalis*, the research interest in *S. Officinalis* plant is still justifiable because of its reputed medicinal value. The purpose of this study is to evaluate the efficacy of the aqueous extract of *S. Officinalis* leaves on blood lipid profile, glycemia, liver enzymes, gastric ulcer, inflammation and bactericidal activity.

After one month of water extract intake (42, 167 and 420 mg/kg body weight) in drinking water in concomitance with a high fat diet, rats showed an increase in serum HDL cholesterol levels, but significance was reached at the highest dose only. This was accompanied with a small but important decrease in LDL cholesterol when the 167 and 420 mg/kg body weight doses were used. The extract also exhibited a dose dependent decrease in serum glucose level, where significance was attained at the highest dose. However, serum insulin was not significantly different from the control group. Extract intake did not appear to affect stool triglyceride and cholesterol content except for a significant decrease in cholesterol observed with the highest dose. At all doses, the extract, reduced significantly the water content in the stools of rats. Assessment of liver enzyme activities assured that the extract has no hepatotoxic effects over the study period.

*S. Officinalis* leaves aqueous extract (100 and 500 mg/Kg body weight) also exhibited a significant anti-ulcerogenic effect against ethanol-induced gastric ulcer, an inhibition
comparable to that exerted by cimetidine, a proton pump inhibitor drug. Also, the extract (100, 250 and 500 mg/kg body weight) showed a significant anti-inflammatory effect in cases of both acute and chronic inflammation induced by carrageenan and formalin respectively. No anti-bacterial effect was observed with the different concentrations used. In conclusion, *S. Officinalis* leaves aqueous extract has a beneficial impact upon lipemia, glycemia, and inflammation, and it plays a minor protective role against gastric ulcer. The extract can also be used as a remedy for the treatment of diarrhea.
Acknowledgments

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Finally, I would like to thank my family for their support and without whom this study couldn’t have been done.
Glossary

ACAT: Acetyl-CoA-cholesterol Acetyltransferase
ADP: Adenosine Diphosphate
AST: Aspartate Aminotransferase
CAD: Coronary Artery Disease
CE: Cholesteryl Esters
CETP: Cholesterol Ester Transfer Protein
CHE: Cholesterol Esterase
CHOD: Cholesterol Oxidase
CM: Chylomicron
ELISA: Enzyme Linked Immuno-sorbant Assay
FA: Fatty Acids
FFA: Free Fatty Acids
GK: Glucose Kinase
GOD: Glucose Oxidase
GPO: Glycerol-3-Phosphate Oxidase
HDL: High Density Lipoprotein
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
MDH: Malate Dehydrogenase
MRASA: Methicillin resistant Staphylococcus aureus
MSSA: Methicillin sensitive Staphylococcus aureus
NADH: Nicotinamide adenine dinucleotide
POD: Peroxidase
SGPT: Serum- Glutamic oxaloacetic- Transaminase
SGOT: Serum- Glutamic Pyruvic- Transaminase
TAG: Triglyceride
VLDL: Very Low Density Lipoprotein
Chapter I

INTRODUCTION AND LITERATURE REVIEW

During the past decades, there has been an increasing interest in the use of traditional medicine. According to the World Health Organization WHO traditional medicine refers to "approaches, health practices, knowledge and beliefs involving mineral, animal, plant based medicines, spiritual therapies, manual techniques, and exercises applied alone or in combination to treat, diagnose and prevent illnesses or maintain well-being". Nowadays, industrialized countries refer to traditional medicine as "Complementary" or "Alternative" medicine (Spot light on traditional med., 2006). Herbal remedies and alternative medicine is used throughout the world where herbs often represented the original source of most drugs. The plant kingdom has provided endless sources of medicinal plants. Plants were first used in their crude forms as food additives, then as a remedy for many diseases starting from very mild to serious illness like heart diseases, high blood pressure, asthma, cancer and others. Stimulated by their safeness, effectiveness, low cost and their mild toxic reactions, the consumption of herbal drugs is rapidly increasing (Saad et al., 2005).

About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants (Nascimento et al., 2000). In addition, the global market for herbal medicines currently stands at over 60 billion dollars annually and is growing steadily (Spot light on traditional med., 2006).

Traditional herbal medicine has always been adopted, despite the significant improvement in modern medicine. Cultural beliefs and practices often lead to home remedies and self-care in rural areas and consultation of traditional physicians. These alternative therapies have been
strongly adopted by Arabs due to their faith in spiritual healers. The Mediterranean region has a very rich tradition in the use of medicinal plants for treating various ailments. According to recent surveys, the Middle Eastern region has more than 2600 plant species of which more than 700 are recorded for their use as medicinal herbs or as botanical pesticides. Currently, fewer than 200 to 250 plant species are still used as traditional medicine by Arabs for various diseases treatments (Saad et al., 2005).

1.1 Plant Taxonomy and Distribution

*S. Officinalis* or as known in Arabic “Ase’en”, “Merameya” or “Ayzaan” belongs to the genus “Lamiaceae” and the family “Salvia”. The pharmaceutical name is “Salviae Folium”. Other names are broad-leaved sage, common sage, Dalmatian sage, garden sage (Newall et al., 1996). The word salvia comes from the Latin word “Salvare” meaning “to cure” (Chevallier, 1996). This name “Salvare” was changed into sauge in France and sawge in old English, and in the long run to sage (Newall et al., 1996).

*S. Officinalis*, native to the Mediterranean rim is now cultivated all around the world especially in Albania, Turkey, Greece, Italy, France, the United Kingdom, and the United States (Wichtl and Bisset, 1994; Bruneton, 1995; Budavari, 1996; Leung and Foster, 1996). The material of commerce comes mainly from southeastern European countries, such as Albania and Yugoslavia (Wichtl and Bisset, 1994; British Herbal Pharm., 1996). Its cultivation in northern Europe dates back to medieval times, and it was introduced to North America during the seventeenth century (Bown, 1995).

This species was used in ancient Egyptian, Roman medicine, India, Germany, Greek, China, the United States (Newall et al., 1996) and in South Africa (Kamatou et al., 2005) as a curable herb for different diseases.
1.2 Plant description and constituents

The medicinal parts of *S. Officinalis* are the dried leaves, the fresh flowering parts, the fresh leaves and the oil removed from both the flowers and the stems (Medical Economics Company, 1998)

*S. Officinalis* as shown in figure 1.1 (Isererre, 2006; Sauge, 2006; Mary H., 2006) is a shrubby plant that grows as a bush up to 60 cm high. The stem is erect and woody at the base with leafy, quadrangular, white-gray tomentose branches. The leaves are tangy, aromatic, astringent, simple, and oblong, narrowed at the base, tough and evergreen. Flowers are of medium size, colored in violet, pink or white and arranged in 6 to 12 false coils above each others in 4 to 8 rows (Medical Economics Company, 1998; Bown, 1995).

Constituents of *S. Officinalis* plant are shown in tables 1.1, 1.2 and in table 1.3 (Medical Economics Company, 1998; Newall et al., 1996; Capasso et al., 2003; Duke, 1985).

![Salvia officinalis](image)

Fig 1.1 “Salvia officinalis” plant (English Country Garden, 2006)
Table 1.1: Volatile oils of *S. Officinalis* and their percentages. (Medical Economics Company, 1998; Newall et al., 1996; Capasso et al., 2003; Duke, 1985).

<table>
<thead>
<tr>
<th>Volatile Oils</th>
<th>Percentages</th>
<th>Volatile Oils</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-thujone</td>
<td>1.5-3.5%</td>
<td>Cis-ocimene</td>
<td>9.3%</td>
</tr>
<tr>
<td>Beta-thujone</td>
<td>20-60%</td>
<td>Gamma terpinene</td>
<td>0.5%</td>
</tr>
<tr>
<td>1,8-cineole</td>
<td>6-16%</td>
<td>Terpinolene</td>
<td>0.4%</td>
</tr>
<tr>
<td>Camphor</td>
<td>14-37%</td>
<td>P.cymene</td>
<td>0.8%</td>
</tr>
<tr>
<td>Linalool</td>
<td>-</td>
<td>Bornyl acetate</td>
<td>1.9%</td>
</tr>
<tr>
<td>Camphene</td>
<td>-</td>
<td>Terpinen-4-ol</td>
<td>4%</td>
</tr>
<tr>
<td>Isobutyl acetate</td>
<td>-</td>
<td>Alloaromadendrene</td>
<td>0.3%</td>
</tr>
<tr>
<td>Borneol</td>
<td>-</td>
<td>Caryophyllene</td>
<td>0.4%</td>
</tr>
<tr>
<td>Alpha and beta pinene</td>
<td>-</td>
<td>Gamma and delta cadinene</td>
<td>0.1%</td>
</tr>
<tr>
<td>Alpha and beta caryophyllene</td>
<td>-</td>
<td>Sabinol</td>
<td>3.4%</td>
</tr>
<tr>
<td>Viridiflorol</td>
<td>-</td>
<td>Alpha-terpineol</td>
<td>3.4%</td>
</tr>
<tr>
<td>Cis-2-methyl-3-methylenhept-5-ene</td>
<td>0.1%</td>
<td>Sabinyl acetate</td>
<td>4.4%</td>
</tr>
<tr>
<td>Methyl isovalerate</td>
<td>-</td>
<td>Alpha humulene</td>
<td>4.4%</td>
</tr>
<tr>
<td>Myrcene</td>
<td>-</td>
<td>Caryophyllene oxide</td>
<td>1.1%</td>
</tr>
<tr>
<td>Limonene</td>
<td>2.2%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2: Minerals and Vitamins found in the whole plant of *S. Officinalis*. (Medical Economics Company, 1998; Newall et al., 1996; Capasso et al., 2003; Duke, 1985).

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td>91 mg Phosphorus</td>
<td>5900 IU Vitamin A</td>
</tr>
<tr>
<td>428 mg Magnesium</td>
<td>0.34 mg Riboflavin (vitamin B2)</td>
</tr>
<tr>
<td>1652 mg Calcium</td>
<td>0.75 mg Thiamine (vitamin B1)</td>
</tr>
<tr>
<td>28.1 mg Iron</td>
<td>5.7 mg Niacin (vitamin B3)</td>
</tr>
<tr>
<td>1070 mg Potassium</td>
<td>32 mg Ascorbic acid (Vitamin C)</td>
</tr>
<tr>
<td>11 mg Sodium</td>
<td></td>
</tr>
<tr>
<td>4.7 mg Zinc</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.3: Other components of *S. Officinalis and their derivatives*. (Medical Economics Company, 1998; Newall et al., 1996; Capasso et al., 2003; Duke, 1985).

<table>
<thead>
<tr>
<th>Other Components</th>
<th>Their Derivatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>Chlorogenic acid + 3-6% rosmarin acid.</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Apigenin</td>
</tr>
<tr>
<td>Luteolin-7-glucosides</td>
<td>Genkwanin + genkwanin-6-methylrther</td>
</tr>
<tr>
<td>Methoxylated aglycones</td>
<td>5% ursolic acid</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>Carnosolic acid + rosmanol + saffricinolide</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>Genkwanin + genkwanin-6-methylrther</td>
</tr>
</tbody>
</table>

Each 100g of the dried herb of *S. Officinalis* contains 315 calories, 8% water, 12.7 g fat of which 7.03 g saturated fatty acids, 1.87 g monounsaturated fatty acids, 1.76 g polyunsaturated fatty acids, 10.6 g protein. Leaves contain 3% tannin, malic acid, oxalic acid, fumaric acid,
saponins, pentoses, a wax, KNO3 and picrosalvin. While the seeds contain 18% protein, 14.2% oleic acid, 34.7% linolenic, 29.2% linoleic and 12% saturated acids (Duke, 1985).

Toxins known to be found in *S. Officinalis* are Cineole, histamine, linalool, borneol, hexanol, limonene, tannic acid, saponin, thujone and camphor (Duke, 1985).

1.3. **Plant Uses and overdose side effects**

There are about 900 *salvia* species around the world (Paton, 1999). *Salvia* family “Lamiaceae” is considered to be an important genus in folk medicine making members of this genus a popular option for researchers (Kamatou et al., 2005). One of these famous family members is “*S. Officinalis*” that has been widely used in folk medicine for the treatment of several disorders such as gastric problems including loss of appetite, bloating, diarrhea, enteritis and flatulence. Other uses of *S. Officinalis* are for treating bleeding gums, skin inflammation, Laryngitis, pharyngitis and firming the gums (Medical Economics Company, 1998). Moreover, *S. Officinalis* stops sudation as in patients suffering from tuberculosis, helps both calm and stimulate the nervous system, treats sore throat, mouth ulcers, asthma, rheumatoid, heart diseases, sexual debility, female sterility, anxiety and depression, encourages a better menstrual blood flow, helps the body adapt to the hormonal changes, lessens sweating, reduces excessive salivation as in Parkinson’s disease, improves liver function and treats irregular periods and symptoms of menopause (Watt and Breyer-Brandwijk, 1962; Chevallier, 1996; Bown, 1995; Baricevic and Bartol, 2000; Guerrera, 2004).

It is also thought that *S. Officinalis* has antiseptic, anti-inflammatory and anti-microbial effects (Bown, 1995). Furthermore, strong *S. Officinalis* tea can dry up the mummeries when a mother wants to wean (Duke, 1985).

On the other hand, there exist medically proved uses of *S. Officinalis* leaves such as their internal use for dyspeptic symptoms and external use for inflammation of the mucous
membranes of the nose and throat. Also, the European scientific cooperative on phytotherapy (ESCOP, 1997) indicates *S. Officinalis* use for stomatitis, gingivitis, pharyngitis, and hyperhidrosis. Whereas the German standard license for *S. Officinalis* infusion indicates its use for pressure spots caused by prostheses and for the treatment of gastrointestinal catarrh (ESCOP, 1997; Newall et al., 1996; Wichtl and Bisset, 1984).

In addition, leaves of *Salvia Officinalis* are generally known for their anti-bacterial (Miski et al., 1983; Nascimento et al., 2000; Abu-Shanab et al., 2004; Pereira et al., 2004), anti-tumor (Liu, 2000), hypoglycemic (Perfumi, 1991; Jouad et al., 2001; Alarcon-Aguilar et al., 2002; Verspohl, 2002; Eidi, 2005), anti-oxidative (Wang et al., 2003), anti-cholestatic (Oh et al., 2002), anti-viral (Monolova et al., 1995) and anti-inflammatory effects (Baricevic and Bartol, 2000). Other studies done on *Sage* oil proved its hypotensive properties, anti-spasmodic effect and central nervous system-depressant activities (Newall et al., 1996).

*S. Officinalis* is contraindicated during pregnancy since it contains alpha and beta thujones that are considered to be abortifacient (Newall et al., 1996). Moreover it is not recommended in epileptic patients (Bown, 1995). *S. Officinalis* may also interfere with hypoglycemic therapies and anticonvulsant and it may potentiate other sedatives (Duke, 2002). Overdose symptoms may include a sense of heat, feeling of vertigo, dizziness, epileptiform convulsions and tachycardia following an extended intake of ethanolic extract or an over dose of *S. Officinalis* leaves i.e. more than 15g (Medical Economics Company, 1998).

1.4. Gastric Ulcer

Gastric mucosal layers acts as a barrier that prevents an exposure of the gastric mucosal cells to different injurious, luminal agents and irritants whether from internal or external sources. Mucosal surface epithelium is a matter is assaulted by microbiological, chemical or physical agents acting from the gastric lumen, which are implicated in multiple diseases, such as peptic
ulcer, gastritis, or gastric cancer (Zayachivska et al., 2005). Ulcer is defined as disruptions of the mucosal integrity of the stomach or the duodenum leading to a local defect due to active inflammation and may lead to hemorrhage and cancer in severe cases (Braunwald et al., 2001). In the United States, acid peptic disorders are affecting around 4 million individuals (Braunwald et al., 2001). Ulcers of the stomach are areas of mucosal ulceration extending to a variable of less than 1 mm degree into the submucosal tissues (Hodgson et al., 2000). The majority of Gastric ulcer can be attributed to either the bacterium *Helicobacter pylori* or Non-steroidal anti-inflammatory Drugs (NSAID)-induced mucosal damage (Braunwald et al., 2001). Stress, smoking and nutritional deficiencies may also contribute to the occurrence of Gastric ulcer (Belaiche et al., 2002). Moreover, Gastric ulcer may be caused by an imbalance between pepsin and acid of the stomach (Baron et al., 1980). On the other hand, lysolecithine, bile acids and pancreatic enzymes may injure the gastric mucosa; however no definite role for these factors has been established in the gastric acid pathogenicity (Braunwald et al., 2001). Although there exist several medications for the treatment of gastric ulcer such as anti-histamines and anti-acids, most of these medications have side effects including impotence, hematopoietic changes and gynecomastia (Arapaho et al., 1986). Thus, there is a demand for anti-ulcer agents with reduced toxicity and more efficacies (Alkofahi and Atta, 1999). In traditional medicine, several plants and herbs extract have been widely used to treat gastric ulcer. So, this is a vital motivation to investigate anti-ulcer outcome of medicinal plants that were traditionally used in folk medicine for gastric disease (Kushima et al., 2005).
1.5. Lipids

1.5.1. Introduction

Lipids are biological molecules that are insoluble in aqueous solutions and soluble in organic solvents. The lipids of physiological importance for humans have four major functions: They serve as structural components of biological membranes, provide energy reserves mainly in the form of triacylglycerols, and serve as vitamins, hormones and lipophilic bile acids. Types of lipids include: fatty acids (saturated and unsaturated), glycerides such as triglycerides, complex lipids such as lipoprotein and glycolipids, nonglycerides such as sphingolipids, steroids and waxes (Vance and Vance, 2004).

1.5.2. Triglycerides

Triglycerides are the main storage form of lipids that constitute about 95% of fatty tissues. Triglycerides consist of one molecule of glycerol bounded to three molecules of fatty acids (Loeb, 1994). They are found in the bloodstream with normal blood levels between 40 to 160 mg/dl in adult men and 35 to 135 mg/dl in adult women. Elevations of the triglyceride level (particularly in association with elevated cholesterol) have been correlated with the development of atherosclerosis, the underlying cause of heart disease and stroke. High blood levels of triglycerides result from different conditions found mainly in carbohydrate-sensitive people whose bodies have trouble breaking down fats or carbohydrates, in obese people, diabetic patients especially those having type II diabetes or in heavily alcoholic people (Loeb, 1994).

1.5.3. Cholesterol

Cholesterol is a structural component in cell membranes and plasma lipoproteins. It is absorbed from the diet and synthesized in the liver and other body tissues. It is then
metabolized to steroid hormones including mineral corticoids and glucocorticoids produced in the adrenal cortex and sex steroid hormones synthesized in the female and male gonads: estrogen and testosterone. Cholesterol is also a substrate for the formation of bile acids in the liver cells. It is also considered as an essential nutrient contributing for the formation of vitamin D (Ger and Van der Vusse, 2004). High serum cholesterol levels may be associated with an increased risk of coronary artery disease (CAD) (Loeb, 1994).

1.5.4. lipoproteins

Lipoproteins can be described as inner core of hydrophobic lipids (triglycerides and cholesteryl esters) within a membrane of proteins (apoprotein) associated with free cholesterol and phospholipids. So, lipoproteins are soluble complexes of proteins and lipids that function mainly in transporting lipids throughout the body. They are synthesized in the intestines, liver, arise from metabolic changes of precursor lipoproteins, or are assembled at the cell membranes from cellular lipids and exogenous lipoproteins or apolipoproteins. In the circulation, lipoproteins undergo enzymatic reactions of their lipid components, transfer of soluble apolipoproteins, and conformational changes of the apolipoprotein in response to the compositional changes. In the final stages, lipoproteins are taken up and metabolized in the kidney, liver, and peripheral tissues (Vance and Vance, 2004). Table 1.4 illustrates the compositions of major lipoproteins that are classified into five types according to their density and size as follows:

**Chylomicrons (CM):** the lowest density lipoprotein, consist mostly of triglycerides and are in a form in which long chain fats and cholesterol are transported from the intestine to the blood then to the liver, fat cells and skeletal muscles where the enzyme lipoprotein lipase will break down the chylomicron molecules to become chylomicron remnants (Loeb, 1994; Cooper,
1997). They are assembled in the intestinal mucosa as a means to transport dietary cholesterol and triacylglycerols to the rest of the body. Chylomicrons are, therefore, the molecules formed to mobilize dietary lipids. The predominant lipids of chylomicrons are triacylglycerols. The apolipoproteins that predominate before the chylomicrons enter the circulation include apoB-48 and apoA-I, -A-II and IV. ApoB-48 combines only with chylomicrons (Vance and Vance, 2004).

**Very Low Density Lipoprotein (VLDL):** consist mainly of triglycerides, and smaller amounts of phospholipids, cholesterol, and protein (Loeb, 1994). Triacylglycerols are packaged into VLDLs and released into the circulation for delivery to the various tissues mainly muscle and adipose tissue for storage or production of energy through oxidation. VLDLs are, therefore, the molecules formed to transport endogenously derived triacylglycerols to extra-hepatic tissues. Moreover, VLDLs contain some cholesterol and cholesteryl esters and the apoproteins, apoB-100, apoC-I, apoC-II, apoC-III and apoE. Newly released VLDLs acquire apocS and apoE from circulating HDLs. The fatty acid portion of VLDLs is released to adipose tissue and muscle through the action of lipoprotein lipase (Vance and Vance, 2004).

**Intermediate Density Lipoprotein (IDL):** are short-lived and contain almost equal amounts of cholesterol and triglycerides, and smaller amounts of phospholipids and protein. They are converted to LDL by lipoprotein lipase enzyme (Loeb, 1994). IDLs are formed as triacylglycerols are removed from VLDLs. The destiny of IDLs is either conversion to LDLs or direct uptake by the liver. Transformation of IDLs to LDLs occurs as more triacylglycerols are isolated. The liver takes up IDLs after they have interacted with the LDL receptor to form a complex, which is endocytosed by the cell. LDL receptors in the liver require the presence of both apoB-100 and apoE to recognize IDLs (Ger and Van der Vusse, 2004).
**Low Density Lipoprotein (LDL):** often called "bad cholesterol" are about half cholesterol and half protein, triglycerides and phospholipids (Loeb, 1994). 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, chylomicrons and LDLs. The dietary cholesterol that goes into chylomicrons is supplied to the liver by the interaction of chylomicron remnants with the remnant receptor. In addition, cholesterol synthesized by the liver can be transported to extra-hepatic tissues if packaged in VLDLs. In the circulation, VLDLs are converted to LDLs through the action of lipoprotein lipase. LDLs are the primary plasma carriers of cholesterol for delivery to all tissues. 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. The interaction of LDLs with LDL receptors requires the presence of apoB-100 (Vance and Vance, 2004; Ger and Van der Vusse, 2004).

**High Density Lipoprotein (HDL):** referred to as "good cholesterol", are about half protein and half phospholipids, triglycerides and cholesterol (Loeb, 1994). HDLs are synthesized de novo in the liver and small intestine. The newly formed HDLs are nearly devoid of any cholesterol and cholesteryl esters. The primary apoproteins of HDLs are apoA-I, apoC-I, apoC-II and apoE. The major function of HDLs is to act as circulating stores of apoC-I, apoC-II and apoE. HDLs are converted into spherical lipoprotein particles through the accumulation of cholesteryl esters. This accumulation converts nascent HDLs to HDL₂ and HDL₃. Any free cholesterol present in chylomicron remnants and VLDL remnants (IDLs) can be esterified through the action of the HDL-associated enzyme, lecithin-cholesterol acyltransferase (LCA). Cholesterol-rich HDLs return to the liver, where they are endocytosed. Hepatic uptake of
HDLs, or reverse cholesterol transport, 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 also acquire cholesterol by extracting it from cell surface membranes. 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 has the added effect of allowing the excess cellular cholesterol to be returned to the liver through the LDL-receptor pathway as well as the HDL-receptor pathway (Vance and Vance, 2004).
Table 1.4: Composition of the Major Lipoprotein Complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Source</th>
<th>Density (g/ml)</th>
<th>% Protein</th>
<th>%TG*</th>
<th>%PL*</th>
<th>%CE*</th>
<th>%C*</th>
<th>%F FA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicron</td>
<td>Intestine</td>
<td>&lt;0.95</td>
<td>1-2</td>
<td>85-88</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>VLDL</td>
<td>Liver</td>
<td>0.95-1.006</td>
<td>7-10</td>
<td>50-55</td>
<td>18-20</td>
<td>12-15</td>
<td>8-10</td>
<td>1</td>
</tr>
<tr>
<td>IDL</td>
<td>VLDL</td>
<td>1.006-1.019</td>
<td>10-12</td>
<td>25-30</td>
<td>25-27</td>
<td>32-35</td>
<td>8-10</td>
<td>1</td>
</tr>
<tr>
<td>LDL</td>
<td>VLDL</td>
<td>1.019-1.063</td>
<td>20-22</td>
<td>10-15</td>
<td>20-28</td>
<td>37-48</td>
<td>8-10</td>
<td>1</td>
</tr>
<tr>
<td>HDL$_2$</td>
<td>Intestine, liver (Chylomicrons and VLDLs)</td>
<td>1.063-1.125</td>
<td>33-35</td>
<td>5-15</td>
<td>32-43</td>
<td>20-30</td>
<td>5-10</td>
<td>0</td>
</tr>
<tr>
<td>HDL$_3$</td>
<td>Intestine, liver (Chylomicrons and VLDLs)</td>
<td>1.125-1.21</td>
<td>55-57</td>
<td>3-13</td>
<td>26-46</td>
<td>15-30</td>
<td>2-6</td>
<td>6</td>
</tr>
<tr>
<td>Albumin-FFA</td>
<td>Adipose tissue</td>
<td>&gt;1.281</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

* TG: Triglycerides; PL: Phospholipids; CE: Cholesteryl esters; C: Cholesterol; FFA: Free Fatty Acid

1.6. Glycemia

Diabetes Mellitus is a chronic metabolic disease. There exist two types of diabetes: type 1 diabetes (insulin dependent diabetes mellitus or IDDM) that is due to an autoimmune mediated destruction of pancreatic β cell islets, and type 2 diabetes (non-insulin dependent diabetes mellitus or NIDDM) that is caused by low insulin secretion from the pancreas and
low insulin action. The prevalence of diabetes is greatly increasing all over the world. There are an estimated 143 million people in the world with diabetes and this number will probably doubled by the year 2030 (Boyle et al., 2001). Diabetes is associated with high rates of morbidity and mortality secondary to the other serious complications such as myocardial infarction, renal disease, retinopathy, atherosclerosis, neuropathy, stroke, etc...

Diabetes type 1 is treated with consecutive insulin injections depending on the patient's blood glucose level. While diabetes type 2 is treated with several anti-diabetic medicines that improves insulin resistance. In both types of diabetes, a well controlled blood glucose level is considered imperative to either prevent or delay the progression of the disease complications.

In case of diabetes type 2, many medications given nowadays have certain limitations such as high rates of secondary failure and adverse effects (Inzucchi, 2002). Thus, many diabetic patients and healthcare professionals are looking for other alternatives such as the use of herbal medicine specially those herbs that are thought to have anti-hyperglycemic effect.

1.7. Liver Toxicity Assessment

The liver is located in the upper right part of the abdomen. It functions in storing glycogen, helping to process fats and proteins from digested food; making proteins that are essential for blood to clot (clotting factors), processing many medicines, helping to remove poisons and toxins from the body and making bile. As the liver performs its various functions it makes a number of chemicals which pass into the bloodstream and bile. Some of these chemicals may produce substances that harm the liver cells and result in impaired liver functioning. These chemicals can be measured in a blood sample. These tests which are commonly done on a blood sample are called liver function tests 'LFTs' which are mainly ALT, AST, Bilirubin, total protein and albumin (Braunwald et al., 2001).
Aspartate Aminotransferase (AST) or (GOT): is one of two enzymes that catalyze the conversion of the nitrogenous portion of an amino acid to an amino acid residue. It is essential to energy production in the Krebs cycle. AST is found in the cytoplasm and mitochondria of many cells, mainly in the liver, heart, skeletal muscle, pancreas, kidneys and to a lesser extent in the RBCs (Loeb, 1994).

Alanine Aminotransferase (ALT) or (GPT): is the second enzyme that catalyzes a reversible amino group transfer reaction in the Krebs cycle. ALT is primarily found in hepatocellular cytoplasm, with lesser amounts in the kidneys, heart, and skeletal muscles, and it is a relatively specific indicator of acute hepatocellular damage. When such damage occurs, ALT is released from the cytoplasm into the bloodstream, resulting in high serum levels that persist from days to weeks (Loeb, 1994).

Lactate Dehydrogenase (LD): catalyzes the reversible conversion of muscle lactic acid into pyruvic acid. LD is almost found in all cells of the body. It has five isoenzymes LD₁, LD₂, LD₃, LD₄ and LD₅. LD₁ and LD₂ are found primarily in the heart, RBCs and the kidneys. LD₃ is mainly present in the lungs. LD₄ and LD₅ are found in the liver and skeletal muscle. The specificity of LD isoenzymes and their distribution pattern is useful in diagnosing hepatic, pulmonary and erythrocytic damage (Loeb, 1994). Herbal medicines may sometimes cause toxic or adverse effects on the liver. So, liver function tests must be examined before approving any plant extract usage for medical purposes.
1.8. Antimicrobial Effect

Although pharmacological trading companies have created many developed antibiotics in the last three decades, bacterial resistance to these drugs has improved. Generally, bacteria have the genetic capability to acquire and transmit resistance to antibiotics that are used for therapeutic purposes (Cohon et al., 1992). This resistance makes worries, because of the new strains of bacteria which are multi resistant and due to hospitalized patients who have suppressed immunity. Thus, new infections can occur in hospitals resulting in high mortality rates. (Nascimento et al., 2000)

The issue of bacterial resistance is growing and the viewpoint for the use of antimicrobial drugs in the future remains unconfident. Therefore, actions must be taken to lessen this problem. This can be solved in different alternatives for example by doing more research to understand the genetic mechanisms of resistance, by controlling the use of antibiotics, and by continuing studies to develop new drugs either synthetic or natural (Nascimento et al., 2000).

One alternative used is medicinal herbs. The antimicrobial properties of plants have been investigated by a number of researcher’s world wide. New compounds inhibiting microorganisms such as emetine and benzoin have been isolated from plants (Cox, 1994). The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used anti-microbiotics and may have significant clinical value in treatment of resistant microbial strains (Eloff, 1988).

1.9. Anti-inflammatory effect

Inflammation is the response of our body to an infection. Initiation of an inflammatory response begins with an increase in blood flow to infected tissues, opening of the blood vessels or capillaries, followed by emigration of cells into the infected tissues. Signs of
inflammation are manifestation of increased blood flow and infiltration of tissues by inflammatory proteins and cells. Increased blood flow causes redness and increased temperature. The presence of fluid and red blood cells in tissues is recognized by swelling or edema and redness. White blood cells infiltration causes a white color. The cellular progression of an inflammatory response eventually results in the healing or scarring of the lesion (Sell, 2001).

There exist two types of inflammation: acute and chronic inflammation. In acute inflammation, there are four signs of acute inflammation that are redness, swelling, heat and pain. Cells of the acute inflammation are complement, kinin, coagulation, and mast cells. On the other hand, chronic inflammation follows acute inflammation if the acute response is not adequate to stop the infection. Chronic inflammation functions to clear the tissue of necrotic debris provide more powerful defensive weapons against persistent infections and complete the process of wound healing. The cells involved in inflammation response are lymphocytes, macrophages and plasma cells (Sell, 2001).

Inflammatory diseases are associated with the release of reactive oxygen, cytokines and nitrogen species (Conner and Grisham, 1996). Because of the significance of reactive oxygen species in the inflammatory process, numerous natural and synthetic antioxidants derived from plant extracts have been considered as potential therapeutic mediators (Sell, 2001).

1.10. Purpose of the Project

*S. Officinalis* is considered in the folk medicine as one of the medicinal herbs and it is widely used in the Arab countries especially in the Middle East. Although some studies have been done on *S. Officinalis*; however, most of theses studies targeted the oil extracted from the plant and not the plant itself. The present study investigates the effects of the leaves of *S. Officinalis* water extract upon:
• Lipid Profile including: TAG, total cholesterol, LDL-Cholesterol and HDL-cholesterol.

• Liver toxicity by measuring the liver enzymes: GOT, GPT and LDH.

• Glycemia involving both Insulin and Glucose levels.

• Gastric Ulcer.

• Inflammation: both acute and chronic inflammation.

• Anti-microbial activity.
Chapter II

Materials and Methods

2.1. Plant Material

*S. Officinalis* plant was collected from different areas throughout Lebanon between July and September 2005. The plant was identified according to the characteristics described in “Handbook of Medicinal Herbs” book (Duke et al., 2002). Fresh *S. Officinalis* plants were collected from wild bushes, leaves separated, and dried in shade at room temperatures. The dried leaves were then chopped into small pieces and used for the preparation of water extracts.

2.2. Determination of Water Extract Dry Weight

In order to determine the mass of dry water extract per gram of leaves, 3 g of *S. Officinalis* leaves were simmered in 50 ml of pre-boiled distilled water for 30 minutes with occasional stirring. The solution was then filtered using #1 whatman filter paper. The filtrates were then subjected to evaporation and the dry weight of the extract was determined. Results have shown that the dry water extract yield is 8.33% (w/w). All experimental protocols performed in this study were based on this methodology and yield.

2.3. Animals Preparation

Male Sprague-Dawely rats from the Lebanese American University Stock were used. The rats were housed in a controlled environment (21±1°C, 40-60% humidity and 20 air changes per hour with a 12-h light: 12-h dark cycle) with free access to standard rat chow food (Laboratory rodent starter diet no. 1, Hawa chicken Co., Lebanon) and water.
2.4. Animals Treatment

Male Sprague-Dawely rats were divided into four groups each containing ten rats with an average weight of 180 to 240 g. Food was composed of standard rat chow diet to which 5% coconut oil was added. Coconut oil was added to the diet to increase its atherogenicity (Daher et al., 2003). One group served as a control while the other three groups "the treatment groups" received the same food as the control group in addition to the leaves of *S. Officinalis* water extract in drinking water (42mg/kg body weight, G I; 167 mg/kg body weight, G II; and 420 mg/kg body weight, G III). These doses used were based on the fact that a rat consumed 100ml of drinking water per 100 g body weight (Waynfthor and Flecknel, 1992). After one month period of extract intake, fresh feces were collected from the treatment groups as well as the control group then the animals were sacrificed using diethyl ether and blood samples were collected.

2.5. Serum Assays

2.5.1. Samples Preparation and Experimental Protocols

Rats were fasted for 18 hours before being sacrificed then 4 ml of venous blood were drawn from the inferior vena cava using a 10 ml sterile syringe. Blood samples were put in glass tubes for 30 to 45 minutes at room temperature to be able to clot followed by centrifugation (20 minutes at 3000 rpm at 10°C). Serum samples were isolated, divided into eppendorf tubes and kept on ice to be used for the assessment of lipid profile (*Total cholesterol, HDL cholesterol, LDL cholesterol*, and *Triglycerides*), liver enzymes activities (*SGPT, SGOT, ALP* and *LDH*) and glycemic profile (*Glucose and Insulin*).
2.5.2. Determination of Total Cholesterol

*Principle:* Cholesterol is determined enzymatically based on Cholesterol esterase (CHE), Cholesterol oxidase (CHOD) and peroxidase (POD) according to the following reactions:

- Cholesterol esters + H₂O → \( \text{CHE} \) Cholesterol + Fatty acids
- Cholesterol + O₂ → \( \text{CHOD} \) 4-Cholestenona + H₂O₂
- 2 H₂O₂ + Phenol + 4-Aminophenazone → \( \text{POD} \) Quinonimine + 4 H₂O

The resulted Hydrogen peroxide forms a red dye. This color intensity is proportional to the cholesterol concentration in the sample that can be determined using the spectrophotometer at a wavelength of \( \lambda = 505 \) nm.

*Procedure:* 10μl of each sample and standard, which were run in duplicates, are mixed with 1ml of the working reagent (buffer plus enzymes) and incubated for 5 min at 37°. The absorbance (A) of the samples and the standard are measured against the reagent blank at \( \lambda = 505 \) nm (Jenway 6105 spectrophotometer, U.K).

*Calculation:*

- Concentration of cholesterol in the sample (mg/dl) = \( \left( \frac{(A \text{ Sample}}{(A \text{ Standard})} \right) \times 200 \text{(standard concentration)} \).

* Concentration of the standard= 200 mg/dl according to the manufacturer’s leaflet (SPINREACT).

2.5.3. Determination of HDL-Cholesterol

*Principle:* Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the serum are precipitated by phosphotungstate in the presence of magnesium ions. Thus, they
can be removed by centrifugation whereas high density lipoprotein (HDL) remained in the supernatant. Determination of HDL cholesterol is done using the clear supernatant.

**Procedure:** 0.5 ml of the serum sample is mixed with 50 µl of the precipitating reagent (14 mmole/L phosphotungstic acid with 2 mmole/L magnesium chloride). Then, samples were incubated for 10 minutes at room temperature, followed by centrifugation at 12000 rpm for 4 minutes. Absorbance of the supernatant fraction is measured by spectrophotometry at \( \lambda = 505 \) nm (Jenway 6105 spectrophotometer, U.K).

**Calculation:**

- Absorbance of the sample x 320 HDL = HDL Cholesterol (mg/dl) in the sample.

**2.5.4. Determination of LDL-Cholesterol**

**Principle:** Direct determination of LDL-cholesterol takes benefit of the selective micellar solubilization of LDL-cholesterol by a non-ionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and Chylomicrons). When a detergent is integrated in the enzymatic method for cholesterol determination (Cholesterol esterase CE, Cholesterol oxidase CHO coupling reaction), the relative reactivates of cholesterol in the lipoprotein fraction increase in the following manner: HDL< Chylomicron< VLDL< LDL. In the presence of magnesium ions, a sugar compound obviously reduces the enzymatic reaction for the cholesterol measurement in VLDL and Chylomicron. The combination of a sugar compound with detergent allows the selective determination of LDL- cholesterol in serum.

- LDL- cholesterol esters + H₂O \( \xrightarrow{CE \text{ detergent}} \) Cholesterol + Free Fatty acids
- LDL- cholesterol + O₂ \( \xrightarrow{CHO} \Delta^4 \) cholesterol + H₂O₂
- \( 2H₂O₂ + 4\)-amino-antipyrine + HSDA + H⁺ + H₂O \( \xrightarrow{peroxidase} \) Purple-blue pigment + 5H₂O
The color intensity formed is proportional to the LDL-cholesterol concentration and is determined by the spectrophotometer at a wavelength of λ = 505 nm.

**Procedure:** 10 µl of the serum sample or calibrator is mixed with 900 µl of 25 mM Good’s buffer pH: 6.8, 5000 U/L CHE, 5000 U/L CHO, 1000 KU/L catalase and 0.64 mM H-HSDA (sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline) incubated for 5 minutes at 37°C. The absorbance A1 is read at 650 nm against the reagent blank. Now to this mixture, 300 µl of 25 mM Good’s buffer pH: 7, 3.4 mM 4- aminoantipyrine, 20 KU/L POD and sodium azide are added then incubated for 5 minutes at 37°C. After that the second absorbance A2 is read (Jenway 6105 spectrophotometer, U.K).

**Calculation:**
- Concentration of the sample (mg/dl) = \((A2-A1)\) sample - \((A2-A1)\) blank \times \text{Conc. of calibrator} / \((A2-A1)\) sample - \((A2-A1)\) blank).

*The concentration of the calibrator is 108 mg/dl according to the manufacturer’s leaflet (SPINREACT).*

### 2.5.5. Determination of Triglycerides

**Principle:** Triglyceride in serum is determined in accordance to the following enzymatic reactions:

- Triglycerides + H₂O $\xrightarrow{\text{LPL}}$ glycerol + free fatty acids
- Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ G3P + ADP
- G3P + O₂ $\xrightarrow{\text{GPO}}$ DAP + H₂O₂
- H₂O₂ + 4-AP + p- chlorophenol $\xrightarrow{\text{POD}}$ Quinone + H₂O

The intensity of the ended red dye formed is proportional to the concentration of triglycerides in the sample and can be determined by spectrophotometry at a wavelength = 505 nm.
*LPL: lipoprotein lipase; G3P: Glycerol-3-phosphate; ADP: adenosine-5-diphosphate; DAP: dihydroxyacetone phosphate; 4-AP: 4-aminophenazine; GPO: phosphate dehydrogenase; POD: peroxidase.

**Procedure:** 10µl of each sample and standard, which were run in duplicates, are mixed with 1ml of the working reagent (buffer plus enzymes) and incubated for 5 min at 37°C. The absorbance (A) of the samples and the standard are measured against the reagent blank at λ= 505 nm (Jenway 6105 spectrophotometer, U.K).

**Calculation:**

- Conc. of Triglycerides (mg/dl) in the sample = \((A)\) Sample/ \((A)\) Standard \times 200 (Standard Conc.).

* Concentration of the standard = 200 mg/dl according to the manufacturer's leaflet (SPINREACT).

2.5.6. Determination of Serum GOT

**Principle:** Aspartate aminotransferase (AST) or glutamate oxaloacetate (GOT) is one of the liver enzymes that catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate. Then the oxalacetate formed will be reduced to malate by malate dehydrogenase (MDH) and NADH as follows:

- Aspartate + α-ketoglutarate $\xrightarrow{\text{AST}}$ Glutamate + Oxalacetate
- Oxalacetate + NADH +H\(^+\) $\xrightarrow{\text{MDH}}$ Malate + NAD\(^+\)

Using the spectrophotometer, the rate of decrease in concentration of NADH is measured at a wavelength = 340 nm. This decrease in the concentration of NADH is proportional to the catalytic concentration of AST found in the sample.

**Procedure:** For each 100 µl of the serum sample, 1 ml of the working reagent (100mM Tris buffer pH: 7.8, 500 mM aspartate, 0.18mM NADH, 1200U/L malate dehydrogenase and 15
mM α-ketoglutarate) is added. After one minute at a temperature of 25-30°C, the extinction decrease (ΔE) is measured against distilled water every 1 to 3 minutes by spectrophotometry (Jenway 6105 spectrophotometer, U.K).

Calculation:
- \[ \text{SGOT activity (U/L)} = \frac{\Delta E}{(\text{min})} \times 1750. \]

2.5.7. Determination of Serum GPT

Principle: Alanine aminotranferase (ALT) or glutamate pyruvate transaminase (GPT) is another liver enzyme that catalyses the conversion of amino acids to the corresponding α-keto acids through the transfer of an amino group. GPT is determined based on the following enzymatic reactions:

- \[ \text{Alanine} + \alpha\text{-ketoglutarate} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{Pyruvate} \]
- \[ \text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+ \]

Using the spectrophotometer, the rate of decrease in the concentration of NADH is measured at a wavelength = 340 nm. This decrease in the concentration of NADH is proportional to the catalytic concentration of ALT found in the sample.

* LDH: lactate dehydrogenase

Procedure: For each 100 μl of the serum sample, 1 ml of the working reagent (100mM Tris buffer pH: 7.8, 500 mM L-alanine, 0.18mM NADH, 1200U/L lactate dehydrogenase and 15 mM α-ketoglutarate) is added. After one minute at a temperature of 25-30°C, the extinction decrease (ΔE) is measured against distilled water every 1 to 3 minutes by spectrophotometry (Jenway 6105 spectrophotometer, U.K).

Calculation:
- \[ \text{SGPT activity (U/L)} = \frac{\Delta E}{(\text{min})} \times 1750. \]
2.5.8. Determination of Serum LDH

**Principle:** Lactate dehydrogenase is measured based on the following reaction:

\[
\text{P} + \text{NAD} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L} + \text{NAD}^+
\]

**Procedure:** For each 20 μl of the serum sample, 1 ml of the working reagent (50 mM Tris buffer pH: 7.3, 500 mM L-alanine, 0.18mM NADH, 0.6 mM pyruvate) is added. After one minute at a temperature of 37°C, the extension decrease (ΔE) is measured against distilled water every 1 to 3 minutes by spectrophotometry at a wavelength of 340 nm. (Jenway 6105 spectrophotometer, U.K).

**Calculation:**

- \( \text{LDH activity (U/L)} = \frac{\Delta E}{(\text{min})} \times 9690 \)

2.5.9. Determination of Serum ALP

**Principle:** Alkaline phosphatase is measured based on the following reaction:

\[
\begin{align*}
\text{p-nitrophenyl-phosphate} & \xrightarrow{\text{ALF}} \text{p-nitrophenol + phosphate} \\
\end{align*}
\]

**Procedure:** For each 20 μl of the serum sample, 1.2 ml of the working reagent (buffer: 1mmole/L diethylamine buffer pH: 10.4; 0.5 mmole/L magnesium chloride and substrate: 10mmole/L p-nitrophenyl-phosphate) is added. After one minute at a temperature of 25-30°C, the extension increase (ΔE) is measured against distilled water every 1 to 3 minutes by spectrophotometry at a wavelength of 405 nm (Jenway 6105 spectrophotometer, U.K).

**Calculation:**

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

2.5.10. Glucose Determination

**Principle:** Glucose in serum is measured based on the following enzymatic reactions:

\[
\begin{align*}
\text{Glucose + H}_2\text{O + O}_2 & \xrightarrow{\text{GOD}} \text{H}_2\text{O}_2 + \text{Glucose} \\
\end{align*}
\]
- Gluconate + phenol + 4-aminophenazon $\xrightarrow{POD}$ 4-(p-benzo-quinone-monoimino)-phenazone + 4 H$_2$O

The intensity of the red-violet dye formed at the end of the reaction is proportional to the glucose concentration and is measured by spectrophotometry at a wavelength of 505 nm.

*GOD: glucose oxidase; POD: peroxidase

Procedure: 10 µl of each sample and standard, which were run in duplicates, are mixed with 1 ml of the working reagent (buffer plus enzymes) and incubated for 10 minutes at 37°C. The absorbance (A) of the samples and the standard are measured against the reagent blank at $\lambda$ = 505 nm on the spot (Jenway 6105 spectrophotometer, U.K).

Calculation:

- Concentration of the sample (mg/dl) = \( \frac{(A) \text{ of the unknown} \times \text{Conc. of the standard}}{(A) \text{ of the standard}} \)

* Concentration of the standard = 100 mg/dl according to the manufacturer's leaflet (SPINREACT).

2.6. Determination of Serum Insulin

The rat serum insulin is determined using the Rat-insulin ELISA (Enzyme Linked Immuno-Sorbent Assay) kit. This kit is a solid phase two-site enzyme immunoassay based on the direct sandwich technique where two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. During incubation, insulin reacts with horseradish peroxidase conjugated to anti-insulin biotinylated antibodies and anti-insulin antibodies bound to the microtiter wells. Then a washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with the substrate 3, 3', 5, 5'-tetramethylbenzidine. The reaction stops when the acid is added to give colorimetric endpoint results. The increased absorbance is read by the spectrophotometer at a wavelength of 450 nm, corrected from the
absorbance at 590 nm. The increase in absorbance is proportional to the amount of captured insulin in the unknown sample, then this latter can be derived by interpolation from a reference curve created with reference standards of known concentrations of rat insulin.

2.7. Determination of Triglyceride, cholesterol and Water Contents in feces

**Principle:** Few days before scarifying the rats, feces were collected in order to determine their cholesterol, triglycerides and water contents.

**Procedure:** Fresh feces from each rat were weighed immediately, and then kept in the oven at 65°C for 24 hours for drying. To determine water content, feces were reweighed after being dried. Then, feces of different rats were crushed and 0.2 g samples were subjected for lipid extraction and determination of cholesterol and triglyceride content. Each 0.2 g of feces was extracted using 2 ml of N-hexane (a hydrophobic solvent) in a glass test tube by incubating the mixture for 2 hours in a water bath at 40°C with constant stirring. After centrifugation at 12000 rpm for 10 minutes, 300 µl from the supernatant were collected for cholesterol and triglycerides determination. These two latter parameters were assessed using regular cholesterol and triglyceride kits after evaporation of the hexane in a water bath at 70 to 75°C.

**Calculation:**

- % of water content in feces = \( \frac{\text{weight of the fresh feces} - \text{weight of the dried feces}}{\text{weight of the fresh feces}} \times 100 \)

- Concentration of triglycerides/cholesterol (mg/ml) = \( \frac{(A \text{ Sample})}{(A \text{ Standard})} \times 200 \text{(standard concentration)} \).

2.8. Effect of Salvia Officinalis Extract on Ethanol Induced Gastric Ulcer

The effect of aqueous extract of *S. Officinalis* on ethanol induced gastric ulcer was conducted on male Sprague-Dawley rats of an average weight of 235-240 g. The rats were kept in a
controlled environment with free access to food and water. They were fasted 2 days before ulcer induction to ensure an empty stomach. Throughout the fasting period, animals were given 8% sucrose (w/v) solution in 0.2% NaCl (w/v) to avoid excessive dehydration during fasting. This solution was removed one hour prior to treatment with either drug or plant extracts. Animals were divided into 4 groups of six rats each. Group I served as a control group and received only water (10 ml/kg body weight). Group II and Group III were the treatment groups and received the plant extract in two different dosages 100 and 500 mg/kg body weight respectively. Group IV received 10 mg/kg of the reference drug cimetid. Both cimetid and water extract were given by intra-gastric gavage using a stainless steel intubation’s needle. Extract and drug doses were given in two shots in the first day at 08:00 h and 16:00 h. Then a third shot was given in the second day 1.5 h prior to gastric ulceration. To induce stomach ulceration, ethanol 50% (v/v) in distilled water was given orally to all groups in a dose of 10 ml/kg body weight (Alkofahi and Atta, 1998). After one hour of ethanol administration, the rats were sacrificed with an over dosage of ether. Then, stomachs were removed, opened along the greater curvature and gently washed under tap water (Kushima et al., 2005). To determine the ulcerative lesions in the stomach mainly in the glandular region, a stereomicroscope was used. Long lesions were counted and measured using a ruler, while each five petechial lesions were considered as 1 mm of ulcer (Gurbuz et al., 2005).

- The ulcer index (mm) = The sum of the total length of long and petechial lesions (of the group) / number of rats per group

- The curative ratio = ((control ulcer index) – (Test ulcer index)) / (control ulcer index)) \times 100
2.9. Anti-Inflammatory Activity

To study the effect of the leaves of *S. Officinalis* water extract upon acute and chronic inflammation induced by carrageenan and formalin respectively, Sprague-Dawley male rats weighing between 230 to 300g were used. The rats were divided into five groups each of 6 rats. All animals had free access to the regular chow diet (6.5g per 100 g body weight) and water.

2.9.1. Acute Anti-Inflammatory Activity

In this experiment, five groups of 6 animals each were used. All animals were subjected to edema that was induced by injection of 0.02 ml of freshly prepared 1% Carrageenan in normal saline into the sub planter area of the right hind paw of the rat. Three groups of rats were given the water extracts of *S. Officinalis* leaves 30 min prior to inflammation induction at three different concentrations which are 250, 500 and 1000 mg/Kg body weight. One group was injected with Carrageenan alone and was considered as the positive control group and another group received diclofenac (10mg/kg body weight) as a standard reference drug one hour before the carrageenan injection. The paw thickness was measured before and three hours after carrageenan injection using the vernier calipers (Ajith and Janardhanan, 2001). Extracts were sterilized by using syringe filter sterilization.

2.9.2. Chronic Anti-Inflammatory Activity

In chronic inflammation, paw edema was induced by the injection of 0.02 ml of 2% formalin in the right hind paw of rats. Three groups of rats were given the water extracts of *S. Officinalis* leaves at three different concentrations which are 250, 500 and 1000 mg/Kg body weight. One group received diclofenac (10mg/kg body weight) as a standard reference drug intraperitoneal 30 min prior to formalin injection. The control group did not receive any
treatment. Extract or diclofenac treatment was continued once daily for 6 consecutive days. The paw thickness was measured before and six days after formalin injection using the vernier calipers (Jose et al., 2004).

2.9.3. Paw Thickness Calculations

The paw thickness increase in acute and chronic inflammation was calculated based on the following formula: \( P_t - P_0 \). Where \( P_t \) represents the paw thickness at time \( t \) (3 hours after the carrageenan injection and 6 days after the formalin injection in acute and chronic inflammation respectively) and \( P_0 \) represents the paw thickness at time 0.

To calculate the inhibition percentage, the following formula was used: \( (C - T/C) \times 100 \). Where \( C \) is the increase in paw thickness of the control group and \( T \) is the increase in paw thickness of the treatments.

2.10. The Antimicrobial Effect of Salvia Officinalis Water Extracts

The present study investigates the effect of the leaves of *S. Officinalis* water extract upon bacterial growth. The bacterial strains which were obtained from Beirut General Hospital are *Pseudomonas aureginosa*, *MRSA* (*Methicilin resistant staphylococcus aureus*), *MSSA* (*Methicilin sensitive staphylococcus aureus*), *Citrobacter freundii*, *Serratia*, *Ewingella*, *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Salmonella typhi* and *proteus mirabilis*. Each strain was identified in the hospital in a different way as listed in table 2.1.

The disc diffusion method was used to determine the inhibition of bacteria growth. Bacterial strains were grown on Muller Hinton (MH) agar plates and then resuspended in Muller Hinton broth. A pure colony of each of the tested microorganisms was taken from the MH agar, subcultured into a 5ml of nutrient broth, followed by incubation at 37°C for 5h (Barbour et al., 2004). Bacterial suspension was standardized by adjusting its turbidity to 0.5 McFarland that
has absorbance of 0.132 at 600 nm. Each bacterial suspension was spread on the Muller Hinton agar plate. On the other hand, six concentrations of aqueous extracts were prepared as shown in table 2.2.

Table 2.1: Different identification tests done on the 11 bacterial strains used in this study

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Method of Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aureginosa</em></td>
<td>Oxidase +ve; isolated at 42°</td>
</tr>
<tr>
<td><em>MRSA</em></td>
<td>Glucose +ve; Catalase +ve; Coagulase +ve; DNAse+ve; Oxacillin Resistant</td>
</tr>
<tr>
<td><em>MSSA</em></td>
<td>Glucose +ve; Catalase +ve; Coagulase +ve; DNAse+ve; Oxacillin Sensitive</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>API 20 E</td>
</tr>
<tr>
<td><em>Serratia</em></td>
<td>Phoenix</td>
</tr>
<tr>
<td><em>Ewingella</em></td>
<td>Phoenix</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Urease -ve; Indole +ve</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>API 20 E</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>API 20 E</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>API 20 E</td>
</tr>
<tr>
<td><em>proteus mirabilis</em></td>
<td>Urease +ve; Indole -ve</td>
</tr>
</tbody>
</table>
Table 2.2: The six concentrations of aqueous extracts of *Salvia Officinalis* prepared to test for its antimicrobial effect.

<table>
<thead>
<tr>
<th>Tubes #</th>
<th>Plant Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1</td>
<td>400μg/20μl</td>
</tr>
<tr>
<td>Tube 2</td>
<td>200μg/20μl</td>
</tr>
<tr>
<td>Tube 3</td>
<td>100μg/20μl</td>
</tr>
<tr>
<td>Tube 4</td>
<td>50μg/20μl</td>
</tr>
<tr>
<td>Tube 5</td>
<td>20μg/20μl</td>
</tr>
<tr>
<td>Tube 6</td>
<td>10μg/20μl</td>
</tr>
</tbody>
</table>

20 μl of the six extract solutions prepared above were dispensed on sterile 5-mm diameter filter paper disks. On each plate, a blank disc containing sterile distilled water (negative control), a reference drug disc and three water extract containing discs were placed on the surface of the agar as shown in figure 2.2. The reference drugs used for each bacterial strain are shown in table 2.3. All plates were then incubated at 37°C for 24 h after which the antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (Nascimento et al., 2000)

Fig. 2.1: The distribution of the plant extract, antibiotic and the negative control
discs on the Muller-Hinton agar.

Table 2.3: Bacterial strains and their corresponding antibiotic standards.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Reference Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em> auruginosa</td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc)</td>
</tr>
<tr>
<td><em>MRSA</em></td>
<td>Vancomycin (30μg/ disc)</td>
</tr>
<tr>
<td><em>MSSA</em></td>
<td>Vancomycin (30μg/ disc)</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc)</td>
</tr>
<tr>
<td><em>Serratia</em></td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc)</td>
</tr>
<tr>
<td><em>Ewingella</em></td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc), tetraeyehlin(30μg/ disc)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc)</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc)</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc)</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Bactrim (23.75 μg Sulfamethoxazole+ 1.25 μg Trimethoprim)</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Cefepime (30μg/ disc), Imipenem (10μg/ disc)</td>
</tr>
</tbody>
</table>

2.11. Statistical Analysis

The data were expressed as mean values ± S.E.M. The relationship between two groups was done by independent *t* test. An *α* level of 0.05 was considered to be significant for both the *t* test and analysis of variance.
Chapter III

RESULTS

After one month of high fat intake, in the presence or absence of *S. Officinalis* water extract in drinking water, animals were sacrificed and blood samples were tested for their serum Total cholesterol, HDL-cholesterol, LDL-cholesterol, Triglycerides, Glucose and Insulin levels. Liver function was assessed by measuring the activity of serum liver enzymes: SGPT, SGOT, ALP and LDH. Also, stools cholesterol, triglyceride and water content were determined few days before animal sacrifice.

Other groups of rats were used to determine the effects of *S. Officinalis* extract upon ethanol induced gastric ulcer, acute inflammation, chronic inflammation and bactericidal effect.

3.1. Effects of *S. Officinalis* on serum lipid, glycemia and liver enzymes

3.1.1. Serum total cholesterol

There was no significant difference between Serum Cholesterol levels of the control and that of the treatment groups. Data are shown in figure 3.1.

![Graph showing total cholesterol levels for different groups](image-url)
Fig. 3.1: Serum Total cholesterol levels after one month of in-vivo treatment with S. Officinalis water extract in rats fed a high fat diet. Data are presented as mean ± SEM, (n=10).

3.1.2. Serum HDL-cholesterol

The serum HDL-cholesterol levels showed an increase in Group II and Group III as compared with the control and Group I. However, significance was only reached in Group III. Data are shown in Figure 3.2.

![HDL-Cholesterol Graph](image)

Fig. 3.2: Serum HDL-cholesterol levels after one month of in-vivo treatment with S. Officinalis water extract in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10).

3.1.3. Serum LDL-cholesterol

The LDL-cholesterol levels in the different groups did not show any significant changes. However, at high dosages of the plant extract LDL cholesterol levels were relatively reduced. Data are shown in Figure 3.3. Table 3.1 represents the Total cholesterol/HDL cholesterol and LDL/HDL cholesterol ratios.
Fig. 3.4: Serum Triglycerides levels after one month of in-vivo treatment with *S. Officinalis* water extract in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10).

3.1.5. Serum glucose levels

The fasting plasma glucose concentrations of the Sprague-Dawely male rats after one month of treatment with water extract of *S. Officinalis* was gradually decreasing compared with the control group (Fig. 3.5). However, the decrease in the concentration reached significance only at the highest dose used.
3.1.6. Insulin levels

Serum insulin levels of the different groups were measured using ELISA method. Data presented in Figure 3.6 showed that there were no significant changes among the different groups.

![Insulin Chart]

Fig. 3.6: Fasting serum insulin levels after one month of in-vivo treatment with water extract of *S. Officinalis* in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10).

3.1.7. Liver enzymes

The serum liver enzymes activities were measured in all groups. Table 3.2 illustrates the enzymes activities of SGOT, SGPT, ALP and LDH for both control and treatment groups. The serum levels of AST and ALT in the different experimental groups were similar to that of the control. On the other hand, serum LDH levels in Group II and Group III, but not Group I, were significantly lower than that of the control group. Measurement of the serum ALP activity revealed no significant change in Group I and II with respect to the control. However, Group III exhibited a significant increase in serum ALP when compared with the control group.
Table 3.2: SGPT, SGOT, LDH and ALP activities after one month of water extract of *S. Officinalis* in control and treatment groups of rats. Values are expressed as mean ± SEM (n=10).

<table>
<thead>
<tr>
<th>Liver Enzyme (U/L)</th>
<th>Control group</th>
<th>Treatment Group I (42mg/kg body weight)</th>
<th>Treatment Group II (167mg/kg body weight)</th>
<th>Treatment Group III (420mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST(SGOT)</td>
<td>41.5± 1.84</td>
<td>37.4± 2.93</td>
<td>39.9± 3.23</td>
<td>35.1± 2.47</td>
</tr>
<tr>
<td>ALT(SGPT)</td>
<td>23.1± 1.60</td>
<td>21.8± 1.41</td>
<td>18.0± 1.34</td>
<td>20.4± 1.51</td>
</tr>
<tr>
<td>LDH</td>
<td>381.8± 33.53</td>
<td>359.6± 79.62</td>
<td>241.3± 47.32*</td>
<td>219.7± 50.29*</td>
</tr>
<tr>
<td>ALP</td>
<td>141.2± 11.93</td>
<td>148.9± 7.70</td>
<td>134.3± 7.64</td>
<td>181.0± 7.51*</td>
</tr>
</tbody>
</table>

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

3.2. Stools cholesterol, triglyceride and water content levels

3.2.1. Triglyceride and cholesterol levels in stools

Data presented in figure 3.7 shows that after one month of supplementation with water extract of *S. Officinalis*, there were no significant changes in stools triglycerides content of control and treatment groups. On the other hand, stools cholesterol contents in Group I and II are not significantly different from that of the control group. When compared to all other groups, Group III showed significantly the lowest cholesterol concentrations. Data are shown in Figure 3.8.
Fig. 3.7: Triglyceride levels in feces after one month of in-vivo treatment with water extract of S. Officinalis in control and experimental groups of rats. Data are presented as mean ± SEM, (n=12).

Fig. 3.8: Cholesterol levels in feces after one month of in-vivo treatment with water extract of S. Officinalis in control and experimental groups of rats. Data are presented as mean ± SEM, (n=14).
3.2.2. Water Content in Stools

Determination of the stools water content in the different groups revealed that *S. Officinalis* exerted a dose dependent decrease in water content. When compared with the control group, Group I, II and III showed a significant reduction in water content (Table 3.3).

Table 3.3: Water content in stool of rats fed on a high fat diet after one month of in-vivo treatment with water extract of *S. Officinalis* in control and experimental groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Treatment Group I (42mg/kg body weight)</th>
<th>Treatment Group II (167mg/kg body weight)</th>
<th>Treatment Group III (420mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of water content in feces</td>
<td>77.82%± 1.76</td>
<td>67.94%± 3.13*</td>
<td>64.6%± 2.99*</td>
<td>61.45%± 1.97*</td>
</tr>
</tbody>
</table>

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

3.3. Ulcer

The effect of aqueous extract of *S. Officinalis* leaves on ethanol induced gastric ulcer was identified by measuring both long and small lesions in the glandular region of the stomach. Both doses of *S. Officinalis* water extract leaves used resulted in significant anti-ulcerative effect with respect to the control group and similar to that of cimetidine (anti-ulcer medication) where the 100 mg/kg body weight dose produced the most protective effect. Ulcer index and curative ratios are presented in Table 3.4. Figure 3.9 shows the difference between ulcerative and non-ulcerative stomachs.
Table 3.4: Effect of water extract of *S. Officinalis* on ethanol induced gastric damage in rats.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Ulcer Index (mm)</th>
<th>Curative Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>38.6± 1.51</td>
<td>-</td>
</tr>
<tr>
<td>Cimetidine Group</td>
<td>25.5± 3.92</td>
<td>33.6%</td>
</tr>
<tr>
<td>Group I (100mg/kg)</td>
<td>21.0± 5.14</td>
<td>45.5%</td>
</tr>
<tr>
<td>Group II (500mg/kg)</td>
<td>25.7± 7.2</td>
<td>33.5%</td>
</tr>
</tbody>
</table>

Fig. 3.9: Ulcer lesions as shown in the treatment groups receiving *S. Officinalis* extract. a: Stomach ulcerated, b: non-ulcerated normal stomach.

3.4. Anti-inflammatory Effect

In acute inflammation, water extract of *S. Officinalis* was able to significantly inhibit it. On the other hand, the extract was able to significantly inhibit chronic inflammation in all of the treatment groups. Results are shown in tables 3.5 and 3.6.
Table 3.5: Effect of water extract of *S. Officinalis* on carrageenan induced acute inflammation.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Group I (500mg/kg)</th>
<th>Group II (250mg/kg)</th>
<th>Group III (100mg/kg)</th>
<th>Diclofenac Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of inhibition</td>
<td>-</td>
<td>28.47%</td>
<td>24.86%</td>
<td>20.56%</td>
<td>45.53%</td>
</tr>
<tr>
<td>Increase in paw thickness after 6 days (mm)</td>
<td>0.015±0.006</td>
<td>0.012±0.005*</td>
<td>0.007±0.003*</td>
<td>0.004±0.002*</td>
<td>0.029±0.011*</td>
</tr>
</tbody>
</table>

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

Table 3.6: Effect of water extract of *S. Officinalis* on formalin induced chronic inflammation.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Group I (500mg/kg)</th>
<th>Group II (250mg/kg)</th>
<th>Group III (100mg/kg)</th>
<th>Diclofenac Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of inhibition</td>
<td>-</td>
<td>66.42%</td>
<td>60%</td>
<td>42.41%</td>
<td>40.67%</td>
</tr>
<tr>
<td>Increase in paw thickness after 3 h (mm)</td>
<td>0.122±0.00622</td>
<td>0.076±0.00479*</td>
<td>0.041±0.0027*</td>
<td>0.029±0.00151*</td>
<td>0.136±0.011*</td>
</tr>
</tbody>
</table>

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

3.5. Anti-bacterial effect

The antimicrobial effect of aqueous extract of *S. Officinalis* was tested against *Pseudomonas aeruginosa*, MRSA (*Methicillin resistant staphylococcus aureus*), MSSA (*Methicillin sensitive staphylococcus aureus*), *Citrobacter freundii*, *Serratia*, *Ewingella*, *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Salmonella typhi* and *Proteus mirabilis*. Data have shown
that the used concentration of extract showed no inhibitory effect against all bacterial strains used. Figure 3.10 shows the inhibition zone of the antibiotic used while the extract and negative control did not show any inhibition zone. Table 3.7 reveals the antibiotic sensitivity test exhibited by the different bacterial strains.

Fig. 3.10: The inhibition zones induced by the standard antibiotics, negative control and the Salvia Officinalis extract on the Muller-Hinton agar. Where A and F represents the negative control; B, E, D, G, I and J represents the different plant concentrations (10, 20, 50, 100, 200, 400 μg/μl); C and H represents the reference drug (antibiotic).
Table 3.7: Antibiotic sensitivity test on the 11 bacterial strains used to evaluate the antimicrobial effect of *Salvia Officinalis* extract. S: susceptible, R: resistant

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Reference drug</th>
<th>Antibiotic sensitivity</th>
<th>Extract Effect (Zone of inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, S</td>
<td>0</td>
</tr>
<tr>
<td><em>MRSA</em></td>
<td>Vancomycin (30 µg/disc)</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td><em>MSSA</em></td>
<td>Vancomycin (30 µg/disc)</td>
<td>S</td>
<td>0</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc), tetracycline (30 µg/disc)</td>
<td>S, S, R</td>
<td>0</td>
</tr>
<tr>
<td><em>Serratia</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc), tetracycline (30 µg/disc)</td>
<td>S, S, R</td>
<td>0</td>
</tr>
<tr>
<td><em>Ewingella</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc), tetracycline (30 µg/disc)</td>
<td>S, R, R</td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, S</td>
<td>0</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, S</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, S</td>
<td>0</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Bactrim (23.75 µg Sulfamethoxazole + 1.25 µg Trimethoprim)</td>
<td>S</td>
<td>0</td>
</tr>
</tbody>
</table>
Chapter IV

Discussion and Conclusion

Leaves of *S. Officinalis* have been medically proved to have many potent activities. The different effects of *S. Officinalis* evaluated in this study presented further information to support what has been studied before, in addition to other medicinal effects of the plant including the anti-ulcerogenic and anti-hyperlipidemic effects. The present study also took into consideration the safety of water extract intake over a period of one month.

It is well known that excessive intake of fats leads to the accumulation of triglycerides in several tissues mainly in the fat tissues (adipocytes). Whenever circulatory fatty acid increases in association with both high lipolysis in adipocytes and insulin resistance, there will be an overabundance of fatty acids in the non-adipose tissues such as pancreas, liver and muscle (Lebovitz, 2001). Thus, hyperlipidemia can result from the intake of a high cholesterol and lipid diet in human and animals as proved by many researchers (Umeda et al., 1989; Blazovics et al., 1993). In addition, it is very well known that the intake of saturated fat especially the one present in coconut oil increases the atherogenic profile in both human and animal models (Daher et al., 2003). After one month of feeding the rats on a high saturated fat diet, the water extract of *S. Officinalis* leaves appeared to increase significantly the good cholesterol "HDL" and decrease slightly the bad cholesterol "LDL". Since, HDL-cholesterol is the lipoprotein that removes cholesterol from the peripheral tissues whereas LDL-
cholesterol is the other atherogenic lipoprotein that is associated with coronary heart diseases (CHD) (Kingsbury and Bondy, 2003), the significant increase in HDL-cholesterol and the slight decrease in LDL-cholesterol is considered to be an important marker and may confirm on a cardioprotective role of *S. Officinalis* leaves. Moreover, LDL/HDL ratio is another important marker that helps realizing the effect of *S. Officinalis* leaves on lipoproteins. This ratio was decreased in all treatment groups with respect to the control. On the other hand, serum triglyceride and total cholesterol levels were not affected by the leaves of *S. Officinalis* water extract intake over a period of one month. Thus, these findings indicates that water extract of *S. Officinalis* leaves have a positive impact on cardiovascular diseases by promoting a better HDL cholesterol profile and LDL/HDL ratio.

Lectin and saponin are two active components of *S. Officinalis* that can be found in its aqueous extract and are responsible for hypolipidemic effects. Lectin is proved to have a significant effect in lowering both serum and hepatic cholesterol (Okazaki et al., 2005), whereas saponin is able to potentially reduce lipid peroxidase enzyme and LDL-cholesterol indicating decreased potential to atherosclerosis (Rodrigues et al., 2005; Zhao et al., 2005).

Type II Diabetes mellitus is correlated with many serious complications that are mostly related to chronic increase of glucose levels (Sabu et al., 2002). The available therapeutic medicines used to treat type II diabetes such as insulin, oral hypoglycemics and dietary modifications have some restrictions (Vats et al., 2002). Thus, medicinal plants are widely used nowadays to substitute the adverse effect of the therapeutic medicines. A literature review revealed few reports about the hypoglycemic effect of *S. Officinalis* (Jouad et al., 2001; Alarcon-Aguilar et al., 2002; Verspohl, 2002; Eidi et al., 2005). These reports examined the glycemic profile in healthy or diabetic rats (induced by streptozotocin or alloxan) after treatment with water, methanolic or oil extract of *S. Officinalis*. None of the previous reports
studied the hypoglycemic effect of the *S. Officinalis* leaves in rats fed a high fat diet rich in saturated fatty acids. Such an atherogenic diet may also lead to obesity, hyperglycemia, hepatic steatosis and possibly insulin resistance (Thupari et al., 2004; Surwit et al., 1998; Yun et al., 2004). In the present study, leaves of *S. Officinalis* water extract decreased serum glucose levels in a dose dependent manner and reached significance at the 420 mg/kg body weight dose. Although insulin concentrations among the different groups were not statistically significant, the highest dose of extract used showed the highest serum insulin concentration. This finding perhaps suggests that the plant hypoglycemic effect may be a combination of enhanced insulin secretion by the pancreas and/or increased sensitivity of the cells to insulin. Studies conducted on rats by Eid et al. (2005), revealed that methanolic extract of *S. Officinalis* leaves significantly decreased serum glucose in streptozotocin diabetic rats after three hour of intraperitoneal administration of the extract. However, no effect was observed on both serum glucose and insulin concentration in normal healthy rats. Therefore, there exist a difference between methanolic and water extract impact upon blood glucose levels in normal rats. Other studies on mice (Alarcon-Aguilar et al., 2002) revealed that water-ethanol extract of the whole plant significantly reduced blood glucose of normal fasting animals. The dose used in this study was 500 mg/kg body weight, somehow similar to the highest dose used in the present study (420 mg/kg body weight). On the hand, Alarcon-Aguilar et al. (2002) used a water-ethanol solvent where ethanol constituted only 15% of the solvent (which was boiled during extract preparation) meaning that the solvent used is very much close to that used in the present study. Again, this confirms our hypothesis that the hypoglycemic component exists in the water rather than the alcoholic extract. The hypoglycemic activity of the sage plant was also reported in an ethnobotanical survey of medicinal plants conducted on human in Morocco (Jouad et al., 2001). Usually, herbal remedies are taken by human as tea prepared by incubating the plant in hot boiled water. Therefore, this report further support the
hypoglycemic effect of the plant and specifically that the hypoglycemic active ingredient is present in the water extract.

In the last decades, there has been an increasing demand on the use of medicinal plants for the treatment of several disorders (Bolkent et al., 2005). Although the use of therapeutic plants is known to be safe and harmless, liver toxicity assessment must be well investigated to prove the safety of the plant extract since the liver is the essential organ responsible for the metabolism of drugs and any exogenous toxin (Grunhage et al., 2003). The widespread use of *S. Officinalis* in herbal teas and as a food condiment necessitates that studies on its biological effects must be conducted in order to prevent toxic effects on humans. To understand the hepatoprotective role or the hepatotoxic role of the water extract of *S. Officinalis* leaves on the liver of rats, four serum liver enzymes activities were assessed including Lactate dehydrogenase (LDH), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP). A study done on the leaves of *Melissa Officinalis* (a member of Labiatae family) proved its protective effect on the liver of hyperlipidemic rats (Bolkent et al., 2005). However, no earlier study explored the general safety of the *S. Officinalis* leaves extract on rats fed a high fat diet. Serum activity levels of ALT and AST have been found to be a useful indicator of liver cellular damage in the diagnosis and study of acute hepatic diseases. However, these enzymes are not only located in the liver but also in the extrahepatic tissues (He and Aoyama, 2003). Thus, any change in the serum activity levels of the latter enzymes may reflect cell damage in other cells of the body such as heart and muscle (Harold et al., 2002). Since there was no elevation in serum activity levels of both ALT and AST after one month of supplementation of the leaves of *S. Officinalis* water extract in rats fed on atherogenic diet, the intake of *S. Officinalis* leaves water extract appeared to be safe on hepatic cells. Also, serum AST/ALT ratio is another indicator for cellular damage. If this ratio
is less than 2, there will be no toxic effect on liver cells and this was the case in our study (Cohen and Kaplan, 1979). LDH is another marker enzyme of liver damage and tissue breakdown in general since it is present in almost all body cells. Results have shown that LDH levels were significantly reduced in Group II and III receiving 167 and 420 mg/kg body weight of the leaves of *S. Officinalis* water extract respectively; another sign of the safety use of the leaves of *S. Officinalis* water extract. ALP is the liver enzyme that gets elevated in case of cholestatis. In the present study, ALP activity level was only increased at the highest dose (420 mg/kg body weight) by less than one third with respect to the control. Since in toxic situations the increase in ALP levels should be more than three times higher than that of the control, it can be considered that the ALP activity observed at the highest dose is not far a normal value. So, leaves of *S. Officinalis* are proved to be a safe medicinal plant for the treatment of diseases and may contribute to maintain the hepatoprotective activity. Based on the obtained results, further studies are needed to examine the morphological and histological appearance of the liver tissue receiving the water extract of *S. Officinalis* leaves to confirm the general safety of the plant.

Since *S. Officinalis* is mostly known in folk medicine for the healing of gastric disorders (Gali-Muhtasib et al., 2000) mainly diarrhea (Medical Economics Company, 1998), the present investigation studied the effect of the water extract of *S. Officinalis* leaves upon water content in stools. Results are in support to what is commonly known in folk medicine since all doses used were efficient in reducing the water content of stools. This emphasizes the medicinal use of *S. Officinalis* leaves in cases of diarrhea. On the other hand, other experiments were done to investigate the effect of the leaves of *S. Officinalis* water extract upon nutrient absorptive capacity of the gastrointestinal tract. Assessment of triglyceride and
cholesterol absorption revealed the efficacy of the rat digestive system in the absorption of dietary fat in concomitance with the consumption of water extract of *S. Officinalis* leaves.

Gastric ulcer can be induced experimentally in the rats through different ways such as the HCl/ethanol, non steroidal-anti inflammatory drug, ethanol, Shay method (Kushima et al., 2005), restraint water immersion stress, or by indomethacin (Rujjanawate et al., 2005). Since ethanol can easily produce sufficient number of gastric lesions, I chose to follow this procedure over the other ones to test for the anti-ulcerogenic effect of the water extract of the leaves of *S. Officinalis* in rats.

Ethanol-induced damages are caused by disturbance of mucosal microcirculation, appearance of free-radicals, ischaemia, endotelin release, inhibition of prostaglandins and degranulation of mast cells. Moreover, ethanol reduces the defensive factors in the stomach such as the reduction of mucus production and bicarbonate secretion (Marhuenda and Martin, 1993; Samonina et al., 2004). Oral administration of ethanol solution produced several long and small lesions in the control group. While the oral administration of the water extract of *S. Officinalis* leaves in Group I and II at doses of 100 and 500mg/Kg body weight respectively, decreased the ulcer index from 38.6 mm in the control group to 25.5 mm in Group I and 21.5 mm in Group II, whereas the ulcer index in the group receiving Cimetidine (the anti-ulcerogenic medication) was 25.7 mm. Cimetidine protection against gastric ulcer in the present study is consistent with other studies (Kushima et al., 2005). No previous studies have investigated the effect of aqueous extract of *S. Officinalis* leaves on ethanol induced gastric ulcer in rats. Further studies are necessary to find out the mechanism of protection involved, and to isolate the component responsible for this action. However, it is very well known that flavonoids, tannins, mucilage and other plant extract components may be behind this effect.
Another aim of this study is to assess the anti-inflammatory property of the aqueous extract of *S. Officinalis* leaves. Some of the *S. Officinalis* constituents are well known for their anti-inflammatory effect such as ursolic acid, diterpene carnosol and triterpenes oleanolic acid as proved in previous studies (Tokuda et al., 1986; Huang et al., 1994; Liu, 1995). These studies were done on methanolic, n-hexane and chloroform extract of the plant (Baricevic et al., 2000) without further specification on acute and chronic inflammation. In the present study the topical anti-inflammatory effect of aqueous extract of *S. Officinalis* leaves in the hind paw of rats was investigated in acute and chronic models of inflammation induced by carrageenan and formalin respectively. Carrageenan is able to produce edema with plasma exudation and high levels of neutrophils up till 24 hours (Henriques et al., 1987); while formalin drug can be used to induce chronic inflammations. It is interesting to note that aqueous extract of *S. Officinalis* leaves have an effect on both acute and chronic inflammation whereby the inhibition percentages of the plant extract sometimes were higher than that produced by the reference drug diclofenac. Since *S. Officinalis* leaves contains many important constituents such as tannins that are mainly present in the leaves of the plant, the anti-inflammatory activity might be attributed to this agent rather than ursolic acid and oleanolic acid that are other constituents possessing anti-inflammatory properties. This is because tannins can be extracted with water as a solvent while ursolic and oleanolic acids are unlikely present in aqueous solvents (Cowan, 1999). More fractions of the plant components should be isolated to be tested against inflammation. Moreover, mechanism(s) responsible for this pharmacological activity remain to be investigated.

Various studies all over the world have been done to investigate the anti-microbial effect of plant extracts or the natural products of certain plants since the rapid appearance of resistant pathogenic bacteria against certain antibiotics. *S. Officinalis* was commonly known in folk
medicine for its anti-microbial effect. Then this characteristic was proved medically throughout several studies (Miski, 1983; Nascimento et al., 2000; Abu-Shanab et al., 2004; Pereira et al., 2004). The results obtained in this study showed that the aqueous extract of *S. Officinalis* leaves were unable to induce an anti-microbial effect against any of the 11 pathogenic bacterial strains tested. This result is inconsistent with previous studies done by Abu-Shanab et al. (Abu-Shanab et al., 2004) in Palestine which proved the bacterial inhibitory effect of both the aqueous and ethanolic extract of *S. Officinalis* leaves on MRSA and B.subtilis microorganisms. On the contrary, the present results are consistent with another study done in Brazil, which proved that *S. Officinalis* aqueous extract has no anti-microbial activity (Nascimento et al., 2000). Another study done on the essential oil extracted from *S. Officinalis* leaves "1,8-cineol" proved its potent anti-bacterial effect mainly against enterobacteria and Pseudomonas aeruginosa isolated from urinary tract infections (Pereira et al., 2004). The reason of the insensitivity of the water extract of *S. Officinalis* against the 11 hospital isolates of different microorganisms is mainly because the anti-microbial effect comes from the plant components extracted from the ethanol or methanol solvents and not from water (Cowan, 1999). According to Cowan's work, aqueous extracts may contain starches, anthoyanins, tannins, polypeptides, terpenoids, lactins and saponins; while methanol solvents are able to extract polyphenols, tannins, saponins, totarol, xanthoxyllines, terpenoids, quessionoids, flavones, phenones and lactones all of which have antimicrobial effects.

In Conclusion, the present report reveals the safety and importance of the aqueous extract of *S. Officinalis* leaves as an inexpensive treatment for several important diseases that affects the well being of human. The extract showed a good impact on lipidemia, which help in the reduction of cardiovascular diseases, the number one killing disease. Also, the extract helps in reducing serum blood glucose levels, which make it suitable for patients suffering from
diabetes. It aids also in reducing the symptoms of diarrhea by promote more water absorption through the GI tract thereby reducing the chances of dehydration. Furthermore, the water extract of *S. Officinalis* leaves demonstrated its anti-inflammatory potential and at the same time appeared to have an anti-ulcerogenic effect. Thus, to be more precise in knowing which component is responsible for which action, further purification of the water extract of *S. Officinalis* is recommended in future studies.
Chapter V

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