THE EFFECT OF MALVA SYLVESTRIS ON INFLAMMATION, GASTRIC DAMAGE, LIPEMIA, GLYCEMIA AND MICROBIAL GROWTH

By

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ABSTRACT

Medicinal plants have long been used for the treatment of many diseases. Recently, several experimental studies have contributed scientific evidence for the pharmacological effects of these medicinal plants observed in folk medicine. The present work was performed to look at the medicinal effects of the aqueous extract of common mallow (Malva sylvestris) upon blood lipid profile, inflammation, glycemia, ulcer prevention and microbial growth. After a month period of administration of extract via drinking water (95, 380, and 950 mg/kg body weight), only triglyceride content was increased significantly in serum samples. No significant changes were witnessed in any other lipid parameter studied. No changes in serum glucose and insulin levels were recognized in the animals receiving the aqueous extract over the period of study. The extract appeared not to have a negative impact on the hepatocyte integrity assessed through the measurement of transaminases, lactate dehydrogenase and alkaline phosphatase. In addition, an increase and decrease in stool triglyceride and cholesterol content were observed respectively. A 9.5% increase in stool water content was observed at the highest dose. The aqueous extract showed an anti-ulcer activity that was comparable with that of the reference drug (Cimetidine). The aqueous extract revealed an anti-inflammatory activity in the acute and chronic inflammation models induced by carrageenan and formalin respectively. This activity was optimum at the 100 mg/kg body weight dose in both models of inflammation. In addition, the aqueous and methanolic extracts of the dried plant material were tested for any antimicrobial activity against 11 gram-positive and
gram-negative bacterial strains; the methanolic extract was further tested against the yeast *Candida albicans*. None of the extracts exhibited inhibition zones in the Kirby-Bauer method at the tested concentrations.

The present study shows that *Malva sylvestris* has no major impact on blood lipid profile and bacterial growth. Yet, a potent anti-inflammatory and anti-ulcer activity is demonstrated. Further studies on the fractionated extract mode of action, and clinical assessment remain to be explored.
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GLOSSARY

ACAT: Acetyl-CoA- cholesterol AcetylTransferase
ADP: Adenosine Diphosphate
ALP: Alkaline phosphatase
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
CHD: Coronary Heart Disease
CHE: Cholesterol Esterase
CHO: Cholesterol oxidase
CM: Chylomicron
ELISA: Enzyme Linked Immuno Sorbant Assay
GOD: Glucose oxidase
GPO: Glycerol-3-phosphate oxidase
HDL: High Density Lipoprotein
LCAT: Leicithine Cholesterol Acetyltransferase
LDH: Lactate Dehydrogenase
LDL: Low Density Lipoprotein
LPL: Lipoprotein Lipase
MRSA: Methicillin resistant Staphylococcus aurens
MSSA: Methicillin sensitive Staphylococcus aurens
POD: Peroxidase
TG: Triglycerides
INTRODUCTION

Throughout history plants and plant products have been widely used to treat medical problems. The first documented use of medicinal plants can be found in the early Egyptian and Asian cultures (Capasso et al., 2003). Herbal medicines, before appearing in the pharmacies as a medicine, should be required to undergo pharmacological and toxicological testing on animals and clinical trials in humans. Unfortunately, this is not the case. The cost of such an endeavor, especially for products that will not have patent protection, is perhaps the major reason for the lack of research on herbal medicines (Capasso et al., 2003). There has been a global tendency for the revival of interest in the traditional system of medicine for the period of the past several decades. Simultaneously the need for basic scientific investigation of medicinal plants using indigenous medical systems has become ever more interesting and relevant (Gurbuz et al. 2005). Many researchers have discussed the importance of medicinal plants as sources of new therapeutic agents, and others have effectively focused on the potential of specific chemical classes in drug discovery (Lewis et al., 2003).

Consequently, a wide variety of natural products are under scrutiny for their clinical potential, both in terms of disease prevention and treatment.
1.1. Plant Taxonomy and Habitat

_Malva sylvestris_ commonly known as Mallow, Common Mallow or Blue Mallow belongs to the family Malvaceae (Table 1). Plants from this family are noted for their economic, horticultural and medicinal importance (Beer and Howie, 1985). _Malva_ a genus of about 30 species of annuals, biennials, perennials, and sub shrubs distributed throughout Europe, Asia, and Africa and widely naturalized in temperate and tropical regions; _M. sylvestris_ is a native to Europe, northern Africa, and south western Asia.

The plant’s names are derived from the old English _malve_, "soft", and refers to the abundant mucilage in certain species, which softens the skin (Bown, 1995). Today the plant can be found in subtropical and temperate latitudes of both hemispheres. Mallow leaves are harvested from June to the beginning of September. Mallow flowers are harvested without the pedicles from the end of June to October (Medical Economics Company, 1998).

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Table 1. Classification of plant *Malva sylvestris*

<table>
<thead>
<tr>
<th>Kingdom</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta- Vascular plant</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Spermatophyta- Seed plant</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta- Flowering plant</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida- Dicotyledons</td>
</tr>
<tr>
<td>Subclass</td>
<td>Dilleniidae</td>
</tr>
<tr>
<td>Order</td>
<td>Malvales</td>
</tr>
<tr>
<td>Family</td>
<td>Malvaceae- Mallow</td>
</tr>
<tr>
<td>Genus</td>
<td>Malva</td>
</tr>
<tr>
<td>Species</td>
<td>sylvestris</td>
</tr>
</tbody>
</table>
The medicinal parts are the dried leaves, dried flowers and the whole of the flowering fresh plant. The bright purple flowers with long dark stripes are clustered in leaf axils. They have 3 epicalyx leaves, 5 sepals, 5 petals which are much longer than the calyx and have a deep margin. The numerous stamens are fused to a 10 to 12mm column (Medical Economics Company, 1998). Figure 1.1 represents M. sylvestris whole plant and flowers.

Figure 1.1. (a) The whole plant of M. sylvestris and (b) its flower (Hinsley, 1999).

The fruit stems are erect and slanted to one side. The ovaries are made up of a ring of 9 to 11 carpels. The fruit is 7 to 9 mm wide and 2 mm thick disc, which breaks up into mericarps. These are glabrous or covered in a few scattered hairs, sharply angular and punctuate. M. sylvestris is biennial or perennial leafy herb 0.3m to 1.2m high. The stems are branched, prostrate to curved, ascending, slightly woody, and roughly
pubescent. The leaves are alternate, long petioled, reniform orbicular, 5-lobed, and crenate serrate (Medical Economics Company, 1998).

1.1.1 Growth and Harvest

*M. sylvestris* is considered ornamental. It can be found in well-drained to poor soil, in sun or partial shade. Propagate by seed sown in spring or autumn or by division during dormancy or by cuttings of basal shoots in spring or summer. Mallows are prone to tarnish (oxidation). Leaves and flowers are gathered in summer and used fresh for compresses or dried for infusions and liquid extracts. Fruits (seed capsules) are picked when green and used fresh (Bown, 1995).

1.1.2. History and Folklore

*M. sylvestris* has been grown as a medicinal plant and pot herb since Roman times. In the 16th century it was known as a “cure all”, or omnimorbia. Several species have very similar constituents, and *M. sylvestris* is used interchangeably with the medicinally less potent *M. moschata* (musk mallow), and the stronger *M. neglecta* (dwarf mallow). The Musk Mallow is not an uncommon plant in dry pastures and in hedgerows. It grows 2 feet high, with round, thick, erect stems, somewhat hairy, often purple spotted. The foliage is light-green, the lower leaves kidney-shaped, five to seven lobed, those on the stem finely divided into numerous narrow segments. The handsome rose-colored flowers are three times the size of the Common Mallow, crowded towards the summit of the stem. It emits from its leaves a faint, musky odor, especially in warm weather, or when drawn through the hand. The root is white and is the part used. It has the same virtues as the Common Mallow, but is not quite as strong, and the leaves have similar properties (Bown, 1995).
All are regarded as inferior to the closely related *Althea officinalis* (marsh mallow). The great demulcent and emollient properties of Marsh Mallow make it useful in inflammation and irritation of the alimentary canal, and of the urinary and respiratory organs. The stems, which die down in the autumn, are erect, 3 to 4 feet high, simple, or putting out only a few lateral branches. The leaves, shortly petioled, are roundish, ovate-cordate, 2 to 3 inches long and about 1 1/4 inch broad, entire or three to five lobed, irregularly toothed at the margin, and thick. They are soft and velvety on both sides, due to a dense covering of stellate hairs. The flowers are shaped like those of the common Mallow, but are smaller and of a pale colour, and are either axillary, or in panicles, more often the latter (*Grieve*, 1900).

*Malva parvifolia* a prostrate perennial herb belonging to the family Malvaceae is applied as hot poultice to wounds and to draw boils (*Grierson* and *Afolayan*, 1999). Rather different in effects is the Chinese *M. verticillata* (farmer’s tobacco), a soothing diuretic, used for urinary tract infections (*Bown*, 1995). All Mallows contain abundant mucilage, and the Arab physicians in early times used the leaves as a poultice to suppress inflammation (*Grieve*, 1900).

1.2 Plant Traditional and Medicinal Uses.

Common mallow young leaves and shoots are eaten raw in salads or cooked in vegetable dishes. Unripe seed capsules, known as "cheeses" because of their shape, make an unusual addition to salads. Flowers are used to make tea. Traditional herbal medicine continues to regard the plant as a useful anti-inflammatory agent for the respiratory tract, the skin (*Lust*, 1974; *Chevallier*, 1996), and the gastrointestinal tract (*Lust*, 1974) the leaves reduce gut irritation and have a laxative effect in large doses
(Bown, 1995; Chevallier, 1996). The German physician and herbal authority, Rudolf Weiss recommended mallow primarily for irritations of the mouth and throat, as well as for dry, irritating coughs, he also mentions its topical use in cases of eczema and dermatitis (Weiss, 1985). *M. sylvestris* leaves and flowers because of their mucilage content are used against dry cough (Bown, 1995; Classen et al., 1998). Common mallow is an effective demulcent and the root can be used to relieve children teething (Chevallier, 1996). It has also been found to be used as an eye wash and ophthalmic remedy, drops and decoction of the leaves and flowers are instilled into inflamed eyes (Leporatti and Pavesi, 1990). Traditional Egyptian medicine considers *M. sylvestris* for treating hemorrhoids (Nawwar and Buddrus, 1981).

### 1.2.1 Contraindications, Interactions, and Side effects

Mallow can be safely consumed if used properly. Hazards and/or side effects for proper therapeutic dosages are not known. Germany’s Commission E has approved its use for irritations of the mucosa of the mouth and throat and associated dry cough (Duke et al., 2002).

### 1.3. Plant Constituents

Common mallow contains flavonal glycosides (including gossypin-3-sulphate), mucilage and tannins (Chevallier, 1996). Mallow flower contains more than 10% mucilage which on hydrolysis produces galactose, arabinose, glucose, rhamnose and galactouronic acid along with small amounts of tannin. Less than 0.1% leucoanthocyanins, but about 7% (based on dry weight) anthocyanins, about half of which is malvidin 3,5-diglucoside (malvin); delphinidin and malvidin 3-glucosides are also present (Wichtl and Bisset, 1994).
The mallow leaf similarly contains about 8% mucilage which upon hydrolysis arabinose, glucose, rhamnose, galactose and galactouronic acid; small amounts of tannin. The occurrence of flavonoid sulphates is noteworthy; several 8-hydroxyflavonoid glucuronides have been isolated as well, gossypetin 3-glucoside 8-glucurononoid and hypolaetin 8-glucurononide being quantitatively the main ones (Wichtl and Bisset, 1994).

1.3.1. Mucilage

Mucilage is found in the tissues, surface of seeds or in the bark of succulent or non-succulent plants. Mucilage derived from plants is employed in food-processing and pharmaceutical industries and have been found to possess biological activity. Commonly all mucilages have a valuable effect on wounds, burns, ulcers, diarrhea, dysentery along with external and internal inflammations and irritations (Morton, 1990). Mucilages are usually brittle, amorphous, transparent substances that absorb water to form gelatinous masses or viscous colloidal solutions (Ebadi, 2002). Immunomodulatory activities (macrophage and complement system activation, inducement of natural killer cell cytotoxicity) have been exhibited by some of the high molecular weight acidic polysaccharides and glycoproteins of mucilages (Classen and Blaschek, 2002). Mucilage from Malva sylvestris ssp. mauritiana has been shown to be sited in idioblasts, large cavities or also in specialized epidermal cells in case of leaves (Classen et al., 1998).

In the study by Classen and Blaschek, suspension culture media showed the accumulation of arabinogalactan suggested by the dominance of Ara and Gal neutral monosaccharides. This glycosyl composition suggests that some pectic polysaccharides are secreted by suspension culture cells of Malva. However, uronic
acids, rhamnose and galactose are dominant monosacharrides from leaves and flowers of Malva indicating that rhamnogalactan type polysaccharides are the main components of mucilage from leaves and flowers (Classen and Blaschek, 2002).

1.3.2. Flavonoids

The flavonoids constitute a large group of naturally occurring compounds widely distributed in the plant kingdom and display a remarkable array of biochemical and pharmacological actions (Wagner, 1999). The major flavonoid constituents identified in the leaf tissue of Malva sylvestris are gossypetin 3'-sulphate-8-O-β-D-glucoside and hypolaetin 3'-sulphate. A minor flavonoid constituent from the same tissue is 8-O-β-D-glucuronide-3-sulphate (Nawwar and Budrus, 1981; Billiter et al., 1991). An enzyme which catalyzes the final reaction in oxidative degradation of sulphur containing amino acids; sulphite oxidase, was isolated from M. sylvestris leaves and partially characterized (Ganai et al., 1997). Sulphite oxidase has been purified also from an array of bacterial and animal sources (Garrett and Rajagopalan, 1994). It is physiologically important and its absence may lead to death (Ganai et al., 1997).

1.3.3. Anthocyanins

Anthocyanidin (aglycon) is a diphenylpropane- based polyphenolic ring and is limited to a few structure variants as cyanidin, pelargonidin, peonidin, delphinidin and malvidin. These represent the aglycons of the majority of Anthocyanins in plants (Hou et al., 2005). Anthocyanins contribute to the intense color of many fruits and vegetables (Mazza, 1995). Anthocyanins have been detected in several families including Malvaceae. Malva sylvestris petals are a standard source of the malvidin
pigment, malvidin 3, 5-diglucoside; both in cultivated and wild forms (Takeda et al., 1989).

Anthocyanins have both a biological and pharmacological beneficial potential such as: anti-inflammatory (Subarnas and Wagner, 2000), antioxidant (Wang et al., 1997; Moyer et al., 2002) suggesting that they play a role preventing mutagenesis and carcinogenesis (Omenn, 1995), anti-tumor properties (Kamei et al., 1995; Katsube et al., 2003), and reducing cardiovascular disease risk (Wang and Mazza, 2002). Delphinidin alters key protein expression in cell migration and cell proliferation, where it plays an important function as an in vivo anti-angiogenic compound (Favot et al., 2003).

1.4 Lipid Overview

Lipids are a diverse group of molecules characterized by a predominantly hydrophobic nature. Lipids show the propensity to clump together forming either particles like micelles and triacylglycerol-containing lipid droplets or biological membranes such as phospholipid bilayers. Lipids are involved in a plethora of processes required to maintain cellular structures and to execute cellular functions. A major class of biologically important lipids is fatty acids and their derivatives. Fatty acids are required for the formation of phospholipids, the building blocks of cellular membranes, and the coating of lipoproteins, the lipid transporting particles in blood plasma. Fatty acids themselves and their derivatives are information carriers interacting with intracellular signaling pathways and nuclear factors involved in the regulation of DNA transcription. A second class of lipids that play a major role in biological systems are cholesterol and its derivatives and metabolic products (Ger, 2004).
Lipids play an important part in biological structures whose purpose is to provide barriers that protect organisms against their environment. The simplest type of barrier is simply a layer of lipids on the surface of the skin, the surface of leaves in plants or associated with the walls of microorganisms. Fatty acids in the form of simple glycerides constitute an important source of fuel in mammals and in many plants. The triacylglycerols are by far the most important storage form. Lipids not only contribute to the structure of cells and provide an energy store; they also participate in the transmission of chemical messages in living organisms. A variety of lipid molecules take part in diverse aspects of metabolism and its control (Gurr and Harwood, 1991).

1.4.1 Cholesterol

An important lipid constituent of biological membranes is cholesterol, specifically influencing membrane fluidity also a substrate for the formation of bile acids in liver cells. After storage in the gall bladder, bile acids are of paramount importance in the transportation of the digestion products to the epithelial cells of the intestinal wall in addition to facilitating the digestion of dietary lipids. Equally important is the fact that cholesterol is the precursor of various classes of steroid hormones, including mineralocorticoids and glucocorticoids produced in the adrenal cortex and sex steroid hormones synthesized in the male and female gonads (Ger, 2004).

1.4.2 Lipoproteins

Lipoproteins are soluble complexes of proteins (apolipoproteins) and lipids that transport lipids in the circulation. Lipoproteins are synthesized in the liver, in the intestines, 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 are highly dynamic. They undergo enzymatic reactions of their lipid components, facilitated and spontaneous lipid transfers, transfers of soluble apolipoproteins, and conformational changes of apolipoproteins in response to the compositional changes (Vance, 2004).

The major lipoproteins are classified according to density. Since lipids have lower buoyant densities than proteins, lipoproteins that have a high ratio of lipid to protein have lower density than lipoproteins having a lower ratio of lipid to protein. Chylomicrons (CM) are synthesized in the intestines for the transport of dietary triacylglycerols to various tissues. Very low density lipoproteins (VLDL) are synthesized in the liver for the export of endogenous triacylglycerols, while low density lipoproteins (LDL) arise from the metabolic transformation of VLDL in circulation. The function of LDL is to deliver cholesteryl ester to peripheral tissues and to the liver. High density lipoproteins (HDL) are synthesized and assembled in the liver and intestine or are formed from metabolic transformations of other lipoproteins in circulation, and from cellular lipids at the cell membranes. HDL removes excess cholesterol from cells and transports it to liver and steroidogenic tissue for metabolism and excretion (Vance, 2004).

HDL removes excess cholesterol from the blood and body cells and carry it to the liver for elimination. A high HDL level is associated with decreased risk of coronary artery disease because HDLs prevent accumulation of cholesterol in the blood (Tortora and Grabowski, 2003). LDL carries about 75% of the total cholesterol in blood and deliver it to cells throughout the body for use in repair of cell membranes and synthesis of steroid hormones and bile salts. LDLs contain the apoprotein, apo B100; which is the docking protein that bonds to LDL receptors for receptor mediated endocytosis of LDL into a body cell. Excessive numbers of LDL form fatty plaques
that increase the risk of coronary heart disease (CHD) (Tortora and Grabawski, 2003).

1.4.3 Triglycerides

Most serum triglycerides are carried in the VLDL fraction, in the fasting state. Two factors determine serum levels of triglycerides; rates of hepatic secretion of VLDL triglyceride and the capability for hydrolyzing circulating triglyceride. Hepatic overproduction of VLDL triglycerides causes hypertriglyceridemia. This can occur in two ways; first, increase in the total number of VLDL particles secreted by the liver, second, the total number of synthesized particles is not increased but rather there is an increase in the triglyceride content of each VLDL particle. Defective lipolysis of triglyceride-rich lipoproteins is a general reason for hypertriglyceridemia (Grundy and Denke, 1990).

1.5. Inflammation

The body repairs tissues damage and defends itself against infection through a process called inflammation. The process of inflammation is divided into acute and chronic forms. The hallmark of an inflammatory response is the passage of proteins, fluid, and cells from the blood into focal areas in tissues (Seil and Max, 2001). Acute inflammation delivers plasma and cellular components of the blood to extravascular tissue spaces where it causes dilution of toxic materials and increases lymphatic flow. Chronic inflammation follows acute inflammation if the acute response is not satisfactory to clear the tissue of necrotic debris produced by acute necrosis. Chronic inflammation is more defensive against persistent infections, and completes the process of healing (Seil and Max, 2001).
Inflammation involves:

- Vasodilation (↑ in blood flow).
- Vasopermeability (↑ vascular permeability leading to edema).
- Infiltration of polymorphonuclear neutrophilic leukocyte (PMNs).

- Infiltration of lymphocytes and macrophages.
- Restoration of normal structure or scarring.

Important mediators of inflammation and pain are prostaglandins synthesized from arachidonic acid by the rate limiting enzymes cyclooxygenases (COX) which have two isoforms. COX-1 plays a regulatory role in normal physiology and is constitutively expressed. COX-2 is induced by pro-inflammatory cytokines and bacterial toxins in inflammatory cells (Lee et al., 2006). Since non-steroidal anti-inflammatory drugs (NSAIDs) have damaging effects on the kidney and stomach at doses sufficient to suppress prostaglandin production (Lee et al., 2006), thus, efficacious agents are needed to inhibit COX and lipooxygenase pathways that produce PGs, thromboxanes and leukotrienes (Jose et al., 2004).

The *M. sylvestris* leaves are used in the Mediterranean area where they are known traditionally to possess anti-inflammatory properties (Picci, 1980). Plant extracts are being screened for their potential anti-inflammatory activities contributed by anthocyanidins mainly on COX-2. Anthocyanidin inhibited COX-2 expression in lipopolysaccharide activated RAW264 macrophage cells with a structure-activity relationship, and by blocking MAPK-mediated transcription regulation (Hou et al., 2005). *Sida cordifolia* L. (Malvaceae) commonly known as Malva branca (white
mallow) exerts anti-inflammatory and analgesic properties by interfering with the cyclooxygenase pathway (Franzotti et al., 2000).

1.6 Gastric Damage

Gastric and duodenal ulcers affect a great number of the world population and are induced by numerous factors, including stress, smoking, nutritional deficiencies, and intake of non-steroidal anti-inflammatory drugs (Basil and Howard, 1995; Belaiche et al., 2002). Several plants and herbs have been used to treat gastrointestinal disorders in traditional medicine (Toma et al., 2003) and have shown promising results in the treatment of gastric ulcer as being sources of new drugs (Borelli and Izzo, 2000).

A membrane bound enzyme $\text{H}^+, \text{K}^+ - \text{ATPase}$, located in the apical membrane of parietal cells pumps protons into the gastric lumen using energy derived from the ATP hydrolysis. Hydrochloric acid, secreted in the stomach, does indeed play a part in the development of ulcers, but it is not solely responsible. Abnormally large amounts of acid secretion occur in rare situations, such as in the genetic condition known as Zollinger-Ellison syndrome, in which large amounts are stimulated by tumors located in the pancreas or duodenum. To treat acid related problems the $\text{H}^+, \text{K}^+ - \text{ATPase}$ constitutes a great pharmacological target since the inhibition of this enzyme inhibits acid secretion (Reyes-Chilpa et al., 2005).

Several mechanisms have been proposed to explain the gastroprotective effects of flavonoids, including decrease of histamine secretion from mast cells, increase in mucosal prostaglandins content, and inhibition of acid secretion along with the inhibition of Helicobacter pylori growth (Reyes-Chilpa et al., 2005). Flavonoids have been found to be free radical scavengers which play a key role in ulcerative and erosive lesions of the gastrointestinal tract (Borelli and Izzo, 2000; Ebadi, 2002). As a
folk remedy mucilaginous plants are used to treat gastric ulcer since they have the property of covering and protecting the mucosa of the stomach. *Malva neglecta* (Malvaceae) leaf due to its high mucilage content is widely used in many parts of Turkey for the treatment of ulcer (*Gurbuz et al.*, 2003). *M. sylvestris* is traditionally used in several countries for the treatment of gastric ulcer since it contains mucilage (*Ebadi*, 2002).

1.7 Glycemia

Long regarded as a disease of minor consequence to world health, Diabetes Mellitus now occupies one of the main causes of serious maladies (*Zimmet et al.*, 2001). Autoimmune-mediated destruction of pancreatic β-cells islets leads to Type 1 Diabetes mellitus, whereas Type II Diabetes Mellitus is a metabolic disease caused by decreased insulin secretion and action (*Cavaghan et al.*, 2000). Insulin is a potent and critically important hormone. The effect of insulin is to enhance the uptake of glucose into the cells where it is metabolized and stored as glycogen or used as an energy substrate in the synthesis of proteins or fats. Insulin plays an important role in potassium homeostasis (*Hadley*, 2000).

Insulin resistance is a characteristic feature in type II diabetes, and several drugs are currently being used to increase the insulin sensitivity. Adverse effects and high rates of secondary failure are limitations of the currently available drugs for type II diabetes (*Oh et al.*, 2005). The use of medicinal plants has flourished as an alternative for the control and prevention of diabetes intricacy due to the vast costs of modern treatment of diabetes (*Luo et al.*, 1998).
1.8 Liver Assessment

Biotransformation of all drugs and xenobiotics (foreign substances) is central to the liver along with synthesizing an array of body proteins. Toxic metabolite generation within the hepatocyte may create direct cell injury with disruption of intracellular function or immune-mediated membrane damage by indirect injury thus abnormal liver enzyme levels signal liver damage (Lee, 1993). Liver function tests that measure the level of serum liver enzymes reflect hepatocyte integrity or cholestasis (Giannini et al., 2005).

The aminotransferases constitute a group of enzymes that catalyze the interconversion of amino acids and α-oxo-acids by transfer of amino groups (Burris et al., 1994). Alanine aminotransferase (ALT) serum level increases as a result of any form of hepatic cell damage where the cells of the liver have been inflamed or undergone cell death (Braunwald et al., 2001). Aspartate aminotransferase (AST) also reflects liver damage but to lower extent than ALT. Elevated levels of AST are seen following a heart attack or in cases of viral hepatitis and mononucleosis (Braunwald et al., 2001). The enzyme alkaline phosphatase (ALP) transports metabolites across cell membranes. The most common causes of ALP elevation are liver and bone diseases. Hepatic ALP is found on the surface of bile duct epithelia. The synthesis and release of ALP is enhanced by cholestasis and its release from cell surface is amplified by accumulating bile salts (Giannini et al., 2005). In many diseases the enzyme lactate dehydrogenase (LDH) is observed in mild increases such as in leukemia, myocardial and pulmonary infarction also in non-viral hepatitis and hemolytic anemia and in high increases in cases of disseminated carcinoma, shock and megaloblastic anemia (Braunwald et al., 2001).
1.9. Microbial Growth

The use of herbal medicine as a treatment for many infectious diseases is a rich tradition in many parts of the world (Essawi and Srour, 2000). Plant antimicrobial compounds might inhibit bacterial growth by several mechanisms different from the presently used antimicrobials and might be significant in treating resistant microbial strains (Eloff, 1988). The development of multi-drug resistant bacteria, emerging new pathogens and reduction in the new antibacterial drugs has led to reassessment of medicinal plants as models for antimicrobial agents (Cowan, 1999).

The methanolic, acetone and aqueous extracts of *M. parvifolia* showed no visible antibacterial effect (Grierson and Afelayan, 1999). However, the growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* was inhibited by the hexane, methanol and water extracts made from leaves and roots of *M. parvifolia* with the prostrate form. On the other hand, *M. parvifolia* with an upright and erect growth form inhibited only *Staphylococcus aureus* and *Bacillus subtilis* (Shale et al., 2005). *Malva sylvestris* ethanolic extracts showed activity against *Bacillus subtilis* (Izzo et al., 1995), *Pseudomonas aeruginosa* (Alkofahi et al., 1996), and *Escherichia coli* (Izzo et al., 1995; Alkofahi et al., 1996). The methanolic extract of *M. sylvestris* showed activity against *Saccharomyces cerevisiae* (Coelho de Souza et al., 2004).
Purpose of the Project:

In an attempt to investigate the potential medicinal effect of both aqueous and methanolic extracts of *M. sylvestris*, the extracts were tested against the following:

- Blood lipid profile in rats fed a fat-rich diet:
  - Plasma total cholesterol.
  - HDL- cholesterol.
  - LDL- cholesterol.
  - Triglycerides.
- Anti-inflammatory effect in induced acute and chronic inflammation.
- Ethanol induced gastric damage to test preventive effect of aqueous extract.
- Determination of glucose and insulin concentrations in serum.
- Assessment of liver enzymes (transaminases, lactate dehydrogenase, alkaline phosphatase).
- Antimicrobial effect of aqueous and methanolic extracts on several pathogenic species.
Chapter 2

Materials and Methods

2.1 Plant material

*M. sylvestris* as whole aerial plant (leaves, flowers... etc.) was collected and identified from local folk medicine practitioners. The plant was identified according to the characteristics described in “Handbook of Medicinal Herbs” book (Duke et al., 2002). The plant was left for several days to air dry away from sunlight.

2.2 Extract preparation

Dry plant material was soaked in pre-boiled water and left to soak for about 15 minutes. The suspension was filtered (0.45μm pore size; Millipore) and stored.

2.3 Experimental animals

Male Sprague-Dawley rats (250-300g) (Lebanese American University stock) were divided into four groups (10 rats/group) maintained at ambient temperature 20-25°C under a 12 hour photoperiod. The first group served as control and received rat chow diet (Hawa chicken stock) to which 5% coconut oil was added. The other three treatment groups received the same food, in addition; to the plant aqueous extract at different doses of 0.5g, 2g and 5g of plant in 100ml water corresponding to 95, 380, 950 mg/kg body weight. The period of the experiment was for one month, after which the fasted animals were sacrificed using diethyl ether without recovery from anesthesia.
2.4. Samples

About 7 ml of venous blood was drawn from fasted animals anesthetized with diethyl ether for the determination of cholesterol, triglyceride, blood glucose and insulin levels along with liver enzymes.

The drawn blood was deposited into glass tubes placed on ice for 30 minutes using 10ml sterile syringes. The samples were then subject to centrifugation (Sorvall, Kendro Laboratory Products) at 3000 rpm, for 20 minutes at 10°C. After centrifugation the top serum layer was transferred by pipetting into eppendorf tubes and stored at -20°C until used. All serum analysis was run in duplicate.

2.4.1. Serum Determinations

2.4.1.1 Lipemia

i. Triglyceride

Triglyceride concentration was determined using SPINREACT following the manufacturer’s protocol. Triglycerides incubated with lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (\(H_2O_2\)). In the last reaction, hydrogen peroxide (\(H_2O_2\)) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye. The intensity of the color formed is proportional to the triglycerides concentration in the sample.

\[
\text{Absorbance of sample} \times \text{Standard concentration} = \text{mg/dL Triglyceride in the sample} \\
\text{Absorbance of standard}
\]
ii. Total Cholesterol

Cholesterol concentration was determined using SPINREACT following the manufacturer’s protocol. The determination of serum cholesterol is one of the important tools in the diagnosis and classification of lipemia. Hydrogen peroxide ($\text{H}_2\text{O}_2$) formed by the following reactions yields a red color upon its reaction with 4-aminophenazine (4-AP) and phenol, catalyzed with peroxidase (POD). The concentration of cholesterol is determined spectrophotometrically at 505 nm that is proportional to the color intensity. Cholesterol is stable for 7 days at 2-8°C or 3 months at -20°C.

\[
\text{Absorabance of sample} \times \frac{\text{Standard concentration}}{\text{Absorabance of standard}} = \text{mg/dL Cholesterol in the sample}
\]

iii. HDL-cholesterol (HDLc)

The clear supernatant removed by centrifugation containing high density lipoproteins (HDL) is used for the determination of HDL cholesterol. The phosphotungsticin acid and magnesium ions precipitate the very low density (VLDL) and low density (LDL) lipoproteins from serum or plasma. HDL cholesterol is stable for 7 days at 2-8°C. HDL was determined following SPINREACT manufacturer’s protocol.

\[
\text{Absorbance of sample} \times 320 = \text{mg/ dL of HDLc in the sample}
\]

iv. LDL-cholesterol (LDLc)

The LDLc particle is lipoproteins that transport cholesterol to the cells. The previous determinations of Triglyceride, HDL-cholesterol and total cholesterol concentrations
conducted allowed the determination of LDL-cholesterol using the Friedewald formula:

\[
LDLc = \text{Total cholesterol} - \text{Triglyceride} - \frac{\text{HDLc}}{5}
\]

2.4.1.2 Liver Enzymes

i. Aspartate aminotransferase (AST)

The reversible transfer of an amino group from aspartate to \(\alpha\)-ketoglutarate forming glutamate and oxaloacetate is catalyzed by aspartate aminotransferase formerly called glutamate oxaloacetate (GOT). Malate dehydrogenase (MDH) reduces the produced oxaloacetate to malate. The rate of decrease in concentration of NADH, measured photometrically (340 nm), is proportional to the catalytic concentration of AST present in the sample (SPINREACT Kit).

\[
\Delta A/\text{min} \times 1750 = \text{U/L AST}
\]

ii. Alanine aminotransferase (ALT)

The reversible transfer of an amino group from alanine to \(\alpha\)-Ketoglutarate forming glutamate and pyruvate is catalyzed by ALT formerly known as Glutamate pyruvate transaminase (GPT). Lactate dehydrogenase reduces the produced pyruvate to lactate. The rate of decrease in concentration of NADH, measured photometrically (340 nm), is proportional to the catalytic concentration of ALT present in the sample (SPINREACT Kit).
iii. Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is an enzyme with wide tissue distribution in the body. Lactate dehydrogenase (LDH) catalyses the reduction of pyruvate by NADH. The catalytic concentration of LDH present in the sample is proportional to the rate of decrease in concentration of NADPH, measured photometrically at 340nm (SPINREACT Kit).

\[ \Delta \text{E/min} \times 1750 = \text{U/L ALT} \]

iv. Alkaline Phosphatase (ALP)

Alkaline phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in bone, liver, placenta, intestine and kidney. Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphate liberating p-nitrophenol and phosphate. The rate of p-nitrophenol formation, is proportional to the catalytic concentration of alkaline phosphatase present in the sample (SPINREACT Kit).

\[ \Delta \text{E/min} \times 4925 = \text{U/L LDH} \]

\[ \Delta \text{E/min} \times 3300 = \text{U/L ALP} \]

2.4.1.3 Glycemia

i. Glucose

Glucose is a major source of energy for most cells of the body; insulin facilitates glucose entry into the cells. Diabetes is a disease manifested by hyperglycemia;
patients with diabetes demonstrate an inability to produce insulin. The enzyme Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H₂O₂) is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase (POD).

The intensity of the color formed is proportional to the glucose concentration in the sample measured colorimatically at 505nm (SPINREACT).

\[
\text{Absorbance of sample} \times \text{Standard concentration} = \text{mg/dL Glucose in the sample}
\]

\[
\text{Absorbance of standard}
\]

ii. Serum Insulin

The rat serum insulin determination was achieved using the Rat/Mouse insulin ELISA (Enzyme Linked Immuno-sorbent Assay) kit (LINCO Research, USA). This kit is used for the non-radioactive quantification of insulin. The lowest level of insulin that can be detected by this assay is 0.2 ng/ml (35pM) insulin when using a 10 µl sample size. The specificity of this assay for rat insulin is 100%.

This assay is a direct Sandwich technique. The wells of a microtiter plate are coated by pre-titered amount of monoclonal mouse anti-rat insulin antibodies that capture insulin molecules from samples, biotinylated polyclonal antibodies bind to the captured insulin. Unbound material is washed away from the samples. Horseradish peroxidase binds to the immobilized biotinylated antibodies, free enzyme conjugates are washed away. Quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine.

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

2.5. TAG, Cholesterol and H₂O content in Stool

Stool samples were collected from each animal in every group the sample was weighed immediately and then placed for 24 hours in an oven set at 70°C. After removal of the samples from the oven they were weighed and then crushed using a mortar and pestle. An amount of 0.2g were weighed and placed in a glass tube to which 2ml of n-hexane was added. Then the glass tubes were placed in a water bath set at 40°C and stirred continuously for 2 hours. The samples were then centrifuged (Sorvall, Kendro Laboratory Products) at maximum speed for 10-15 minutes. The supernatant was then divided and SPINREACT kit was applied to determine TG and Cholesterol (Daher et al., 2003).

2.6 Inflammation

2.6.1 Experimental animals

Dawely-Sprague male rats (Lebanese American University) weighing between 250 and 300g were used. Each set of 6 rats was assigned to separate groups placed in cages. All rats had open access to water and were fed the regular chow diet. The water extract of M. sylvestris was sterilized by syringe filtration before injection.
2.6.2 Acute Inflammation

Caragenaan induced inflammation was used to determine the acute anti-inflammatory activity of the plant extract at different concentrations. Four groups each group with 6 rats received the water extract of *M. sylvestris* at concentrations of 10, 50, 100 and 250 mg/kg intraperitoneally 30 minutes prior to caragenaan injection. One group with caragenaan served as a positive control. Diclofenac (10 mg/kg) was used as a standard reference drug and was administered intraperitoneally. The subplanter injection of 0.02ml of freshly prepared 1% Caragenaan in normal saline was used to produce acute inflammation in the right hind paw of all groups (*Ajith* and *Janardhanan*, 2001; *Jose et al.*, 2004). Figure 2.1 (A) demonstrates how the aqueous extract was administered intraperitoneally.

2.6.3 Chronic Inflammation

Formalin induced inflammation was used to determine the chronic anti-inflammatory activity of the plant extract at different concentrations. Three groups each group with 6 rats received the water extract of *M. sylvestris* at concentrations of 100, 250 and 500 mg/kg intraperitoneally 30 minutes prior to formalin injection. One group with formalin alone served as a positive control. Diclofenac (10 mg/kg) was used as a standard reference drug and was administered intraperitoneally. The subplanter injection of 0.02ml of 2% formalin was used to produce chronic inflammation in the right hind paw of all groups. For 6 consecutive days the administration of the extract (100, 250 and 500mg/kg) was continued once daily (*Ajith* and *Janardhanan*, 2001; *Jose et al.*, 2004).
2.6.4 Measurements and Calculations

The paw thickness measurements were carried out using vernier calipers. For both acute and chronic inflammation the right hind paws of all the rats were measured before inducing inflammation. For the acute inflammation the measurement was taken 3 hours after carrageenan injection. On the other hand, the paw thickness for the formalin induced inflammation was measured after 6 days (Ajith and Janardhanan, 2001; Jose et al., 2004).

\( P_0 \): the paw thickness at time zero (before inflammatory induction).

\( P_t \): the paw thickness at time \( t \) (after administration of inflammatory causing agent).

Increase in paw thickness: \( P_t - P_0 \)

Percent inhibition: \[ \frac{(C - T)}{C} \times 100 \]

* \( C \): increase in paw thickness of the control.

** \( T \): increase in paw thickness of the treatments.

2.7 Gastric Damage

2.7.1 Experimental animals

Dawley-Sprague male rats (Lebanese American University) weighing between 250 and 300g were used. The animals were divided into 4 groups 6 rats each. All rats had open access to water and were fed the regular chow diet. However, to guarantee an empty stomach all animals were starved 48 hours before use. To prevent coprophagy, they were kept in cages of raised floors with wired mesh. In order to prevent excessive dehydration during starvation, the rats were supplied with 8% sucrose (w/v) solution in NaCl 0.2% (w/v) which was taken away 1 hour before carrying out the assessment (Alkofahi and Atta, 1999; Gurbuz et al., 2005).
2.7.2 Ulcer formation

Group I: Control, was given distilled water (10ml/kg).

Group II: received 100mg/kg plant extract.

Group III: received 500mg/kg plant extract.

Group IV: Reference drug, was given Cimitrel 10mg/kg (Cimitidine).

The distilled water, plants extract and reference drug were delivered orally via an intubation needle. On the first day of the experiment two doses were given, the first at 08:00 h and the second at 16:00 h. On the second day, a third dose was given 1.5 hours before inducing the gastric ulcer by ethanol 50% (w/v) (in distilled H₂O) orally in a dose of 10 ml/kg. All rats were killed by an overdose of chloroform after 1 hour of ethanol administration. The stomachs were quickly removed rinsed underneath running tap water and opened along their greater curvature (Alkofahi and Atta, 1999).

2.7.3 Measurements and Calculations

In the glandular part of the stomach the lesions were counted and measured under an illuminated magnifying microscope (10x), 1 mm of measurement was taken for 5 petechial lesions. To calculate the ulcer index, the summation of the total length of long ulcers and petechial lesions was divided by its number. To determine the curative ratio the following formula was used: (Alkofahi and Atta, 1999)

\[
\text{Curative ratio} = \frac{(\text{Control ulcer index}) - (\text{Test ulcer index})}{\text{Control ulcer index}} \times 100
\]
2.8 Microbial Growth

2.8.1. Plant extract preparation

*M. sylvestris* aqueous extract was prepared by placing the dried plant material in boiling distilled water and left to steep for about 15 minutes to a final concentration of 20mg/ml. The obtained solution was filtered by Whatman filter paper. The extract was sterilized by passing the filtered solution through a 0.45μm membrane filter (*Barbour et al.*, 2004; *Romero et al.*, 2005).

Methanolic extract was prepared by placing air dried plant in methanol for 48 hours. Extraction was repeated twice. The filtrate was then taken and subjected to rotary evaporation in order to remove the organic solvent. The remainder was then placed in an oven set at 40°C for about 48 hours to ensure the removal of methanol (*Tadeg et al.*, 2005).

2.8.2 Test organisms

Test organisms in all microbial assays were strains of bacteria and the yeast *Candida albicans*. The pathogenic bacterial strains used were identified at and obtained from Beirut Governmental Hospital and included; *Pseudomonas aeruginosa*, *Staphylococcus aureus* methicillin sensitive, *Staphylococcus aureus* methicillin resistant, *Citrobacter freundii*, *Serratia*, *Ewingella*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Salmonella*, *Proteus mirabilis*. The *C. albicans* CAI12 wild type strain used was a gift from Dr. Roy A. Khalaf (Lebanese American University; Byblos, Lebanon). The microorganisms were maintained on Trypton soy agar and *C. albicans* on Sabauraud dextrose agar with frequent sub-culturing.
Table 2.1 Methods used for the identification of the pathogenic microbial strains used in the antimicrobial screening activity of *Malva sylvestris*.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Method of Identification</th>
</tr>
</thead>
</table>
| *Candida albicans* | Germ tube formation  
Light green (Chrom agar)  
Biolog              |
| *Citrobacter freundii* | API 20E                  |
| *Enterobacter cloacae* | API 20E                  |
| *Escherichia coli* | Urea negative/ Indole positive |
| *Ewingella americana* | Phoenix                  |
| *Klebsiella pneumonia* | API 20E                  |
| MRSA *            | Oxacillin resistant      |
| MSSA *            | Oxacillin sensitive      |
| *Proteus mirabilis* | Urea positive/ Indole negative |
| *Pseudomonas aeruginosa* | Oxidase positive/ Isolation at 42 °C |
| *Salmonella typhi* | API 20E                  |
| *Serratia marcesens* | Phoenix                  |

* Glucose positive, Catalase positive, Coagulase positive, and DNase positive

**2.8.3 Disk Diffusion Method**

To assess the occurrence of antimicrobial activities in the methanolic and water extracts of *M. sylvestris* a single-disk diffusion method was used. A pure colony from each of the test organisms was taken and placed in 5ml of 0.85% NaCl then the turbidity of each was adjusted according to 0.5 McFarland Standard and through spectrophotometry to 0.125 at 550nm corresponding to 150 x 10⁶ cells/ml. Four blank sterile disks (Mast Group Ltd.) were placed on Muller-Hinton agar to which 0.1
ml of the bacterial preparations was spread. One disk had 20μl water applied and served as a control. The plant extract was applied at a volume of 20μl but at different concentrations 400, 200, 100, 50, 20, and 10 μg and in duplicate. According to each bacterial strain a reference antibiotic which served as a positive control was placed at the center of the plate imipenem (10 μg/ disc), cefepime (30 μg/ disc), vancomycin (30 μg/ disc), bacitracin (25 μg/ disc), and tetracycline (30 μg/ disc). The plates were incubated for 24 hours at 37°C. Antimicrobial activity was assessed by measuring the zone of inhibition from the extract against the tested organism and compared to the reference antibiotic (Romero et al., 2005; Tadeg et al., 2005; Barbour et al., 2004).

2.9 Statistical Analysis
Experimental data were expressed as mean ± SEM. All determinations were subject to statistical analysis using the student TTEST to determine the significant difference between the treatment groups and the control. All values were considered significant with a value of p < 0.05.
Chapter 3

Results

After administering *M. sylvestris* aqueous extract for a one month period along with rat chow diet supplemented with 5% coconut oil, the animals were sacrificed and blood samples were collected which served for the determination of triglyceride, total cholesterol, HDLc, LDLc, liver function tests and glycemic profile. Another set of rats served for the purpose of determining the effect of the aqueous extract on both chronic and acute-induced inflammation at diverse doses. In addition, the aqueous extract was tested for any effect on induced gastric damage. Finally, the antimicrobial effect using several pathogenic strains was assessed in vitro using the aqueous and methanolic plant extract.

3.1 Blood Parameters

3.1.1 Total, HDL and LDL cholesterol

No significant changes were observed in the mean total cholesterol, HDL and LDL concentrations among the different tested groups compared with the control. Results are shown in table 3.1.
3.1.2 Triglycerides

No significant changes in serum triglycerides were observed between the control and the low dose extract group. However, a significant increase in the TG concentration was seen in the GII (380 mg/kg body weight) and GIII (950 mg/kg body weight) which were 22.1% and 28.8% respectively. Results are shown in table 3.1.

**Table 3.1** Serum total cholesterol (mg/dl), TG (mg/dl), HDL-cholesterol (mg/dl), LDL-cholesterol and Triglycerides (mg/dl) in control and experimental groups of rats (n=10). The ratio of HDL/LDL is also shown. Values denote mean ± SEM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>65.30 ± 3.45</td>
<td>61.02 ± 4.30</td>
<td>67.74 ± 3.80</td>
<td>66.46 ± 5.52</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>26.91 ± 1.87</td>
<td>26.00 ± 1.71</td>
<td>26.94 ± 1.62</td>
<td>25.33 ± 1.72</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>29.56 ± 1.59</td>
<td>26.10 ± 3.14</td>
<td>26.40 ± 2.26</td>
<td>27.54 ± 2.50</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>44.1 ± 2.64</td>
<td>44.7 ± 4.92</td>
<td>56.6 ± 5.43 *</td>
<td>61.9 ± 3.70 *</td>
</tr>
<tr>
<td>HDL/LDL</td>
<td>0.910</td>
<td>0.996</td>
<td>1.020</td>
<td>0.919</td>
</tr>
</tbody>
</table>

* Significant difference (p<0.05)
  Group I: 95 mg/kg body weight
  Group II: 380 mg/kg body weight
  Group III: 950 mg/kg body weight
3.2 Liver function tests

In order to study the effect of the aqueous extract of *M. sylvestris* on hepatocyte integrity the activities of the hepatic enzymes were measured in both control and treatment groups. Determination of serum levels of all the hepatic enzymes AST, ALT, LDH and ALP after one month administration with *M. sylvestris* aqueous extract at the three different doses GI (95 mg/kg of body weight), GII (380 mg/kg of body weight) and GIII (950 mg/kg body weight) expressed no significant changes from the control group. The results are shown in table 3.2. Values denote mean ± SEM.

**Table 3.2** Determination of serum hepatic enzyme levels (U/L) in serum after one month administration with *M. sylvestris* aqueous extracts in control and treatment groups at three different doses.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>39.11 ± 1.25</td>
<td>39.03 ± 1.75</td>
<td>36.75 ± 3.36</td>
<td>41.30 ± 2.72</td>
</tr>
<tr>
<td>ALT</td>
<td>22.14 ± 1.28</td>
<td>21.44 ± 1.01</td>
<td>20.83 ± 2.36</td>
<td>18.73 ± 1.05</td>
</tr>
<tr>
<td>LDH</td>
<td>180.50 ± 18.82</td>
<td>168.19 ± 23.83</td>
<td>188.56 ± 19.85</td>
<td>195.52 ± 21.97</td>
</tr>
<tr>
<td>ALP</td>
<td>142.63 ± 11.10</td>
<td>139.76 ± 9.26</td>
<td>130.19 ± 11.25</td>
<td>151.64 ± 10.21</td>
</tr>
</tbody>
</table>

Group I: 95 mg/kg body weight  
Group II: 380 mg/kg body weight  
Group III: 950 mg/kg body weight
3.3 Glycemia

Both glucose and insulin parameters were measured in the serum of the rats placed on extract treatment. The serum glucose and insulin concentrations of the rats (n =10/group) after one month period of extract intake, were similar in all groups as shown in figure 3.1 and 3.2.

Figure 3.1 After one month supplementation with *M. sylvestris* aqueous extract serum glucose concentrations were determined in control and treated groups of rats. Control, GI (95 mg/kg body weight), GII (380 mg/kg body weight), and GIII (950 mg/kg body weight) presented respectively.
Figure 3.2 Serum insulin levels at the end of a 4-week treatment with the water extract of *M. sylvestris* were determined in control and treated groups of rats. Control, GI (95 mg/kg body weight), GII (380 mg/kg body weight), and GIII (950 mg/kg body weight) presented respectively.
3.4 Stool samples Determinations

3.4.1 Triacylglycerol

Determination of the triglyceride content in stools revealed that all groups receiving the extract in drinking water for a period of one month had a significant increase in triglyceride content with respect to the control group. Data are shown in figure 3.3.

![Graph showing triglyceride in stool](image)

**Figure 3.3** Triglyceride (mg/gram of feces) in stool samples for control and experimental groups of rats (n=10). Values denote the mean ± SEM.

* Significant difference (p< 0.05)
3.4.2 Cholesterol

Determination of the stools cholesterol content revealed that the plant extracts at three different concentrations of 95, 380, 950 mg/kg of body weight administered in drinking water reduced cholesterol in the stools in a dose dependent manner. However, the decrease reached significance only with G II and G III with respect to the control. Data is shown in figure 3.4.

![Figure 3.4 Cholesterol (mg/g of feces) in stool samples for control and experimental groups of rats (n=10) at three different concentrations administered in drinking water 95, 380, 950 mg/kg of body weight. Values denote the mean ± SEM.](image)

* Significant difference (p< 0.05)
3.4.3 Water content of Stools

Figure 3.4 shows the water content of the stool samples. Results indicate as the dose of the extract increases there is an increase in the water content. This increase showed significance in G III (950 mg extract / kg body weight) administered in drinking water for a period of one month.

![Stool Water Content Diagram](image)

**Figure 3.5** The effect of *M. sylvestris* aqueous extract on stool water content at three different concentrations 95, 380, 950 mg / kg of body weight compared to the control. Values denote the mean ± SEM.

* Significant difference with respect to the control p < 0.05.
3.5 Anti-inflammatory activity

The anti-inflammatory activity of *M. sylvestris* aqueous extract was conducted in both chronic and acute induced inflammation models. In the acute inflammation model induced by carrageenan the plant extract exhibited significant anti-inflammatory effect which in the 100 and 250 mg/kg body weight doses exceeded that of Diclofenac. The optimum dose appeared to be the 100 mg dose. In the chronic inflammation model induced by formalin the plant extract produced a significant anti-inflammatory effect which peaked at the 100 mg/kg body weight dose. Higher doses reduced drastically the anti-inflammatory effect exerted by the plant. Data is shown in Table 3.3.
Table 3.3 *Malva sylvestris* aqueous extract effect at different concentrations 10, 50, 100, 250, 500 mg/kg of body weight and diclofenac (10 mg/kg) as reference drug on acute (carageenan) and chronic (formalin) induced inflammation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Acute Increase in paw thickness (mm) after 3 hours</th>
<th>% Inhibition</th>
<th>Chronic Increase in paw thickness (mm) after 6 days</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>1.300 ± 0.111</td>
<td>---</td>
<td>1.410 ± 0.088</td>
<td>---</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>10</td>
<td>0.788 ± 0.159</td>
<td>39.3</td>
<td>0.823 ± 0.089</td>
<td>41.7</td>
</tr>
<tr>
<td><em>M. sylvestris</em> aqueous extract</td>
<td>10</td>
<td>0.840 ± 0.097</td>
<td>35.4</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.835 ± 0.213</td>
<td>35.8</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.530 ± 0.123 *</td>
<td>59.2</td>
<td>0.605 ± 0.064 *</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.514 ± 0.118 *</td>
<td>60.5</td>
<td>1.095 ± 0.066 *</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>---</td>
<td>---</td>
<td>1.152 ± 0.050 *</td>
<td>20.2</td>
</tr>
</tbody>
</table>

* P < 0.05 significant with respect to the control.
3.6 Ulcer Prevention Study

The presence of extended and petechial lesions was characteristic of ethanol induced ulcer. In both treatment groups of aqueous extract 100 and 500 mg/kg body weight, the ulcer index was lower than the control group. The 500 mg/kg aqueous extract of *M. sylvestris* showed an ulcer index lower than that of the reference drug Cimetrid (Table 3.5). Figure 3.11 shows the percentage of ulcer prevention in the treated groups.

Table 3.4 The ulcer index value (mm) in the two experimental groups and the reference drug (Cimetrid). (n = 6). Values refer to the mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>G I (100 mg/kg)</th>
<th>G II (500 mg/kg)</th>
<th>Cimetrid (10 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>24 ± 0.53</td>
<td>18.7 ± 0.42</td>
<td>15.2 ± 0.33</td>
<td>17 ± 0.41</td>
</tr>
</tbody>
</table>

![Ulcet Curative Ratio](image)

**Figure 3.7** The ulcer curative ratio in the 100 and 500 mg/kg doses of the *M. sylvestris* aqueous extract. The values indicate the percentage of protection against ethanol induced ulcer.
3.7 Microbial Growth

3.7.1 Water and Methanolic extract

The antimicrobial effect of *Malva sylvestris* aqueous and methanolic extracts were tested against 11 pathogenic bacterial strains at six different concentrations 400, 200, 100, 50, 20 and 10 μg/20 μl on each disc. In addition, the methanolic extract was tested against *C. albicans*. Antibiotics known for inhibiting each kind of bacteria was used as reference drug respectively. After observing the microbial plates, growth of the tested microorganisms was not inhibited by any of the tested aqueous nor methanolic extract concentrations and hence no effect was revealed since no inhibition zones were observed. Data is shown in table 3.5.

![Diagram of bacterial growth inhibition](image)

**Figure 3.8** *Escherichia coli* after incubation for a period of 24 hours with three of the different aqueous extract concentrations (400, 200 and 100 μg/20μl) and reference antibiotic cefepime (30μg/disc) along with a control (sterile distilled water).
Table 3.5 The antimicrobial activity of *Malva sylvestris* aqueous and methanolic extract against the bacterial strains based on disc diffusion method.

<table>
<thead>
<tr>
<th>Bacteria Tested</th>
<th>Reference Drug</th>
<th>Antibiotic sensitivity</th>
<th>Extracts zone of inhibition**</th>
</tr>
</thead>
<tbody>
<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>-</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, S</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, S</td>
<td>-</td>
</tr>
<tr>
<td><em>Ewingella americana</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, R</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>Imipenem (10 µg/disc), Cefepime (30 µg/disc)</td>
<td>S, S</td>
<td>-</td>
</tr>
<tr>
<td>MRSA</td>
<td>Vancomycin (30 µg/disc)</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>MSSA</td>
<td>Vancomycin (30 µg/disc)</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>Imipenem (10 µg/disc), Tetracycline (30 µg/disc), Cefepime (30 µg/disc)</td>
<td>S, R, S</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc)</td>
<td>S, S</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>Bactrine (25 µg/disc) *</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Cefepime (30 µg/disc), Imipenem (10 µg/disc), Tetracycline (30 µg/disc)</td>
<td>S, S, R</td>
<td>-</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Ketoconazole (30 µg/disc)</td>
<td>S</td>
<td>-</td>
</tr>
</tbody>
</table>

* Bactrime consists of: 23.75 µg Sulfamoxazole & 1.25 µg Trimethoprim.
** The aqueous extract showed no inhibition in all the concentrations tested.
The methanolic extract showed no inhibition zone in all tested concentrations.
S: susceptible, R: resistant
Chapter 4

Discussion & Conclusions

Originating from Asia and Europe \textit{Malva sylvestris} has been used traditionally to treat cough, rashes and burns, and its abundant mucilage content forms a protective layer on inflamed mucous membranes (Chevallier, 1996). Despite the popular use of this species as a medicinal plant, there are no data about its pharmacological effect. The aim of the present study was to investigate whether \textit{M. sylvestris} after a one month period administration \textit{in vivo} via drinking water in male Sprague-Dawley rats has a beneficial effect on lipid profile, hepatic enzymes and glycemia. The anti-inflammatory activity in acute and chronic inflammation models \textit{in vivo} and the antimicrobial activity against hospital isolated pathogenic strains were also evaluated. The present study also investigated the protective role of the plant water extract upon ethanol-induced gastric ulcer. Scientific evidence is scarce for most of the above mentioned activities in scientific journals though the plant and its extracts have been used extensively in traditional medicine.

The drying process may trigger a conformational change to occur in some of the chemical constituents found in the herbs. Thus, both fresh and dried plant material should be tested to determine if such a difference exists. However, the active product is expected to be more concentrated in dried preparations then in fresh plant material (Romero et al., 2005). In order to minimize variation due to geographic factors plant material should be collected from the same area (Ellof, 1999). This may be difficult if the species is not available in an enough amount at a certain location and hence,
collection from other sites may not result in the presence of the biologically active compounds (Nigg and Seigler, 1992). Scientific studies aimed at the isolation and identification of active substances could also disclose compounds at more amounts with better therapeutic value.

Experimentally the administration of high cholesterol and lipid diet can produce hyperlipidemia and thus the protecting effect of some plant extracts has become center of attention for researchers (Blazovics et al., 1993). Of the natural compounds found in plant-derived human diet are flavonoids, which have been shown to protect low density lipoproteins from oxidation and thus prevent atherosclerotic plaque formation (Gryglewski et al., 1987). Decreased incidence of cardiovascular diseases is correlated with flavonoid-rich foods where they have a beneficial effect on lipid metabolism (Hertog et al., 1993). As a pioneer study, the present investigation revealed the effect of M. sylvestris (at three different doses and for a month period) water extract upon blood lipid profile. Animals were fed a diet rich in coconut oil to increase their atherogenic profile. Although the plant is a rich source of flavonoids (Chevallier, 1996; Wichtl, 1994), data have shown that the extract did not have a significant effect on total cholesterol, HDL-cholesterol and LDL-cholesterol. However, a significant increase in triglycerides was observed in the treatment groups GII (22%) and GIII (40%) with respect to the control. Although high levels of serum triglyceride is among the dangerous risk factors of cardiovascular diseases (Vance, 2004), the intake of plant extract should not be considered as a risk factor for increasing cardiovascular disease since the increase in serum triglyceride concentrations observed with extract intake was within the allowable serum triglyceride range in the blood of rats (Dubey et al., 2004). The present investigation
is in contradiction with previous studies (Gryglewski et al., 1987; Hertog et al., 1993) showing the importance of flavonoids in improving blood lipid profile. This discrepancy in results may be attributed to the fact that different plant species may contain different types and concentrations of flavonoids. Also, not all flavonoids are water soluble to be extracted when incubated in pre-boiled water. Therefore, the solvent being used for extraction may play a key role in this respect.

In order to investigate the potential effect of the plant upon the normal digestive system function in term of triglyceride, cholesterol and water absorption, the latter parameters were assessed in the stools of the animals few days before the end of the study. Eating of mallow leaves has been known to ease gut irritations and give a laxative effect (Chevallier, 1996). From the stool sample analysis it was validated that the *M. sylvestris* aqueous extract had a dose dependent increase in stool water content when administrated. The increase of water content in the stools was not significant in GI and GII but significance was only reached with GIII where a 9.5% increase in water content was observed. A possible explanation for the increase in stool water content is due to the plants mucilage content since they adsorb water. Therefore, the aqueous plant extract can be suggested for use in case of constipation. On the other hand, the plant extract significantly increased the triglyceride concentration in the stool samples irrespective of the dose used. Elevated fecal amounts of TG might reflect incomplete fat hydrolysis. Such an increased triglyceride excretion in the stool, however, showed no serious impact on the plasma triglyceride. Within the intestinal lumen biliary cholesterol is the principal source of cholesterol. Cholesterol concentration in the stools showed a dose dependent decrease with extract intake
where significance was reached in group II and III. The decreased absorption of cholesterol by the digestive tract did not affect the plasma cholesterol concentration.

It is not possible to ascertain medicinal plants preparations as absolutely safe even though herbal treatments are generally perceived as harmless (Fransworth, 1993). Drugs or herbal remedies can cause damage to the liver since it is responsible for the metabolism of exogenous toxins (Pak et al., 2004). The effect of the aqueous plant extract on liver function was validated through the activities of the liver transaminases (AST and ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) after a one month administration period of the plant aqueous extract. After cellular damage there is an increase in the level of serum transaminases reflecting damage to the structural integrity of the liver (Sallie et al., 1991). The aqueous extract of *M. sylvestris* had no significant effect on the integrity of the liver and was therefore preserved. All the hepatic enzyme levels measured showed no significance compared with the control group indicating the harmless side effects of the plant extract to the liver when taken for a period of 30 days.

Due to absolute or relative lack of insulin, high blood glucose is a characteristic of the chronic disease diabetes mellitus (Villasenor and Lamadrid, 2005). A high fat ingestion and increased circulation of free fatty acids might lead to insulin resistance, and in due course to diabetes mellitus in genetically prone subjects by mechanism of lipotoxicity (Manco et al., 2004). Several complications are associated with diabetes mellitus such as atherosclerosis, myocardial infarction, nephropathy, neuropathy etc. (Sabu and Kuttan, 2002). Currently available pharmacological agents for the treatment of diabetes mellitus have a number of limitations and various adverse
effects such as bloating, flatulence, and other gastrointestinal (GI) complaints, due to these factors alternative approaches such as medicinal herbs with anti-hyperglycemic properties have been considered (Inzucchi, 2002). Tannins are compounds that may exert glycemia reduction in response to carbohydrate rich foods in humans, are present in high concentrations in some plant extracts (Gin et al., 1999). Tannins being a constituent of *M. sylvestris* led to validate the use of this plant as an anti-diabetic agent. The results demonstrated that the aqueous extract at all concentrations of *M. sylvestris* (95, 380 & 950 mg/kg body weight) showed no significant changes in plasma glucose and insulin levels. The obtained result might be due to the use of the crude aqueous extract and thus, the tannin levels were not sufficient to produce a noticeable effect in reducing glucose levels. Therefore, the use of pure active components suppresses any other ingredient activity interference and modification. This report of *M. sylvestris* on the glycemic activity requires further comprehensive pharmacological and chemical research.

*In vivo* models for acute and chronic inflammation induced by carageenan and formalin respectively were used to investigate the anti-inflammatory activity of the aqueous extract of *M. sylvestris*. A useful model to assess the involvement of mediators in vascular changes associated with acute inflammation is the carageenan-induced paw edema (Kaur et al., 2004). Reduction of edema was detected in carageenan induced inflammation in the hind paw of male Sprague-Dawley rats, with different results according to the dose used. The optimum dose appeared to be at 100 mg/kg body weight. Increasing the dose to 250 mg/kg did not improve further the edema reduction. Since the 10 and 50 mg/kg body weight doses were much less effective than the 100 and 250 mg/kg body weight doses in the acute inflammation
model, it was decided that in the chronic inflammation model, induced by formalin, doses \( \geq 100 \, \text{mg/kg body weight} \) ought to be used. Similar to the acute inflammation study the 100 mg/kg body weight dose was found to be the optimum dose in the chronic inflammation model. However, it happens that increasing further the doses to 250 and 500 mg/kg body weight reduced drastically the potential anti-inflammatory effect of the plant. This finding supports the fact that acute and chronic inflammations may have different mechanisms of response to anti-inflammatory agents (refer to page 14), or could be due to the complex composition of the extract. Possibly at doses of 250 mg/kg body weight and above certain components in the extract may accumulate in the body (after 6 days) and thereby become effective in reducing the potency of the aqueous extract as an anti-inflammatory agent. Flavonoids are known to modify eicosanoid biosynthesis and thus anti-inflammatory responses (Kuo et al., 1995). Thus, the presence of certain flavonoids in the extract can explain in part the anti-inflammatory effect observed. *M. sylvestris* leaves are considered one of the plant species used in the Mediterranean area in traditional medicine with anti-inflammation properties (Picci, 1980) and in European folk medicine since it contains the active compounds 8-hydroxy-flavonoid sulphates and flavonoid glycosides (Cordell, 1995; Kujumgiev et al., 1999).

Besides flavonoids *M. sylvestris* contains mucilage and tannins which are known for exerting a valuable effect on inflammation (Morton, 1990). Having a wide range of pharmacological activities, tannins, which are water soluble polyphenols, possess an anti-inflammatory activity perceptible by inhibiting the transcription of nuclear factor Kappa B (NF-Kappa B), inhibiting the expression of inflammatory mediators such as cytokines, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (Erdelyi et al., 2005).
A previous anti-inflammatory study on *Sida cordifolia* L. belonging to the family Malvaceae commonly known as malva-branca (white mallow) or malva-branca-sedosa (silky white mallow) showed that the white mallow interfered with the cyclooxygenase pathway (prostaglandin biosynthesis) rather than the lipoxygenase pathway. The aqueous extract showed a significant inhibition at a dose of 400 mg/kg of carrageenan-induced rat paw edema. This fact strongly suggested that *S. cordifolia* extract exerts anti-inflammatory and analgesic properties by interfering with the cyclooxygenase pathway (*Franzotti et al.*, 2000). Possibly because *M. sylvestris* and *Sida cordifolia* belong to the same family Malvaceae the anti-inflammatory effect observed might be attributed to interference with prostaglandin biosynthesis.

The gastrointestinal irritant properties of nonsteroidal anti-inflammatory drugs (NSAIDS) are the major impediments to their use for anti-inflammatory treatment (*Kushima et al.*, 2005). Therefore, the ulcerogenic side effect of NSAIDS can be reduced or antagonized with least side effects if combined with a certain plant extract known to possess antiulcer activity or by using the plant extract alone (*Munniapan and Sundraraj*, 2003). The aqueous extract of *M. sylvestris* was administered orally at 100 and 500 mg/kg body weight before gastric lesions induction by the action of ethanol. Ethanol induces disturbance of mucosal microcirculation, ischemia, free radical appearance, release of endotelin, degranulation of mast cells, inhibition of prostaglandins and decrease in mucosal production (*Samonnia et al.*, 2004).

Antiulcerogenic activity has been reported in plants containing tannins (*Yesildag and Gurbuz*, 2003) and flavonoids (*Alarcon et al.*, 1994). Oral pretreatment administration to of *M. sylvestris* aqueous extract reduced ethanol induced lesions and thus the ulcer index from 24 mm in the control group to 18.7 and 15.2 mm in the 100 and 500
mg/kg doses respectively. The group receiving Cimetrid (10mg/kg) as reference drug showed an ulcer index of 17mm which is equivalent to a curative ratio of 29%, a value coherent with data reported previously regarding cimetidine (Xu et al., 1998). Cimetrid is an anti-secretory agent which lowers gastric acid secretion thereby reducing the chance of gastric ulcer. Common mallow contains anti-oxidants, including polyphenols and anthocyanins, which explain its beneficial effect on gastroenteritis (Chevallier, 1996). Mucilaginous plants are used for the treatment of gastric ulcer since they have the property of protecting and covering the stomach (Gurbuz et al., 2005). Malva neglecta leaves are used widely for the treatment of stomach ache in many parts of turkey due to its high mucilage content (Gurbuz et al., 2005). Also, tannins along with flavonoids found in plants have been shown to contribute to the anti-ulcerogenic effect (Zayachkivska et al., 2005; Ramirez, 2003). The outermost layer of the mucosa is known to be concealed by tannins and hence render it less permeable to mechanical and chemical injury (Borreli and Izzo, 2000). Progress continues to be made to understand the mechanism through which the gastric mucosa can be protected against damage induced by exogenous agents.

Several studies have recognized constituents within herbal plants that are effective antibiotics (Basile et al., 2000). The need to discover new antimicrobial substances for use in combating the emergence of resistant bacteria to multiple antimicrobial drugs and the continual demand for new antibiotics led to investigate the antimicrobial activity of the crude water or methanolic extracts of Malva sylvestris. Gram-negative bacteria are commonly reported to have developed drug resistance of which Escherichia coli is the most illustrious (Allonso et al., 2000; Sader et al., 2002). Difference in sensitivity between gram-positive and gram-negative bacteria
might be due to differences in structural constitutions. Gram-negative bacteria unlike gram-positive have an outer phospholipidic membrane carrying the structural lipopolysaccharide components which acts as a diffusional barrier and thus makes them less susceptible to antimicrobial agents then gram-positive bacteria (Hodges, 2002). Strains from both gram-positive and gram-negative bacteria were used in this study for that purpose.

The methanol solvent is well known for its ability to isolate antimicrobials from plants including tannins, polyphenols, terpenoids, xanthoxylines, flavones, and phenones. On the other hand, water solvent extracts contain anthocyanins, starches, tannins, saponins, terpenoids, lecin and polypeptides (Cowan, 1999). *Malva parvijflora* at a concentration of 100 mg/ml (10,000 μg/disc) demonstrated comparable activity to the standard ketoconazole (0.3 mg/ml) against *Trychophyton mentagrophytes* (Tadeg et al., 2005). The antimicrobial screening tests done in this study of the dried plant material extracted both with water or methanol solvent and at the concentrations tested (highest 400 μg/disc) exhibited no antimicrobial activity against the selected bacterial strains nor *C. albicans*. In previous studies ethanol extracts of *Malva sylvestris* at a concentration of 50 mg/ml were active against *Bacillus subtilis* (Izzo et al., 1995) *Pseudomonas aeruginosa* (Alkofahi et al., 1996) and *Escherichia coli* (Izzo et al., 1995; Alkofahi et al., 1996). A reason behind such non-coherent results might be that the active components known to exhibit antimicrobial activity were not present in sufficient quantities and thus higher concentrations of the extract are needed in order to induce an antimicrobial activity. The reason of not using such high concentrations in the present study (50 vs. 20 mg/ml) is that our aim is to find if any effect is exerted at low concentrations similar
to what would be administered through a water extract preparation and drunk not highly concentrated. Hence, the use of higher concentrations is not favorable if a certain drug is to be used for treatment in human at such very high concentrations. Also the difference in results may be attributed to the use of different extraction solvents (ethanol versus methanol).

Briefly, this study provides preliminary data on all tested parameters. Therefore, further fractionation and purification of the therapeutic components from the crude extracts is recommended in order to specifically isolate the therapeutic agent for each condition and thus reduce risk factors and side effects. The plant aqueous extract appears to have a promising effect at the 100 mg/kg body weight dose in acute and chronic inflammation along with its protective ulcerogenic activity compared to standard reference drugs. Both results are of immense importance since most anti-inflammatory drugs lead to ulcer development. Therefore, the pursuit for non ulcerogenic anti-inflammatory drugs is of great clinical importance. No change in serum insulin and glucose levels was witnessed in the animals tested at the end of the study. The safe use of the aqueous plant extract was demonstrated with no increase in any of the hepatic enzymes measured. The atherogenic risk of *M. sylvestris* water extract administration for a one month period along with a high fat diet appeared to be non significant. Neither aqueous nor methanolic extract of *M. sylvestris* produced zones of inhibition in the Kirby-Bauer analysis. Further research is necessary to determine the full spectrum of efficiency in medicinal plants.
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