Effect of water extract of *Althaea officinalis* flowers on inflammation, gastric ulcer, bacterial activity, platelet aggregation, glycemia, and lipidemia

by

Rouba Hage-Sleiman

A thesis submitted in partial fulfillment
of the requirements for the degree of

Master of Science

Molecular Biology

Lebanese American University

May 2006

Under the supervision of Dr. Costantine Daher

Date of the Thesis defense public announcement: *May 26, 2006*

LEBANESE AMERICAN UNIVERSITY

Thesis approval Form

Student Name: *Rouba Hage-Sleiman* I.D. #: *200301942*

Thesis Title: *Effect of water extract of Althaea officinalis flowers on inflammation, gastric ulcer, bacterial activity, platelet aggregation, glycemia, and lipidemia*

Program : *Molecular Biology*

Division/Dept : *Natural Sciences Division*

School : *School of Arts and Sciences*

Approved by

Thesis Advisor: *Dr. Costantine Daher* [redacted]
Chairperson of Supervisory Committee

Member : *Dr. George Baroody* [redacted]

Member : *Dr. Roy Khalaf* [redacted]

Date **June 2006**

I Grant to the **LEBANESE AMERICAN UNIVERSITY** the right to use this work, irrespective of any copyright, for the University's own purpose without cost to the University or to its students, agents and employees. I further agree that the University may reproduce and provide single copies of the work, in any format other than in or from microfilms, to the public for the cost of reproduction.

Rouba S. Hage-Sleiman

May, 2006

I dedicate my thesis to:

My beloved parents Saleh and Hiba Sleiman

My only brother Rida

My second-half Dr. Jalil Sleiman

Who have always been there for me sources of inspiration, moral support and hope

I love you so much

God bless you real good

The possession of knowledge does not kill the sense of wonder and mystery. There is always more mystery.

Anais Nin

Beware of false knowledge; it is more dangerous than ignorance.

George Bernard Shaw

Science is organized knowledge. Wisdom is organized life.

Immanuel Kant

A little knowledge that acts is worth infinitely more than much knowledge that is idle.

Gibran Khalil Gibran

Effect of water extract of *Althaea officinalis* flowers on inflammation, gastric ulcer, bacterial activity, platelet aggregation, glycemia, and lipidemia

ABSTRACT

Nowadays, the consumption of herbal remedies is becoming more popular based on the fact that herbal products are relatively safe, effective and inexpensive. The present investigation was undertaken to explore and shed light on possible medicinal effects of the water extract of common marshmallow (*Althaea officinalis*) flowers upon blood lipid profile, glycemia, inflammation, platelet aggregation, ulcer and bacterial activity. After one and two months of chronic extract intake via drinking water (59, 230 and 590 mg of extract / kg body weight), a drastic increase in HDL-cholesterol was observed, along with sharp decreases in LDL-cholesterol and mild increases in triglyceride. At the same time, the water extract showed no adverse effect on liver function. An increase in both serum glucose and insulin levels was observed in animals receiving the extract for one month, which raise the possibility of development of insulin resistance syndrome. The effect of water extract of the flowers of *Althaea officinalis* (50, 100 and 250 mg water extract / kg body weight) on ethanol-induced gastric ulcer showed a remarkable anti-ulcerogenic activity that appeared to be better than antisecretory drug such as Cimetril. The water extract of *Althaea officinalis* flowers (100, 250 and 500 mg of flowers / kg body weight) exhibited substantial antiinflammatory effects *in vivo* in both acute inflammation and chronic inflammation induced by carrageenan and formalin respectively. In addition, 500 µg of the pure extract showed a time-dependant inhibition of platelet aggregation thus protecting from thrombosis and shock. The water extract of the flowers of *Althaea officinalis* (200, 100, 50, 20 and 10 µg / 20 µl) didn't have any potential antibacterial effect against the hospital bacterial isolates studied. In conclusion, the water extract of the flowers of *Althaea officinalis* showed anti-ulcerogenic, anti-inflammatory and platelet antiaggregant activities. The extract had also a positive effect on blood lipid profile with no adverse effect on the liver which shed the light on the importance of *Althaea officinalis* water extract as a remedy for treating patients with hypercholesterolemia. Only one draw back of the extract was observed due to the hyperglycemic and hyperinsulinemic effects it caused.

TABLE OF CONTENTS

LIST OF FIGURES

Number **Page**

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and gratitude to my supervisor Dr. Costantine Daher, for all the helpful advice, support and directions he gave me from the writing of the proposal till the completion of this thesis.

I would like to thank the members of the committee, Dr. George Baroody and Dr. Roy Khalaf for their directions and valuable comments and Dr. Sima Tokajian for sharing her knowledge.

I am so grateful to Dr. Fouad Hashwa, Dean of Arts and Sciences for all the facilities he is providing us with and without whom all our researches could not have been accomplished.

Special thanks to:
Miss Helena Bou Farah for her continuous support and facilitations
Mr. Jean Karam for taking care of my experimental rats and for assisting in many experiments
Mrs. Marie-Therese Mitri for providing me with references and articles
My friend Hamdan Ghazi Hamdan for his help and support

Last but not least, I would like to thank GOD for giving me faith, strength and persistence to employ my knowledge for the best of me.

ABBREVIATIONS

ADP	Adenosine 5'- diphosphate
Apo	Apolipoprotein
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
CETP	Cholesteryl ester transfer protein
CHE	Cholesterol esterase
CHO	Cholesterol oxidase
CM	Chylomicrons
ELISA	Enzyme Linked Immuno- Sorbent Assay
ET-1	Endothelin 1
g	Gram
x g	Times gravity (units of gravity)
GK	Glycerolkinase
GOD	Glucose oxidase
GPO	Glycerol phosphate dehydrogenase
HDL	High Density Lipoprotein
IDL	Intermediate Density Lipoprotein
LCAT	Lecithin cholesterol acyltranferase
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
LPL	Lipoprotein lipase
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*

µg	Microgram
µl	Microliter
mm	Millimeter
min	Minute
ng	Nanogram
NAD^+	Nicotinamide adenine dinucleotide
NADH	Redued nicotinamide adenine dinucleotide
p	Probability
POD	Peroxidase
K_2HPO_4	di-Potassium hydrogen Orthophosphate anhydrous
PRP	Platelet rich plasma
r.p.m	Round Per Minute
SGOT	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
SEM	Standard Error Mean
NaH_2PO_4	Sodium Dihydrogen Orthophosphate 2-hydrate
Na_2HPO_4	di-Sodium hydrogen Orthophosphate anhydrous
NaCl	Sodium chloride
TAG	Triacylglycerol or triglyceride
TBS	Tris Buffered Saline
VLDL	Very Low Density Lipoprotein

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

Herbal medicine is an aspect of folk medicine which refers to unwritten and orally transmitted traditional procedures used for treatment of different diseases and disorders. In different cultures, at the herbal level, medicines were developed by personal experience and passed from one generation to generation simply by word of mouth. They consist of gathered plants used most of the time as infusions or ointments of one type of plant or combination of plants. Herbal treatment did not have to work miracles but to cure simple disorders, including headaches, digestive complaints, antiseptic needs, ophthalmia, fever, rheumatic pain, skin eruptions, respiratory complaints and dysentery, etc... The medical practices of many cultures in both past and present are shrouded in mystery. Critics accuse many of these practices as chicanery and many are attacking the folk medicine for becoming a business. This type of medicine is nowadays practiced by anyone without any control and is found to become spread everywhere. Many folk remedies are certainly based upon primitive beliefs of the cause of disease but also based on variety of folklore, sorceries and superstitions. In order to improve the efficacy of this medicine, ethnobotanists, biologists, pharmacologists and chemists are increasingly working to separate science from sorcery and folklore from pharmacology giving more importance to folk medicine. To benefit the most from the pharmacy of nature where men can find for every disease a cure, people must learn how to distinguish between different types of plants, how to collect them, how to use them and especially what parts (flowers, stems, leaves and roots) to consume since some parts might be poisonous. This science should be given importance and must be preserved, improved and not lost year after year (Steiner, 1986).

1.1. Plant distribution and taxonomy

Althaea officinalis belongs to the genus *Althaea* of the family *Malvaceae* and subfamily *Malvaideae*, which includes as many as 300 species. It is commonly known as "marshmallow" and popularly known as "khitmi" or "khitmiyeh" in Arabic. The generic

name Althaea is derived from the Greek althos "a cure" and althaimo "to heal". The name of the order, Malvaceae is derived from the Greek malake "soft" from its special qualities in softening and healing (Mouterde, 1970; Juneidi, 1999; Quattrocchi, 2000). The medicinal parts of this plant are the flowers that appear between July and September, the roots and the leaves. The plant is 60 to 120 cm high hardy, velvety plant with 50 cm long by a few cm thick erect root with secondary roots. Its flowers are reddish-white flowers with 5 heart-shaped petals and numerous stamens fused together with the anthers to a column. The 6 to 9 sepals are fused at the base, pointed and 8 to 10 mm long. *Althaea officinalis* grows in salty lands, in damp meadows, by the sea and on the banks of tidal rivers (Culberth, 1927; Medical Economics Company, 1998). This plant is most abundant in Lebanon, Syria, Turkey, Iraq, Palestine, Iran, Armenia, China, Italy, Romania, Greece, Spain, USA and France. In the Arab world *Althaea officinalis* is consumed mainly for its flowers as "zhourat" a mixture of plants consisting of Chamomile, Zoufa, Rosemary, Zayzafoun and Melissa unlike in the occident where the roots are the most important. The marshmallow must not be confused with the *Malva silvestris* known in Lebanon as "khibayzet barieh" because of the high similarity in the shape (Mouterde, 1970; Juneidi, 1999). For further information concerning the classification, common names and illustrations of Althaea officinalis refer to the Appendix I and II.

1.2. The traditional recipes and prescriptions of Mallows

Althaea officinalis as most of the mallows has been used in many different cultures for so many reasons. It is a rich source of fibers used in salads and in paper-making (Mabberley, 2000). The Romans and Egyptians considered it as a luxurious plate and one of their delicacies. It is much mentioned in their myth and books that mallows were used as laxative and suppressor of inflammation as well as used to decorate the graves of friends. The Chinese use it in the food as well as the poor people of Syria, especially the farmers that depend on the roots as herbal source of food. They eat it boiled than fried along with onions and butter and sometimes served with fried eggs. Arab physicians used the leaves as a poultice to suppress inflammation. In France, the leaves of marshmallows are eaten uncooked in spring salads because they are thought to stimulate kidneys .The flowers boiled in oil and water along with honey are used as gargle for sore throats and a remedy

for colds. The emperor Charlemagne ordered marshmallows cultivation in monasteries. (Cornucopia, 1990)

The roots of *Althaea officinalis* are always given in decoction or infusion. The crushed root boiled in milk forms an admirable body for poultices in irritable swellings and sores and in bruises, scalds and burns. The roots' dust has been used as an absorbent in making pills (Cook, 1869).

1.3. Studies done on certain Althaea species

Many Althaea species are identified and used for their medicinal effects even though no studies were done on most to scientifically prove these practices. *Althaea rosea, Althaea nudiflora* and *Althaea armeniaca* were the only few species that were studied but still not all for their medicinal effects (Chladek and Patakova, 1968; Franz and Chladek, 1973; Recio et al., 1989; Wang et al., 1989; Sagdullaev et al., 2001; Papiez et al., 2002).

Althaea rosea also known as *Alcea rosea* is commonly known as hollyhock and is much cultivated in the gardens for its large and beautiful flowers (Cook, 1869). The flowers are commercially used to give color to wines and juices. Along with roots, the flowers are demulcent and their mucilaginous constituents are good for irritations of cough, stomach, bowels, bladder and urethra (Hocking, 1997).

The ethanolic extract of the flower of *Althaea rosea* were found to inhibit significantly both the twisting movements of mice and increase in permeability of abdominal blood capillaries induced by acetic acid. In addition, these latter also inhibited the heat induced (tail) flicking of rats and the edema of the rat paw induced by carrageenan or dextran and finally the release of prostaglandins from inflammatory tissue (Wang et al., 1989).

In another study, the flowers of *Althaea rosea* were proved to be source of flavonoids and anthocyanins. These belong to phytoestrogens that can play the role of both agonists and antagonists of estrogen and modify the reproductive function of the male. The study focused on investigating whether aqueous hollyhock extract affects the process of aromatization in the testes and in cultured Leydig cells. It was found that a weak antiestrogenic activity of flavonoids compounds found in the plant is mediated through

aromatase and estrogen receptor beta and this means that *Althaea rosea* affect the aromatization in rat testicular cells in vivo and in vitro (Papiez et al., 2002).

Another study was done on Spanish Mediterranean plants including *Althaea rosea* and other 58 plants. This study documented that the chloroform and methanol extracts of this whole plant have antimicrobial activity against *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Staphylococcus aureus*. The study included both disc diffusion and MIC method for detection of antibacterial activity. Bioautography showed that the antimicrobial activity is probably due to the flavonoids, terpenoids and phenolic acids (Recio et al., 1989).

Lipids from roots of *Althaea nudiflora* and *Althaea armeniaca* were shown to contain 22.3 and 12.6 %, respectively of cyclopropenoid fatty acids (Sagdullaev et al., 2001). Two studies were done on the crossbred descendants of *Althaea officinalis* and *Althaea armeniaca* concerning their roots, leaves and flowers morphologies and the comparative composition of their mucus. All the polysaccharides including galactose, glucose, arabinose, xylose and rhamnose were found in a very good percentage in the mucilage of the crossbred descendants. The morphology of all parts was different from *Althaea armeniaca* and *Althaea officinalis* and their shape was between these two for the leaves were big and heart shaped and the flowers were bigger than the two original strains (Chladek and Patakova, 1968; Franz and Chladek, 1973).

1.4. *Althaea officinalis* constituents and their medicinal effects

The most important constituent of *Althaea officinalis* is mucilage (10-20%), which is found the most in the roots and had been investigated quantitatively and qualitatively. The analytical detection of these polysaccharides is very time consuming and indirect (Schulz and Albroscheit, 1988). The mucilage content of the roots is highest in the fall and winter (11%) and lowest in spring and summer (6%) (Basch et al., 2003). It is a mixture of collodially soluble polysaccharides particularly galacturonic rhamnans, arabinogalactans, arabans and glucans that soak up water giving a sticky jelly-like structure (Tomoda et al., 1980; Blaschek and Franz, 1986; Capek et al., 1987). It is a thick and glutinous substance secreted by the seed covers of plants including

marshmallows and flaxes. It is the chief constituent of agar and employed as an adhesive. Mucilages line the mucous membranes of the digestive track and respiratory system protecting from irritation and acidity. In addition, because of their smooth and slippery property, mucilages were found to play a hypoglycemic activity and an antitussive activity (Tomoda et al., 1987; Nosal'ova et al., 1992). In addition to all this, it is suspected to be responsible for the anti-inflammatory effect of the roots of *Althaea officinalis* (Scheffer and Konig, 1991).

In addition, *Althaea officinalis* contains starch (37 %), pectin (11 %), oil, sugar (11%), asparagin (1-12%), alanine, asparaginic acid, cellulose, fat (1.7%), calcium oxalate and glutinous matter (Barnes et al., 2002; Basch et al,, 2003). Pectin is responsible for the hypoglycemic effect of this plant (Siddhu et al., 1989). In the roots of this latter plant, flavonoids, phenolic acids and coumarins were identified. Coumarins are toxic, fragrant and organic compounds (Gudej, 1991; Cowan, 1999). Flavonoids are anti inflammatory useful in maintaining healthy circulation and are protective against gastric ulcer (Izzo et al., 1944; Zayachkivska et al., 2005). Phenolic acids are antiseptic and reduce inflammation when taken internally (Gudej, 1991; Cowan, 1999). Marshmallow is high in aluminum, iron, magnesium, selenium, tin and calcium (Basch et al., 2003).

1.5. The roots of *Althaea officinalis* used as medicinal agents

The marshmallow roots are known scientifically as *Althaea radix*. The roots are used as demulcent , sedative in respiratory diseases as in cough, hoarseness , bronchitis , asthma as well as for skin burns and in cosmetics, plastic masses and previously in manufacturing the marshmallow candies now replaced by mixture of gelatin , sugar and other constituents. The Althaea roots are called *Althaea unguentum* when they are prepared as an ointment consisting of finely powder of marshmallow root with pine resin, yellow wax turpentine and olive oil (Hocking, 1997).

Due to its importance in the medicinal and industrial field, a study was done on the roots of *Althaea officinalis* in an aim to find out the stimulators of root growth. It was found that when naphthylacetate was added an increase in root growth was obvious compared to the unstimulated control (Chladek, 1959).

One study was performed to evaluate the antibacterial activity of *Althaea officinalis L.* roots along with other plants' flowers and leaves. The effect of these medicinal plants were assessed on periodontopathic facultative aerobic and anaerobic bacteria including *Porphyromonas gingivalis*, *Prevotella spp.*, *Fusobacterium nucleatum*, *Capnocytophaga gingivalis*, *Veilonella parvula*, *Eikenella corrodens*, *Peptostreptococcus micros* and *Actinomyces odontolyticus*. In this study, methanol extracts were found to be active against *P.gingivalis*, *Prevotella spp.* and *Actinomyces spp.* The water extracts were less active than methanol extracts because the active constituents are more soluble in methanol. Given that all periodontal infections can cause more or less serious damage, from gum inflammation to the loss of alveolar bone and eventually the loss of a tooth, it is very important to note that *Althaea officinalis* methanol extracts inhibits the activity of these bacteria that have intrinsic resistance to many antibiotics used in odontotherapy, such as Peptostreptococcus spp. *Althaea officinalis* will serve as mouthwashes to reduce microbial populations from the mouth (Iauk et al., 2003).

Vibrio cholerae, the bacterium responsible for the cholera and the major cause of diarrhea in humans was studied using many plants. The water extract of the roots of *Althaea officinalis* were one of these plants of interest that showed no "in vitro" effect against *V. cholerae* (Guevara et al., 1994).

Intragastric administration of polysaccharides isolated from aqueous extract of the roots of *Althaea officinalis* to cats showed that this plant has antitussive action. It suppressed the intensity and frequency of coughs mechanically induced by irritation of laryngopharyngeal and tracheobronchial mucosa (Nosal'ova et al., 1992).

In addition, the polysaccharides extracted from the roots of *Althaea officinalis* have anti-inflammatory activity. Aqueous extracts stimulated phagocytosis and induced release of oxygen radicals, cytokines, interleukins 6 and tumor necrosis factors from human monocytes in vitro thus exhibiting anti-inflammatory and immunostimulant activity (Scheffer and Konig, 1991).

Another study also approves the above study where mucilage polysaccharides of *Althaea officinalis* were administered to mice and revealed a 2.2 fold increase in the phagocytic activity of macrophages (Wagner and Proksch, 1985).

A biological screening of Italian medicinal plants for anti-inflammatory activity was done and revealed no activity for ethanol extract of the roots of marshmallow in the carrageenan induced rat paw edema test (Mascolo et al., 1987).

The mucilage has demonstrated considerable hypoglycaemic activity in non-diabetic mice. At doses of 10 mg/ kg, 30 mg / kg and 100 mg / kg, it reduced fasting plasma glucose levels to 74 %, 81% and 65% of prior values, respectively, after seven hours of extract intake (Tomoda et al., 1987). Precautions in *Althaea officinalis* consumption must be taken when diabetic drugs are administered.

No information is available concerning the drug and laboratory test interaction, carcinogenesis, mutagenesis, infertility, teratogenic ad non-teratogenic effects in pregnancy or nursing mothers. Therefore, precautions should also be taken for pregnant and lactating mothers as well as for children.

Combinations of marshmallow preparations with steroids have been used in the management of dermatological conditions and its anti-inflammatory activity potentates other topical steroids. Marshmallow was reported as being superior to dexamethasone monotherapy in a study conducted to assess the anti-inflammatory properties of marshmallow alone and in combination with dexamethasone in rabbits in response to irritants (Beaume and Balea, 1966; Huriez and Fagez, 1968).

Last but not least, a study was done on the inhibitory mechanism of an extract of *Althaea officinalis* on endothelin 1-induced melanocyte activation. In this research, the roots were found to inhibit both the secretion of endothelin 1 (ET-1) from normal human keratinocytes and the action of ET-1 on normal human melanocytes after UVB radiation. This suggests that the extract inhibited the pigmentation and thus can be considered a useful ingredient for a whitening agent (Kobayashi et al., 2002).

1.6. Lipids

1.6.1. Introduction to lipids: general overview

Lipids are essential components of all living organisms. They are composed of many types of molecules; the majors of these classes are fatty acids, steroids, lipid vitamins and terpenes. Fatty acids are generally stored as neutral lipids called triacylglycerols or triglycerides. Most lipids in the human diet are triglycerides that are broken down in the

small intestine by the action of lipases. Most of the lipids are stored in adipose tissues as energy source and as thermal insulation (Horton et al., 2002).

Many diseases are directly related to increased levels of triglycerides and cholesterol. Although it was thought that diets have less influence on plasma triglycerides levels and consequently on coronary heart disease, recent studies revealed that elevated triglyceride serum concentration is significantly correlated to coronary heart disease risk (Grundy and Denke, 1990).

The steroid cholesterol is an important component of animal plasma membranes, rarely found in plants and never in prokaryotes, protists or fungi (Alberts et al., 2002). Despite its role in cardiovascular diseases, cholesterol plays an important role in mammalian biochemistry. It is a component of certain membrane; it repairs it by modulating and stabilizing its fluidity and thus facilitating the transport across it. In addition, cholesterol is a precursor for steroid hormones and bile salts synthesis. These latter are crucial for the uptake of dietary fats by the small intestine (Horton et al., 2002; Kingsburg and Bondy, 2003).

Cholesterol is a principal manufacturing factor of vitamin D in the skin's surface. A recent study showed that cholesterol plays a role in cell connections in the brain which implies that it is good for learning and memory abilities (Ger Van and Bittar, 2004). Even though cholesterol is very important and essential for many functions, one can not neglect the bad consequences of consuming cholesterol, in what it affects the human well-being. Approximately ninety years ago, Anitschkow and Chalatow (1913) showed that feeding cholesterol to rabbits caused atherosclerosis.

Since then, many studies revealed a relation between high intake of dietary cholesterol, severe hypercholesterolemia and atherosclerosis in many animals. Few studies suggested that dietary cholesterol has almost no influence on serum cholesterol (Slater et al., 1976; Porter et al., 1977; Flynn et al., 1979) but others reported that vegetable oils reduce serum cholesterol levels (Kinsell et al., 1952; Ahrens et al., 1957).

In order to diagnose any disease related to cholesterol, it is mandatory to evaluate the levels of LDL and HDL in the blood, which are commonly known as "bad and good" cholesterol, respectively.

1.6.2. Blood Lipoproteins

Since both triglycerides and cholesterol serve several important functions within the body but are insoluble, they must be packaged into complexes called lipoproteins. These complexes are composed of a core of triglycerides and cholesterol ester, enveloped by phospholipids, free cholesterols and special proteins called apolipoproteins. They are identified as five lipoproteins, which are chylomicrons, very low-density lipoprotein cholesterol (VLDL), intermediate-density lipoprotein cholesterol (IDL), low- density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL) (Kingsbury and Bondy, 2003). These lipoproteins differ by their size and density and are functionally two main classes. The first consists of particles whose main role is to deliver triglycerides from the liver or intestine to peripheral, extrahepatic tissues and includes: chylomicrons (the largest in size and lowest in density), VLDL and IDL. The second consists of cholesterol carrying particles and includes LDL and HDL (P.E. Fielding and C.J. Fielding, 2002) (Table 1.1).

Table 1.1 Major lipoprotein classes (CM, VLDL, IDL, LDL, HDL), their constituents and their source (Kingsbury and Bondy, 2003)

Table I. Lipoprotein Classification

LIPOPROTEIN	MAJOR LIPID COMPONENT	MAJOR APOLIPOPROTEINS	SOURCE
Chylomicrons	TG	ApoA-I, A-II, A-IV; ApoC-I, C-II, C-III; ApoB-48; ApoE	Intestine
Very low-density lipoprotein (VLDL)	TG	ApoB-100; ApoC-I, C-II, C-III; ApoE	Liver
Intermediate-density lipoprotein (IDL)	CE	ApoB-100; ApoE, ApoC	Catabolism of VLDL
Low-density lipoprotein (LDL)	CE	ApoB-100	Catabolism of IDL
High-density lipoprotein (HDL)	CE, PL	ApoA-I, A-II, A-IV; ApoC-I, C-II, C-III; ApoE	Liver, intestine, other

TG=triglyceride; CE=cholesterol ester; PL=phospholipid
Data derived from Dominiczak MH. Apolipoproteins and lipoproteins in human plasma. In: Rifai N, Warnick GR, Dominiczak MH, eds. *Handbook of Lipoprotein Testing*. Washington, DC: AACC Press; 1997:1–17 and Gotto A, Pownall H. *Manual of Lipid Disorders: Reducing the Risk for Coronary Heart Disease*. 2nd ed. Baltimore, MD: Williams & Wilkins; 1999:2–10.

1.6.2.1. Chylomicrons (CM)

Chylomicrons are produced in the intestinal lumen and serve to transport dietary triglyceride to peripheral tissues and cholesterol to the liver. They are the largest lipoproteins and are rich in triglycerides. Because of their size, chylomicrons have more scattering ability for light and therefore after meals, the serum appears turbid or cloudy (Kingsbury and Bondy, 2003).

As mentioned before, lipoproteins in general contain apolipoproteins A and B. since ApoB does not exchange between lipoprotein particles during recirculation, ApoB-48 found in chylomicrons is a perfect marker for the chylomicrons particles (P.E. Fielding and C.J. Fielding, 2002).

In humans with diabetes, renal failure and familial combined hyperlipemia and type III hyperlipidemia, a delayed removal of chylomicrons remnants occurs. The remnants are the results of the hydrolysis of chylomicrons triglycerides by lipoproteins lipase that leads to the delivery of free fatty acids to be oxidized in muscle to generate energy or stored in adipose tissues or used in hepatic VLDL synthesis. Finally the triglyceride depleted chylomicrons need to be removed from the circulation by the liver, endocytosed by hepatic receptors. The chylomicrons remnants retain almost the whole of their original cholesteryl and retinyl ester content which will be cleared by the liver along with remnant triglycerides (Cooper, 1997).

1.6.2.2. Very low density lipoproteins (VLDLs)

Very low-density lipoproteins are similar to chylomicrons and contain high amount of triglycerides. It is produced from free-fatty acids formed in the breakdown of chylomicrons in the liver or from triglycerides synthesized endogenously by the liver or intestine. The VLDLs transport the triglycerides that will undergo hydrolysis later by capillary lipoprotein lipase (LPL) and release fatty acids to adipose and muscle tissue for storage and energy, respectively. Once processed by LPL, the VLDLs become a VLDL remnants, most of which are taken up by the liver via LDL receptor and the remaining

50-70 % remnants form IDL. The metabolism of VLDL remnants is more complex than that of chylomicrons (Kingsbury and Bondy, 2003).

The triacylglycerols-rich core of VLDLs contains high levels of hepatic cholesteryl esters which play very important role for a successful secretion. In addition, VLDLs contain molecules of apoB100, apoC-I, apoC-II, apoC-III as well as apoE. Newly synthesized plasma VLDLs have apoC apoproteins and little apoE, which will be further enriched in the plasma (P.E. Fielding and C.J. Fielding, 2002).

1.6.2.3. Intermediate Density Lipoproteins (IDLs)

When the VLDL is hydrolyzed and fatty acids are released, the remaining lipid portion is called intermediate density lipoprotein (IDL), a smaller and denser lipoprotein. Some IDLs are reabsorbed by the liver by LDL receptors through ApoB-100 that acts as a ligand. Other IDLs are hydrolyzed in the liver by hepatic triglyceride lipase forming LDL, a smaller and denser particle that IDL (Kingsbury and Bondy, 2003).

1.6.2.4. Low-density lipoproteins (LDLs)

The low-density lipoproteins, commonly known as "bad cholesterol", contain 25% proteins, 5% triglycerides, 20% phospholipids and 50% cholesterol (Tortora and Grabowski, 2003). The LDL particles are the major carriers of cholesterol in the blood that will be cleared once the LDLs bind their receptors in the peripheral cells or in the hepatocytes (Rifai et al., 1997). The cholesterol is used by the cells for the buildup of the membranes and for the synthesis of hormones (Kingsbury and Bondy, 2003). The LDLs in the circulation with a plasma half-life of two days contain the apoB-100 particles which are recognized by LDL receptors distributed in the liver as 60 to 70 %. Once endocytosed in the liver, they are degraded and used either for bile acids production or for other lipoproteins synthesis. It is also noted that apoE mediate the recognition of lipoprotein by LDL receptors at the level of liver cells, but in human, LDLs are free of apoE (P.E. Fielding and C.J. Fielding, 2002).

The low-density lipoprotein is an atherogenic type of lipoproteins since its high level is commonly associated with high risk for cardiovascular diseases and shock (Kingsbury and Bondy, 2003). The pathogeneity of high levels of LDLs depends on its heterogeneity. The multiple LDLs result from many metabolic pathways. There are seven types of LDL grouped based on their density into four classes (I through IV) from the largest, most buoyant to the smallest, most dense (Krauss and Burke, 1982; Krauss, 1987). The most pathogenic and atherogenic LDL is the one small, dense and containing more cholesterol ester. This is found to be due to its high binding affinity to arterial proteoglycans, its high oxidative modification and its high toxicity. Once LDL clearance mechanism is impaired, the deposition of excess cholesterol in the arteries, skin, tendons and corneas will be a major cause of diseases. This is the case in familial hypercholesterolemia where LDL receptors are produced in deficiency due to a gene mutation. In addition, the small and large LDL particles have both less binding affinities to the LDL receptors when compared to the intermediate normal sized LDL particles (Berneis and Krauss, 2002).

Many studies were done in a purpose of detecting the effect of total cholesterol, LDL and HDL- cholesterol on many cardiovascular diseases as coronary heart disease and atherosclerosis. One study revealed that active women had low total serum cholesterol, oxidized LDL cholesterol, triglyceride and high level of HDL cholesterol, when compared with inactive women. Similar results were observed for men but were not statistically significant. These results didn't show any significant effect of age, smoking and body mass index on the lipidemic levels (Skoumas et al., 2003).

1.6.2.5. High Density Lipoproteins (HDLs)

The high-density lipoproteins, commonly known as "good cholesterol" are formed in the extracellular space and contain apoA1, apoAII, apoC, apoE and apoD (unique to it) and not apoB- as other lipoproteins. They contain 40-45% proteins, 5-10 % triglycerides, 30 % phospholipids and 20 % cholesterol and are the smallest and most dense lipoproteins (Tortora and Grabowski, 2003). The mature spherical HDLs are formed when lipid-poor apoA1 discoidal HDLs become associated with cell-derived phospholipids and cholesterol. ApoA1 synthesized in the liver and small intestine

becomes bound to the surface of lymphatic triglyceride-rich lipoproteins (P.E. Fielding and C.J. Fielding, 2002).

High levels of HDLs are associated with decreased risk of cardiovascular diseases, especially coronary heart disease because the reaction by which cholesterol is removed from tissues and returned to the liver is mediated by HDL particles and called "reverse cholesterol transport" (Genest, 1990). The precursor nascent HDL particles secreted by the liver and intestine undergo a cycle. Two enzymes play important roles in the reactions involving HDLs: cholesteryl ester transfer protein (CETP) and lecithin- cholesterol acyltransferase (LCAT). Lecithin- cholesterol acyltransferase in plasma promotes the uptake of free cholesterol by HDL by esterification of cholesterol to cholesterol esters, forming a denser and more hydrophobic spheroid HDL particle. Cholesterol ester-rich HDLs may also be taken up directly by receptors in the liver, HDL-specific apoA1 receptor or through lipid-lipid interactions. In addition HDLs can be phagocytosed by macrophages through interactions with apoA1 receptors. The HDL secreted back by macrophages is enriched by cholesterol and thus is more ready to enter the liver (Schaefer et al., 1978; Krauss and Burke, 1982; Gurr and Harwood, 1991).

Logically, a low HDL level signifies that other atherogenic lipoproteins are present in excess, like VLDL remnants, IDLs and small dense LDLs. The ratio between LDL and HDL cholesterols is a precise indicator of atherogenesis. When the ratio is high, it is indicative of high risk of coronary heart disease. In addition to all its features, HDL particles proved to have antioxidant properties and help prevent oxidation of LDL (Abbott et al., 1988; Grundy and Denke, 1990).

As for LDLs, the same study showed that high HDL level was related to physical activity in women and men and not related to age, smoking and weight (Skoumas et al., 2003).

1.7. The activity of hepatic enzymes

The liver is the heaviest and the second largest organ of the body, after the skin. It weighs about 1.4 kg in an average adult. Besides secreting bile, which is essential for the absorption of dietary fats, the liver performs many other vital functions. In addition, the liver processes drugs and hormones by detoxifying and altering them, respectively (Ghany and Hoofnagle, 2001).

Nowadays, the use of herbal medicine is very popular and common, especially that people prefer to use natural products and remedies. The medicinal plants are being sold almost everywhere in a random manner and affordable prices. It was proved that the plants consumed can be detoxified by the liver and the second metabolites issued might cause liver injury that will either accumulate and elevate liver enzymes or cause complete damage of the liver (Grunhage et al., 2003; Pak et al., 2004). Because of its important functions, the liver represents an essential organ that needs to be always controlled and checked for toxicity. For this reason, all plants need to be tested for liver toxicity before use or prescription in drugstores. The liver toxicity is assessed by measuring the amount of its enzymes. The hepatic enzymes measured are lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT)/ aspartate aminotransferase (AST), serum glutamate pyruvate transaminase (SGPT)/alanine aminotransferase (ALT), alkaline phosphatase (ALP). The AST is a cellular enzyme found in high concentrations in heart muscle and cells of the liver and skeletal muscle. The ALT is found in high concentrations in liver and kidneys. High levels of these enzymes in serum are the best diagnosis for hepatic disease like hepatitis. Although an elevated level of AST in the serum is not specific of the hepatic disease, it is used to diagnose and verify the disease with other enzymes like ALT and ALP. The AST: ALT ratio > 2:1 is suggestive and the ratio > 3:1 is highly suggestive for the liver disease. The alkaline phosphatase (ALP) is an enzyme that reflects cholestasis. It is elevated usually in cholestasis and represents a great indicator. High levels of LDH are observed in cases of myocardial infarction, leukemia, hemolytic anemia and non viral hepatitis (Cohen and Kaplan, 1979; Pratt and Kaplan, 2001).

1.8. Inflammation and bacterial growth

Inflammation is a non-specific, defensive response of the body to tissue damage. This response is triggered by chemical irritations, pathogens, disturbances of cells and extreme temperatures. Redness, pain, heat and swelling are four signs and symptoms of inflammation. The inflammatory response has three basic stages: vasodilation and increase permeability of blood vessels, emigration of phagocytes from the blood into interstitial fluid and ultimately tissue repair. Histamine, kinins, prostaglandins and

leukotrienes are the substances involved in vasodilation and increased permeability. Histamine is released by basophils, platelets, neutrophils and macrophages once attracted to the site of injury. Prostaglandins are lipids released by damaged cells and play a very important role in the emigration of phagocytes through capillary walls. Heat and redness are due to the large amount of blood supply in the damaged area. The swelling is due to the high permeability of blood vessel that transport more fluid from the blood plasma to the tissue damaged. The swelling is accompanied by pain because of the pressure it causes. In addition, pain is increased by toxic chemicals released by microbes and by damage of nerve endings (Tortora and Grabowski, 2003).

Many studies were done on plant's extracts in order to assess their anti-inflammatory effects. It was proved that suppression of inflammation is achieved by inhibition of the vasodilator nitric oxide production (Salvemini et al., 1996; Choi et al., 2003).

Previous *in vitro* studies showed that mucilage (Wagner and Proksch, 1985) and aqueous extracts (Scheffer and Konig, 1991) of *Althaea officinalis* stimulate phagocytic activity of macrophages. Recently, a pectic polysaccharide named comaruman was extracted from the aerial part of *Comarum palustre* and was found to reduce a paw edema observed 24 h after injection of 2% formalin in mice (Popov et al., 2005). This study supports the fact that polysaccharides in general have anti-inflammatory activity *in vivo*. Since *Althaea officinalis* is rich in mucilage polysaccharides, the plant may have a possible anti-inflammatory role *in vivo*.

In addition to inflammation, the antibacterial effect of plants is an interesting field of research especially when it comes to pathogenic microbial species. Traditional antibiotics used to treat many pathogenic bacteria have limited lifespan and adverse effects. When it comes to natural products, no one will hesitate to use plants as antibacterial agent as long as the effect is interesting (Avani and Neeta, 2005). Bacterial isolates and fungi provided from hospital can be manipulated and grown to look for any antimicrobial activity of the plant studied. Many previous studies were done on plants and antimicrobial compounds were identified against *salmonella, S. aureus*, fungi, *E. coli*, viruses, *cocci*, etc… They belong to polyphenols, alkaloids and monosaccharide classes and include latex, tannins, essential oils, salicylic acids, cocaine, colchicine, fructose and piperine, and many others.

The mechanism of action of phenols is through inhibiting the enzymes activity of the bacteria. The flavonoids are known to bind to bacterial cell walls and destroy them (Cowan, 1999).

1.9. Gastric ulcer

An ulcer is a crater-like lesion in a membrane caused by a lack of equilibrium between the mucosal protection and the amount of aggressive acidity caused by acid-pepsin. One common ulcer is the gastric ulcer occurring in the stomach due to the high acid-pepsin amounts, impaired bicarbonate neutralization and impaired mucus secretion (Cho and Wang, 2002). Drugs used in the treatment of ulcer are targeted at either counteracting the aggressive factors (acid plus pepsin, active oxidants, leukotrienes, bile or exogenous factors) or stimulating the mucosal defenses (mucus, bicarbonate, prostaglandins or nitric oxide) (Tepperman and Jacobson 1994). Botanical compounds were found to play a role as anti-ulcer remedies and include flavonoids, saponins, tannins, gums and mucilages. Flavonoids, gums and mucilages protect against ulcer through increasing mucosal prostaglandins content and decreasing histamine secretion from mast cells by inhibition of histidine decarboxylase (Capasso and Grandolini, 1999; Borrelli and Izzo, 2000). Another study reveled that flavonoids increase gastric microcirculation by the stimulation of afferent nerves and the release nitric oxide (Zayachkivska et al., 2005). Many studies are being done in order to find protective and healing agents against ulcer and proved that the epidermal growth factor is an inhibitor of gastric acid secretion (Xu et al., 1998).
In a purpose of studying the gastroprotective effect of any plant on ulcer, ulcer may be induced by ethanol in animals previously receiving the plant extract. This method will assess the preventive and protective effect of the plant rather than its healing effect. Studies may be done either on the whole plant or on specific compounds of the plants' extract (Zayachkivska et al., 2005).

1.10. Platelet aggregation activity

The effect of plant's extract on platelet aggregation is very important. Platelet aggregation is essential in preventing blood loss in small vessels and in controlling bleeding of wounds and preventing thrombus formation. The platelets are gathered after being

activated and recruited further by ADP secreted by primarily adhered platelets (Tortora and Grabowski, 2003). The platelets are one of the major blood components, and their dysfunction results in hemorrhage. cAMP is known to be a potent suppressor of platelet aggregation. It was proved that adenylate cyclase toxin suppresses platelet aggregation in vitro through increase of intracellular cAMP due to its catalytic activity and that it induces prolongation of bleeding time in vivo (Iwaki et al., 1999). Many studies were done on plants and showed that some plant products have antiaggregant properties where as others stimulate aggregation. Tannins, for example were shown to be potent stimulators for platelets aggregation *in vivo* (Lauque et al., 1991). Recent study showed that flavonoids inhibit platelet aggregation through binding to the thromboxane A2 receptor (Guerrero et al., 2005). Since *Althaea officinalis* contains flavonoids and other compounds it is important to test for platelet aggregation.

1.11. Laxative effect and lipids excretion

It is commonly known that many plants are used by folk medicine as laxative and are prescribed by herbalists in a random manner. Chemically, feces consist of water, inorganic salts, sloughed-off epithelial cells from the mucosa of gastrointestinal tract, bacteria, unabsorbed digested materials and indigestible parts of food. Of the 0.5 – 1.0 liter of water that enters the large intestine, all but about 100-200 ml is absorbed via osmosis. Sometimes, an increased in the frequency, volume and fluid content of feces occurs because of increased motility and decreased absorption by the intestines. This is called diarrhea and its opposite is the constipation. Laxatives are substances used in case of constipation and induce defecation (Tortora and Grabowski, 2003).

Biliary cholesterol hypersecretion is an important indicator of cholesterol gallstone formation. Cholesterol absorption is a multistep process and any factor that changes the transportation of cholesterol from the intestinal lumen to the lymph may influence intestinal cholesterol absorption efficiency. The same thing applies to triglyceride. For this reason, it is important to monitor if the plant consumed affects cholesterol and triglyceride absorption by measuring their amounts in stools (Wang et al., 2001).

1.12. Glucose and insulin assays

Many studies were done on insulin in order to monitor the effect of plants on insulin secretion and activity. One study showed that tea enhances insulin activity (Anderson and Polansky, 2002). It is well known that insulin secreted by beta cells of the islets of Langerhans regulates the blood glucose level by lowering it. The level of glucose should also be monitored in order to make sure that the plant studied doesn't increase or decrease the glucose level randomly and without control. Glucose is the most important monosaccharide in the blood and glucose substrate is an indispensable energy supplier, which supports cellular function (Tortora and Grabowski, 2003).

It is necessary to mention that various hypoglycemic carbohydrates have been proposed as an alternative in the diabetes mellitus control. In one study done on the roots of *Psacalium peltatum*, peltalosa, a carbohydrate, was proved to have a hypoglycemic activity on mice with mild diabetes, but this activity diminished on mice with severe diabetes. It is likely that the hypoglycemic effect is due to an enhanced secretion of insulin from the islets of Langerhans or an increased utilization of glucose by peripheral tissues (Contreras et al., 2005). Similar studies were done on different compounds in order to find out natural products that can help treating diabetes mellitus, rather than the use of traditional drugs. Mucilages and pectin were found to exert hypoglycemic activity (Tomoda et al., 1987; Siddhu et al., 1989). These two compounds are found in high amounts in *Althaea officinalis* in addition to other compounds that might also be hypoglycemic.

1.13. Purpose of the project

No previous studies were done on flowers of *Althaea officinalis* and most researches were done on the roots of this plant. Thus, this present investigation was undertaken on rats in order to evaluate the effects of the flowers of *Althaea officinalis* water extract upon

- Acute and chronic inflammation induced by carrageenan and formalin respectively
- Protection against ethanol-induced gastric ulcer
- The level of cholesterol and triglyceride in feces

- Laxative effect
- Platelet aggregation
- Antibacterial activity
- Blood lipid profile (TAG, total cholesterol, LDL-cholesterol and HDL-cholesterol), glycemic profile (glucose and insulin) and liver enzymes activities (GOT,GPT,ALP and LDH) in rats put on lipid diet

MATERIALS AND METHODS

2.1. Plant collection and water extract preparation

The flowers of *Althaea officinalis* were collected from the Bekaa valley, specifically from Bedneyel, a town in the middle Bekaa. The flowers were left in the shade until they dehydrated and became dry. The taxonomic identification of the plant material was confirmed through botanic and plant taxonomy books (Juneidi, 1999; Mouterde, 1970). The dry weight of the flowers' extract was determined by weighing 3 g of the flowers and adding them to pre-boiled distilled water. The flowers were left 30 minutes and then the extract was filtrated using a filter paper into a pre-weighed beaker. The water was left to evaporate slowly on a mild flame and it appeared that every 1 g of plant gives 0.118 g of pure extract. From this result, all the experimental doses were calculated and computed. The needed quantity of flowers was put in pre-boiled water and left for half an hour and then filtrated. When the extract was used for intraperitoneal injections and in the experiment of antibacterial activity, syringe filtration using Millipore filters units of 0.45 µm pores size was done otherwise the use of filter paper was enough.

2.2. Animals on lipid diet

Male Sprague-Dawley rats (n=32) (Lebanese American University stock) were randomly divided into four groups comprising 8 animals each with an average weight of 300 g. All the rats received 10 ml of drink per 100 g body weight and an isocaloric diet of 6.5 g of food per 100 g body weight but it was made sure that all the food was eaten and no food was left. In case of food left, the amount of food was diminished for all groups. Food consisted of rat chow to which 5% coconut oil was added. Coconut was used to make the diet atherogenic (Daher et al., 2003). The first group (control) was given only water. The second, third and fourth groups referred to as the treatment groups received respectively 59 mg, 236 mg and 590 mg of extract / kg body weight that were prepared by adding 1.5 , 6 and 15 g of the flowers of *Althaea officinalis* in 300 ml drinking water. Animals were maintained at an ambient temperature of 20-22°C. This experiment was done twice, first the experiment lasted

for one month and second one for two months after which fasted animals were sacrificed using diethyl ether and approximately 8 ml of blood were collected.

2.2.1. Stool analysis

2.2.1.1 Water content of the stools

After one month of lipid diet intake, the rats' feces were taken at day 30 from each rat and weight of each was recorded. Stools were left overnight at 60°C in an oven in order to ensure complete drying. The next day, the dried stools were weighed again and the percentage of water content was computed.

Stools were then crushed into powder using the mortar and pestle. 0.2 g were taken from each sample and put in a glass tube to which 2 g of n-hexane was added. The n-hexane is used as a hydrophobic solvent that extracts lipids from the feces. Tubes were left 2 hours in a water bath of 40 °C with a non-stop stirring with a glass rod. Once done, the tubes were centrifuged at 3000 x g (5000 r.p.m) for 15 min. After centrifugation, 300 µl of the supernatant was taken for the triglyceride test and another 300 µl was taken for the cholesterol test. Tubes were placed in a water bath at 75°C until the n-hexane evaporates. The left-over lipids in the tubes were then tested for triglyceride and cholesterol using the corresponding "Spinreact" kits.

2.2.1.2 Determination of Triglyceride

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

$$\text{TAG} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

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

$$\text{Glycerol- 3-P} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone-P} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{p-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{quinone} + H_2O$$

***Procedure*:** 1 ml of the working reagent (buffer solution and enzymes: LPL, GK, GPO and POD) was added to the 32 triglyceride tubes and incubated 10 min at room temperature. 1 ml of the working reagent was added also to 10 µl of the standard solution. The absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ = 505 nm using a Jenway 6105 U.K spectrophotometer.

Calculation:

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

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

2.2.1.3 Determination of total cholesterol

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

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

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

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-cholestenona} + H_2O_2$$

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

$$2\,H_2O_2 + 4 - \text{aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{quinonimine} + 4H_2O$$

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

Procedure: 1 ml of the working reagent (buffer solution and enzymes: CHOD, CHE and POD) was added to the 32 cholesterol tubes and incubated 10 min at room temperature. 1 ml of the working reagent was added also to 10 µl of the standard solution. The absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ = 505 nm using a Jenway 6105 U.K spectrophotometer.

Calculation:

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

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

2.2.2 Serum assays

2.2.2.1 Samples preparation:

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

2.2.2.2 Determination of Triglyceride

10 µl of the standard and of each serum samples were mixed with 1 ml of the working reagent (buffer solution and enzymes) and incubated 10 min at room temperature. The absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ = 505 nm using a Jenway 6105 U.K spectrophotometer.

2.2.2.3 Determination of total cholesterol

10 µl of the standard and of each serum samples were mixed with 1 ml of the working reagent (buffer solution and enzymes) and incubated 10 min at room temperature. The absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ = 505 nm using a Jenway 6105 U.K spectrophotometer.

2.2.2.4 Determination of HDL-Cholesterol

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

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

Calculation:

Concentration of HDL-cholesterol

in the unknown sample (mg/dL) = $\frac{\text{Abs. of unknown x Conc. of standard}}{\text{Abs. of standard}}$

2.2.2.5 Determination of LDL – Cholesterol

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

LDL cholesterol = total cholesterol – $\frac{\text{Triglycerides}}{5}$ – HDL cholesterol

2.2.2.6 Determination of Serum Glutamate Oxaloacetate Transaminase (SGOT)

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

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

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

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

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

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

Calculation:

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

according to Spinreact procedure

2.2.2.7 Determination of Serum Glutamate Pyruvate Transaminase (SGPT)

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

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

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

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

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

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

Calculation:

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

according to Spinreact procedure

2.2.2.8 Determination of Lactate Dehydrogenase

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

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

Procedure: 100 µl of each serum samples were mixed with 3 mL of the working reagent (Tris buffer pH = 7.5, NADH and pyruvate). After 1 minute of incubation at room temperature 25-30 °C, the absorbance was read at one minute interval for 3 minutes against distilled water using Jenway 6501 U.K spectrophotometer at a wavelength of 340 nm. The difference between absorbances and the average absorbance differences per minute were calculated.

Calculation:

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

according to Spinreact procedure

2.2.2.9 Determination of Alkaline Phosphatase

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

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

Procedure: 50 µl of each serum samples were mixed with 3 mL of the working reagent (diethanolamine buffer pH 10.4, magnesium chlorure and p-nitrophenyl-phosphate). After 1 minute of incubation at room temperature 25-30 °C, the absorbance was read at one minute interval for 3 minutes against distilled water using Jenway 6501 U.K spectrophotometer at a wavelength of 405 nm. The difference between absorbances and the average absorbance differences per minute were calculated.

Calculation:

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

according to Spinreact procedure

2.2.2.10 Determination of serum glucose

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

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

$$H_2O_2 + \text{phenol} + \text{aminophenazone} \xrightarrow{\text{POD}} \text{quinone} + H_2O$$

Procedure: 10 µl of the standard and of each serum samples were mixed with 1 ml of the working reagent (buffer solution and enzymes) and incubated 10 min at room temperature. The absorbances of the standard sample and the unknowns were then measured against the reagent blank at λ = 505 nm using a Jenway 6105 U.K spectrophotometer.

Calculation:

Concentration of glucose

in the unknown sample (mg/dL) = $\frac{\text{Abs. of unknown x Conc. of standard}}{\text{Abs. of standard}}$

The concentration of the standard is 100 mg/dl according to the SPINREACT kit.

2.2.2.11 Determination of serum insulin

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

Procedure:

First, the plate was washed with the washing buffer (TBS buffer) diluted 10 times. 10 µl Serum samples of control and experimental groups as well as the standards and quality controls were dispensed in the appropriate wells of the

microtiter assay plate after the 80µl biotinylated anti-insulin antibodies, 10µl matrix solution (only for standards and quality controls) and assay buffer (only for unknowns) were added. The plate was then incubated at room temperature for 2 hours on an orbital microtiter shaker at about 500 rpm.

Following incubation, solutions were decanted from the plates, which were tapped to remove residual fluid. Wells were then washed 3 times with diluted TBS wash buffer, 300 µl per well per wash. Again the fluid is removed by decantation and tapping firmly against absorbent paper. Finally, 100 µl of enzyme solution were added to each well, plates were shaken for additional 30 minutes. Wells were then washed 6 times with diluted TBS wash buffer, 300 µl per well per wash. Again the fluid is removed by decantation and tapping firmly against absorbent paper. Finally, 100 µl of substrate solution were added to each well, plates were shaken for additional 15 minutes. A blue color should develop in the wells of insulin standards with intensity proportional to increasing concentrations of insulin. Sealer is removed and 100 µl of stop solution were added after which plates were shaken to ensure complete mixing of solution in all the wells. The blue color should turn into yellow after acidification obtained by addition of the stopping reagent and absorbance was read at 450 nm and 590 nm using the Spectra Max Plus ELISA reader.

Calculation:

A standard curve is constructed using all standard points for which absorbances are less than 1.5 OD units. The insulin concentrations of the samples are determined as extrapolation using a computerized data reduction of absorbance of the standards versus their concentration using a correlation graph and a linear equation.

2.2.3. Body weight change

In order to assess the effect of the *Althaea officinalis* on body weight changes of the rats, animals body weight was measured weekly for a period of two months. Rats were given 6.5 g of food per 100 g body weight and their eating behavior was monitored. Every week, the rats were weighed and given food according to their body weight.

2.3. Antiinflammatory activity

Acute and chronic antiinflammation activities were assessed respectively using carrageenan and formalin induced inflammation in mice hind paw (Jose et al., 2004). These two experiments were done on 5 groups of 6 male Sprague-Dawley rats (n=30) weighing between 200 and 250g. The five groups consisted of the control, three treatment groups and the reference drug group (10 mg diclofenac / kg body weight). All animals were fed the regular rat chow diet (6.5 g per 100 g body weight) and had free access to water.

2.3.1. Carrageenan induced paw edema

The right paw thickness of all the animals (n=30) was measured with a vernier caliper in the beginning and noted. Edema was induced by subplantar injection of 0.02 ml of 1% freshly prepared carrageenan in the right hind paw of the control group rats and the paw thickness was measured after 3 hours. The treatment groups I, II and III were administered with 0.02 ml carrageenan after 30 min of injection with 100, 250 and 500 mg of water extract of *Althaea officinalis* flowers / kg body weight, respectively and the paw thickness was measured after 3 hours. The reference drug group was administered with the reference drug diclofenac intraperitoneally at a dose of 10mg/kg, after 30 min it was injected with 0.02 ml carrageenan and the paw thickness was measured after 3 hours (Jose et al., 2004).

2.3.2. Formalin induced paw edema

The right paw thickness of all the animals (n=30) was measured with a vernier caliper in the beginning and noted. Edema was induced by subplantar injection of 0.02 ml of 2% freshly prepared formalin in the right hind paw of the control group rats. The treatment groups I, II and III were administered with 0.02 ml formalin after 30 min of injection with 100, 250 and 500 mg water extract of *Althaea officinalis* flowers / kg body. The reference drug group was administered with the reference drug diclofenac intraperitoneally at a dose of 10mg/kg, after 30 min it was injected with 0.02 ml formalin. The administration of the extracts and reference drug was continued once daily for 6 consecutive days. The paw thickness was measured using vernier calipers the 6th day after 3 hours of injection (Jose et al., 2004).

2.3.3. Calculations

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

2.4. Effect of water extract of *Althaea officinalis* flowers on ethanol induced gastric ulcer in rats

The effect of water extract of the flowers of *Althaea officinalis* on ethanol-induced gastric ulcer was performed in male rats. The rats weighing between 250-300 g were randomly assigned to 6 groups of 6 rats each. The six groups (I to VI) consisted of the control, reference group, three treatment groups (50, 100 and 250 mg water extract / kg body weight) and reference drug Cimetril (11.5 mg / kg body weight). Animals were housed at an ambient temperature of 23 ± 1°C and 65-70% relative humidity. Forty-eight hours before use, animals were fasted to ensure an empty stomach. Furthermore, they were kept in cages with raised floors of wide wire mesh to prevent coprophagy (eating of excrement). To prevent excessive dehydration during the fasting period, all groups were supplied with sucrose 8% (w/v) in NaCl 0.2% (w/v). This fluid was removed 1 hour before experimentation (Alkofahi and Atta, 1999; K. Gharzouli et al., 1999). The Group I (reference group) received 2 ml of distilled water (10ml/kg) as the other groups but had no ulcer induction. Group II (control) was given 3 ml of distilled water (10ml/kg). The treatment groups III, IV and V received 4 ml of the extract prepared respectively according to the doses 50 mg/kg, 100 mg/kg and 250 mg/kg of water extract of *Althaea officinalis* flowers. The group VI received 4 ml of the reference drug Cimetril. (Xu et al., 1998) Doses were administrated orally via a stainless steel intubation needle. Two doses were given on the first day at 9:00 h and 17:00 h; a third dose was given on the second day 1.5 h before induction of gastric ulceration. To induce gastric ulcer, the control (group II) as well as the treatment groups III, IV and V received by gastric gavage 10ml/kg body weight ethanol 50% (v/v) in distilled water. One hour after ethanol administration, all animals were sacrificed by an overdose of chloroform; stomachs were rapidly removed, opened along their greater curvature and rinsed under running tap water.

2.4.1. Calculations

Using an illuminated microscope (10 x), long lesions were counted and measured along their greater length. Petechial lesions (very small lesions) were also counted and each five were taken as a 1 mm of ulcer. The sum of the total length of long ulcers and petechial lesions in each group of rats was divided by its number to calculate the ulcer index (mm) (Alkofahi and Atta, 1999).

The curative ration was determined by the formula:

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

2.5. Platelet aggregation activity

In this experiment, 8 ml of fresh blood from healthy rats was collected in 1.6 ml of anticoagulant solution in a way that the ratio of blood to anticoagulant solution was 5:1. The anticoagulant solution consisted of sodium citrate 2.4% (w/v), citric acid 1.5% (w/v) and D-glucose 1.8% (w/v). Blood was then centrifuged at 1900 rpm for 7 minutes to obtain the supernatant platelet rich plasma (PRP), which was then centrifuged at 4500 rpm for 18 minutes. The supernatant was discarded and the pellet was dissolved in 3 ml of the washing buffer I .The washing buffer I consisted of 113 mM NaCl, 4.3 mM K_2HPO_4, 4.3 mM Na_2HPO_4, 24.44 mM NaH_2PO_4 and 5.5 mM D-glucose (pH 6.5). Pellet platelets were collected after centrifugation at 4000 rpm for 10 minutes and were suspended in 7 ml of buffer II composed of 113 mM NaCl, 4.3 mM K_2HPO_4, 16 mM Na_2HPO_4, 8.3 mM NaH_2PO_4 and 5.5 mM D-glucose (pH 7.5). The suspension was adjusted to a final optical density of 0.5/ml at 600 nm (Jose et al., 2004).

The water extract of *Althaea officinalis* (500 µg) was incubated with a volume of 1 ml washed platelets in 3 siliconized tubes for 5, 10 and 20 minutes in a waterbath at 37°C. 20 µl of 1 mM freshly prepared ADP was added at the end of the incubation period and mixed thoroughly with the washed platelets then the optical density at 600 nm was measured at 1 min intervals up to 5 minutes. For each of the three tubes, a normal and control tube was prepared. A solution of washed platelets incubated with buffer II and to which ADP was added served as a control. A normal solution was also prepared where the washed platelets and extract were incubated alone and no ADP was added while measuring the optical density (Jose et al., 2004).

2.5.1. Calculations

A graph was plotted for the relative optical density versus time for the control, normal and extract. The percentage inhibition was calculated using the formula:

Percent inhibition= % aggregation of control – % aggregation of platelets by test samples

The percentage aggregation of control ADP was assumed as 100 %.

2.6. The antibacterial activity of *Althaea officinalis* flowers water extract

The water extract of *Althaea officinalis* flowers was tested for its antibacterial activity on 11 hospital isolates from different patients using the disc diffusion method. The bacterial strains used were *Enterobacter cloacae*, *Proteus mirabilis*, *Salmonella typhi*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcesens*, *Ewingella americana*, *Staphylococcus aureus* methicillin-resistant (MRSA), and *Staphylococcus aureus* methicillin-sensitive (MSSA). The bacterial strains were provided from the bacteriology laboratory of the Governmental Hospital of Beirut and their identification tests are indicated in the table 2.1 according to the hospital.

Table 2.1 List of bacterial strains tested and their identification tests used in the hospital.

Bacteria tested	Identification test
1. *Citrobacter freundii*	API 20E
2. *Enterobacter cloacae*	API 20E
3. *Escherichia coli*	Urea: negative / Indole: positive
4. *Serratia marcesens*	Phoenix®
5. *Klebsiella pneumoniae*	API 20E
6. *Proteus mirabilis*	Urea : positive / Indole : negative

7. *Pseudomonas aeruginosa*	Oxidase positive / Isolation at 42°C
8. *Salmonella typhi*	API 20E
9. *Staphylococcus aureus methicillin resistant (MRSA)* *	Oxacillin resistant
10. *Staphylococcus aureus methicillin sensitive (MSSA)* *	Oxacillin sensitive
11. *Ewingella americana*	Phoenix*

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

* Phoenix: instrument for automated and rapid identification of gram-negative and gram-positive bacteria of human origin

Disc diffusion assay

The water extract of *Althaea officinalis* flowers was prepared by adding 6.780 g of this plant into 40 ml pre-boiled distilled water. This original extract had a concentration of 400 µg / 20µl. After 30 minutes, the extract was filtrated first by filter paper then by syringe filtration. Different concentrations (200, 100, 50, 20 and 10 µg / 20 µl) of the extract were prepared from the original solution by serial dilutions and added to the blank discs in a volume of 20 µl (Fig. 2.1.). The bacteria were grown and maintained on tryptone soy agar. In order to make sure that the inoculum size of each bacterial strain that was spread on Muller-Hinton agar was the same in all plates, the number of bacteria / ml was standardized. The standardization was done first by adding an inoculum of bacteria to 10 ml of sterile NaCl (0.85 %) and comparing its turbidity to that of the mcfarland standard tube of 0.5 by naked eye. Second the optical density was measured and adjusted to 0.125 at 505 nm that corresponds to 10^8 bacteria / ml of suspension (Basri and Fan, 2005; Rios et al., 1987; Voravuthikunchai and Kitpipit, 2005).

Each bacterial strain was spread on 4 Muller-Hinton agar plates and 4 sterile 5-mm diameter filter paper disks were placed on the surface. The filter papers were soaked with 20 µl of sterile water and of the different concentrations of extract. The sterile water was used as control. A reference antibiotic was used for each type of bacteria

(figure 2.1 and table 2.2) All the plates were then incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone (Barbour et al., 2004).

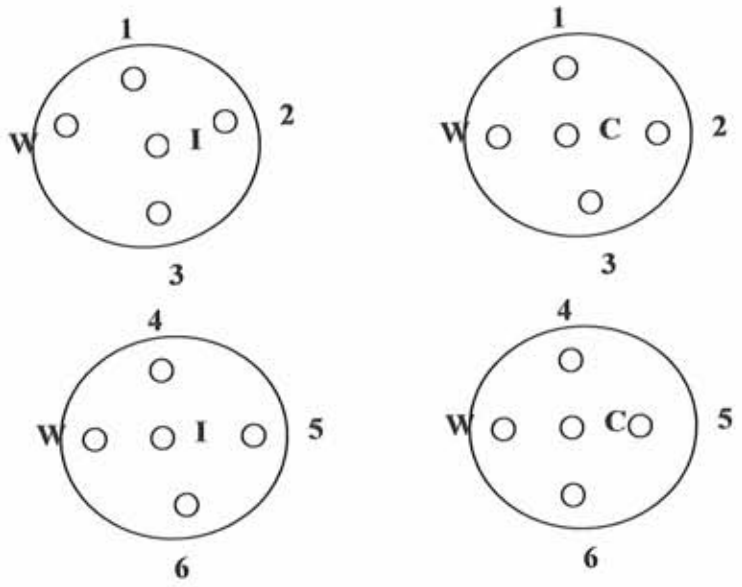


Figure 2.1 The scheme followed in order to prepare the plates for antibacterial activity test. **1**: 400 µg / 20 µl , **2**: 200 µg / 20 µl , **3**: 100 µg / 20 µl , **4**: 50 µg / 20 µl , **5**:20 µg / 20 µl, **6**: 10 µg / 20 µl, **W**: sterile water as control, **I:** Imipenim or Vancomycin or Bactrim and **C**: Cefepime or Vancomycin or Bactrim.

Table 2.2 List of bacterial strains tested and reference drugs used to screen the antibacterial activity of water extracts of *Althaea officinalis* flowers.

Bacteria tested	Reference drug
12. *Citrobacter freundii*	Cefepime / Imipenim (30/10 µg disc)
13. *Enterobacter cloacae*	Cefepime / Imipenim (30/10 µg disc)
14. *Escherichia coli*	Cefepime / Imipenim (30/10 µg disc)
15. *Serratia marcesens*	Cefepime / Imipenim (30/10 µg disc)

16. *Klebsiella pneumoniae*	Cefepime / Imipenim (30/10 µg disc)
17. *Proteus mirabilis*	Cefepime / Imipenim (30/10 µg disc)
18. *Pseudomonas aeruginosa*	Cefepime / Imipenim (30/10 µg disc)
19. *Salmonella typhi*	Bactrim* (25µg/disc)
20. *Staphylococcus aureus methicillin resistant (MRSA)*	Vancomycin (30 µg/disc)
21. *Staphylococcus aureus methicillin sensitive (MSSA)*	Vancomycin (30 µg/disc)
22. *Ewingella americana*	Cefepime / Imipenim (30/10 µg disc)

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

2.7. Statistical analysis:

Values are presented as means ± S.E.M. The comparison between two groups was made by independent *t* test. An α level of 0.05 was considered significant for the *t* test.

RESULTS

3.1. Stool analysis

3.1.1. Water content of stools

The effect of the water extract of the flowers of Althaea officinalis on the water content of stools was monitored in 32 rats divided into four groups. The group III which received 590 mg of extract / kg body weight showed a significant decrease in water content when compared with control. The two other groups were very close to control (Figure 3.1).

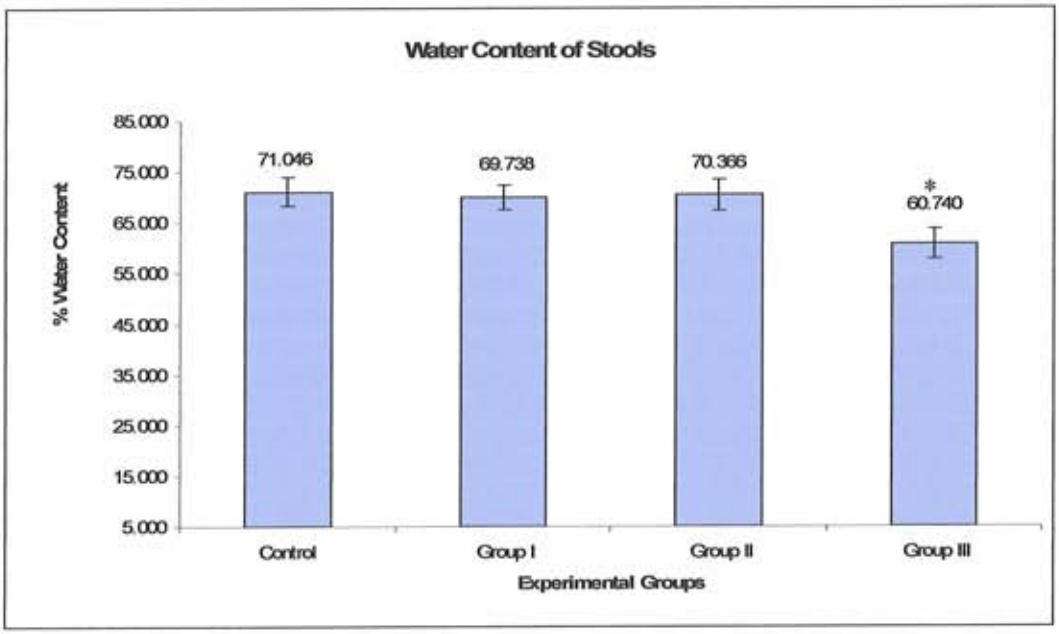


Figure 3.1 The percent water content of stools collected at day 30 after one month of lipid diet and extract intake (59, 236, 590 mg of extract of *Althaea officinalis* flowers / kg body weight to the treatment groups I, II and III respectively). *Significant difference with respect to the control ($p < 0.05$). Bars denote mean ± SEM (n=8)

3.1.2. Determination of Triglyceride

The effect of the water extract of the flowers of Althaea officinalis on the absorption efficiency of triglyceride was monitored. The three groups receiving the extract didn't show any significant difference with respect to the control (Figure 3.2).

3.1.3. Determination of total cholesterol

The effect of the water extract of the flowers of Althaea officinalis on the absorption efficiency of cholesterol was investigated. The three groups receiving the extract didn't show any significant difference with respect to the control (Figure 3.2).

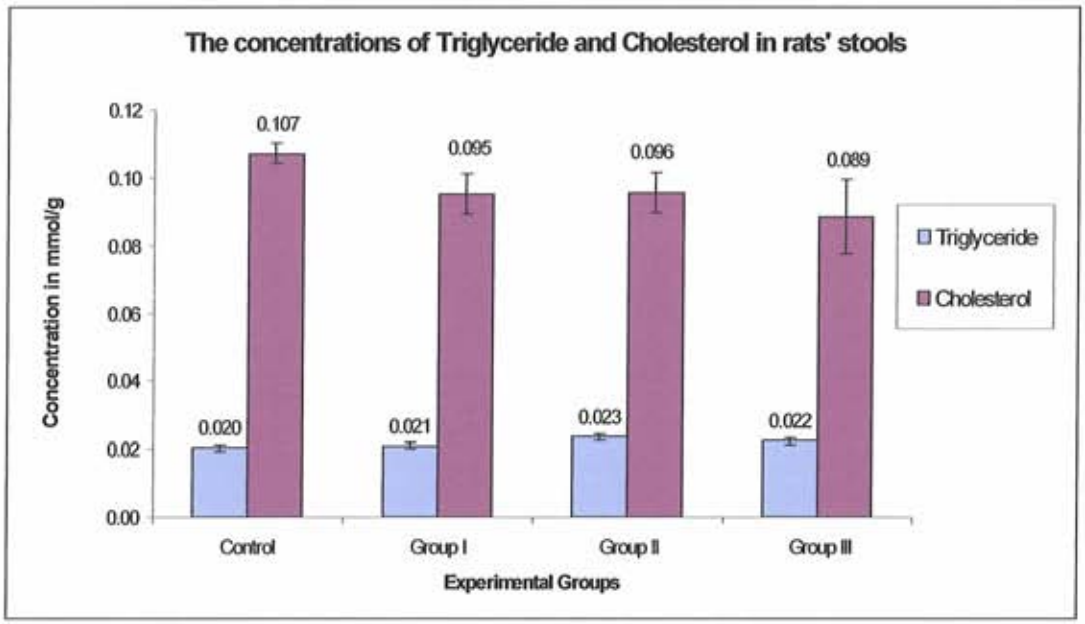


Figure 3.2 The concentrations of triglyceride and cholesterol in stools (mmol/g) collected at day 30 after one month of lipid diet and extract intake (59, 236, 590 mg of extract of *Althaea officinalis* flowers / kg body weight to the treatment groups I, II and III respectively). All the rats were given an isocaloric diet that consisted of rat chow to which 5% coconut oil was added. Bars denote mean ± SEM (n=8)

3.2. Serum lipid profile

The tables 3.1 and 3.2 summarize the mean serum concentration in mg/dL of total cholesterol, TAG, LDL-cholesterol, and HDL-cholesterol in control and treatment groups after 1 month and two months of lipid diet and extract intake, respectively.

3.2.1. Total cholesterol

The cholesterol levels showed a significant increase in the treatment groups I, II and III with respect to the control group, when exposed both to one month and 2 months of treatment. The cholesterol levels of group II and III were increased with respect to group I; however the increase reached significance only after 2 months of extract intake (Table 3.1 and 3.2).

3.2.2. TAG

The triglyceride levels showed a significant increase in the treatment groups I, II and III with respect to the control group, when exposed both to one month and two months of treatment. The TAG levels in all treatment groups were similar (Table 3.1 and 3.2).

3.2.3. LDL- cholesterol

The LDL-cholesterol levels showed a significant decrease in the treatment groups I, II and III with respect to the control, when exposed both to one month and 2 months of treatment. In the first experiment, group II showed a significant decrease in LDL-cholesterol level with respect to group I and III (Table 3.1 and 3.2).

3.2.4. HDL-cholesterol

The HDL-cholesterol levels showed a significant increase in the treatment groups I, II and III with respect to the control, when exposed both to one month and 2 months of treatment. In both experiments, the group II and III showed drastic increase in HDL-cholesterol level which appeared to bee significantly higher than group I (Table 3.1 and 3.2).

3.2.5. Cholesterol/HDL and LDL/HDL ratios

The ratios of total cholesterol / HDL and LDL/HDL of the treatment groups I, II and III were highly decreased with respect to the control group when exposed both to one month and 2 months of treatment. Best ratios were observed in groups II and III in both studies (Figure 3.3 and 3.4).

Table 3.1 Cholesterol (mg/dl), Triglyceride (mg/dl), LDL-Cholesterol (mg/dl) and HDL-Cholesterol (mg/dl) measured in control and treatment groups after 1 month of supplementation with water extract of *Althaea officinalis* flowers (59, 236, 590 mg of extract / kg body weight) to the treatment groups I, II and III respectively, with 5 % lipid diet. Values are expressed as mean ± SEM (n=8).

Parameter	**Control (n=8)**	**Treatment groups (n=8 each)**		
		GI	GII	GIII
Total cholesterol	124.97 ± 3.17	131.69 ± 3.81*	138.97 ± 2.04*	137.95 ± 1.33*
TAG	82.17 ± 1.83	111.17 ± 1.50*	115.38 ± 2.40*	113.78 ± 2.81*
LDL-Cholesterol	75.56 ± 3.56	52.84 ± 1.68*	38.23 ± 2.75*	46.99 ± 2.26*
HDL- Cholesterol	39.56 ± 2.33	46.44 ± 2.8*	77.85 ± 2.28*	68.2 ± 3.26*

* Significant difference with respect to the control

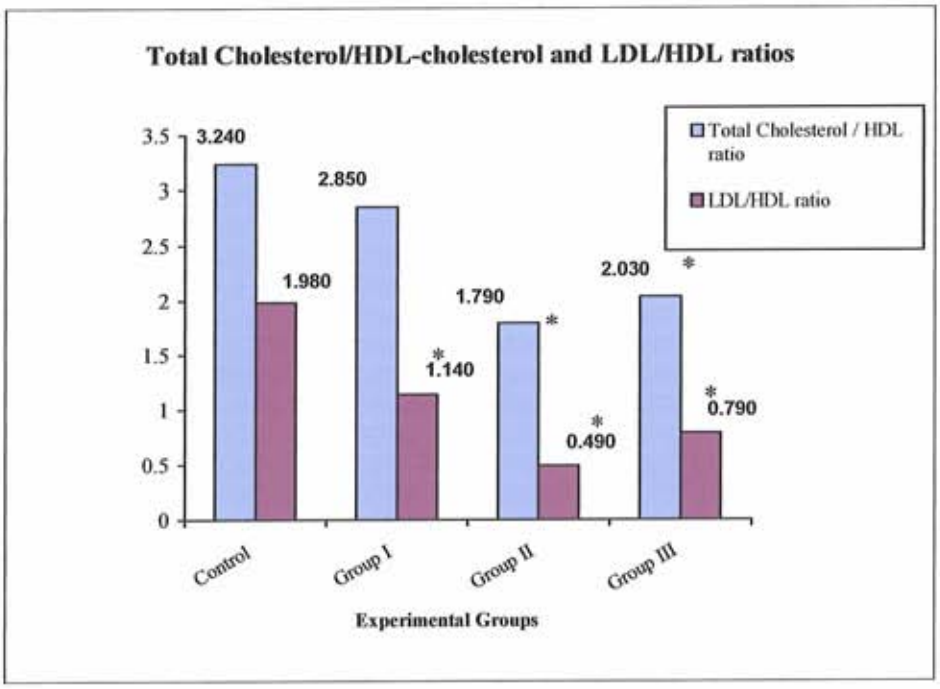


Figure 3.3 The ratios of Total cholesterol / HDL and LDL/HDL in the Control and groups I, II and III after 1 month of *Althaea officinalis* administration (59, 236, 590 mg of extract / kg body weight to the treatment groups I, II and III respectively), with 5 % lipid diet. * Significant difference with respect to the control

Table 3.2 Cholesterol (mg/dl), Triglyceride (mg/dl), LDL-Cholesterol (mg/dl) and HDL-Cholesterol (mg/dl) measured in control and treatment groups after 2 months of supplementation with water extract of *Althaea officinalis* flowers (59, 236, 590 mg of extract / kg body weight) to the treatment groups I, II and III respectively, with 5 % lipid diet.. Values are expressed as mean ± SEM (n=8).

Parameter	**Control (n=8)**	**Treatment groups (n=8 each)**		
		GI	GII	GIII
Total cholesterol	118.5 ± 3.1	128.9 ± 2.1*	156 ± 2.9*	155 ± 4.5*
TAG	80.1 ± 3.51	113.7 ± 4.1*	112.4 ± 3.8*	106.2 ± 4.5*
LDL- Cholesterol	66.36 ± 3.54	55.06 ± 3.62*	53.22 ± 3.61*	51.26 ± 3.88*
HDL- Cholesterol	36.12 ± 2.1	51.1 ± 2.8*	80.3 ± 3.1*	82.5 ± 4.5*

*Significant difference with respect to the control

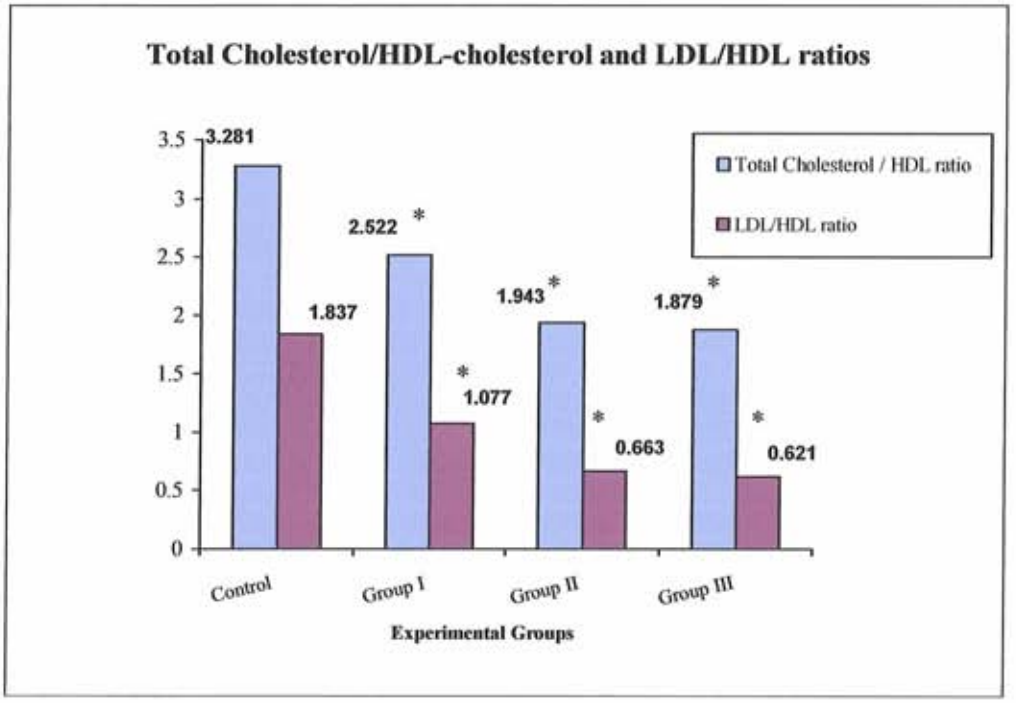


Figure 3.4 The ratios of Total cholesterol / HDL and LDL/HDL in the Control and groups I, II and III after 2 months of *Althaea officinalis* administration (59, 236, 590 mg of extract / kg body weight to the treatment groups I, II and III respectively), with 5 % lipid diet. * Significant difference with respect to the control

3.3. The effect of *Althaea officinalis* on the activity of liver enzymes

The activity of liver enzymes was computed from the extinction decrease measured by the spectrophotometer for 3 minutes at an interval of 1 min. These results were obtained after 1 month (Table 3.3).

SGOT/AST

All experimental groups showed a significant decrease in the activity of SGOT with respect to the control group. No significant changes in SGOT activities were observed among the experimental groups.

SGPT/ALT

All experimental groups showed a decrease in SGPT activities with respect to the control group. However, significance was only reached with group I and III.

LDH

The groups I and II showed a significant increase in the activity of LDH with respect to the control group while group III was similar to the control.

ALP

The group I showed a significant decrease in the activity of ALP with respect to the control group while group II and III were similar to the control.

AST: ALT ratio

Calculation of the AST: ALT ratio revealed that all experimental groups exhibited a lower ratio with respect to the control group.

Table 3.3 SGOT, SGPT, LDH, ALP (U/L) and SGOT/SGPT ratio in serum in control and treatment groups after 1 month of supplementation with water extract of *Althaea officinalis* flowers (59, 236, 590 mg of extract / kg body weight to the treatment groups I, II and III respectively), with 5 % lipid diet. Values are expressed as mean ± SEM (n=8).

Parameter	**Control (n=8)**	**Treatment groups (n=8 each)**		
		GI	GII	GIII
SGOT	183.88 ± 8.90	124.91 ± 5.26*	111.45 ± 11.59*	131.75 ± 6.11*
SGPT	123.38 ± 3.79	89.80 ± 5.59*	113.09 ± 4.46	98.33 ± 3.98*
LDH	360.93 ± 17.78	419.86 ± 16.39*	581.77 ± 29.28*	343.40 ± 11.89
ALP	359.70 ± 8.46	256.16 ± 14.33*	340.73 ± 16.50	350.63 ± 5.28
SGOT/SGPT	1.50 ± 0.01	1.43 ± 0.12	0.99 ± 0.1	1.378 ± 0.06

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

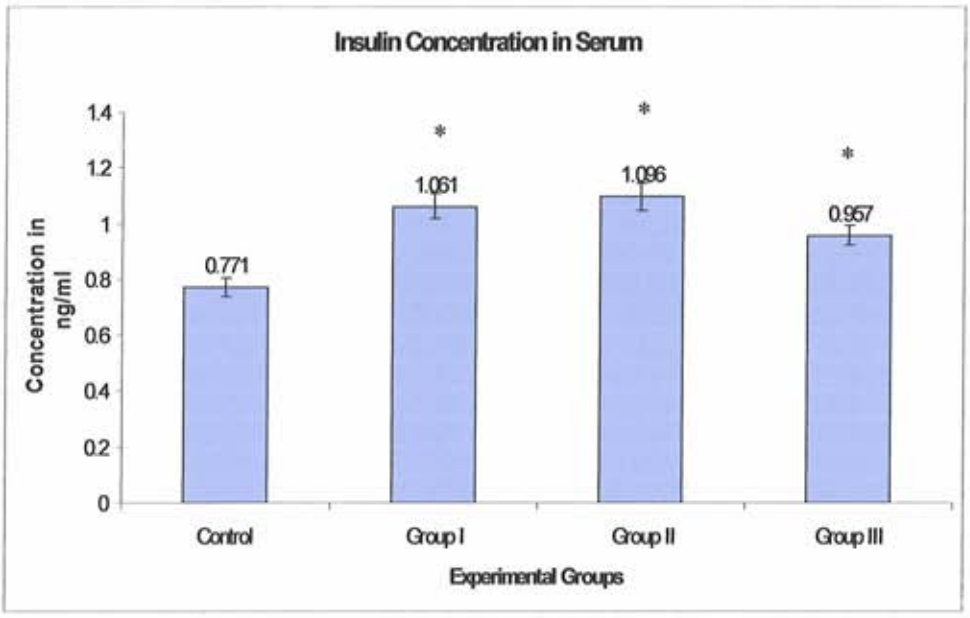
3.4. Serum glycemic profile

3.4.1. Insulin

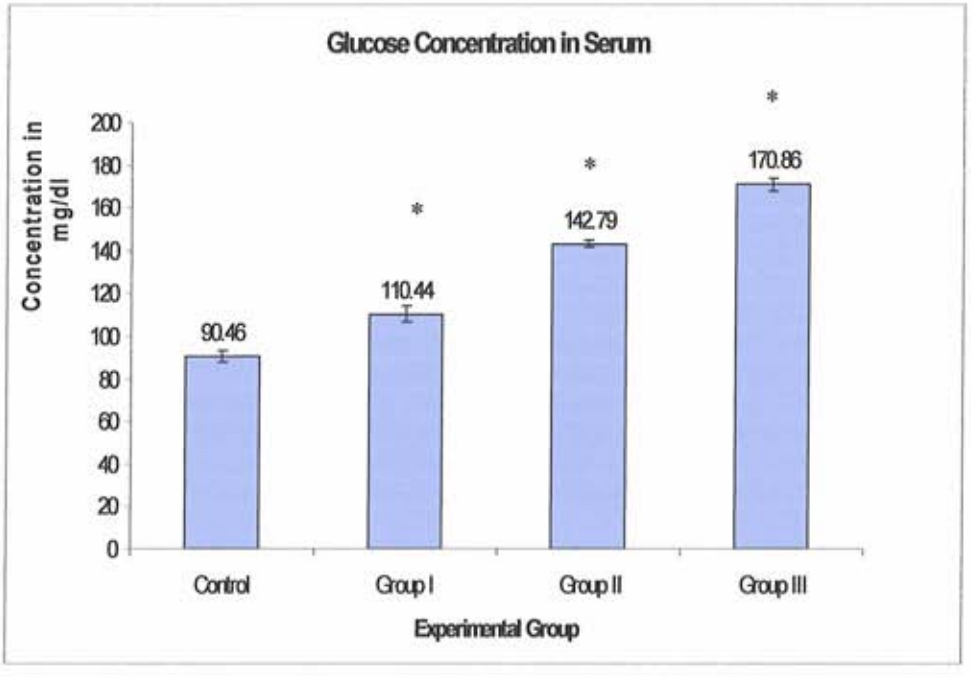
The fasting serum concentrations of insulin increased significantly in the groups I, II and III with respect to the control group when exposed to 2 months of treatment. The increase of insulin observed was not dose-dependant (Figure 3.5).

3.4.2. Glucose

The fasting serum concentrations of glucose increased significantly with respect to the control group when exposed to 2 months of treatment. The increase in serum glucose concentration appeared to be dose-dependant (Figure 3.5).



a



b

Figure 3.5 a. Serum insulin concentrations (ng/ml) in control and experimental groups following two months of intake of water extract of *Althaea officinalis* flowers.

b. Serum glucose concentrations (mg/dl) in control and experimental groups following two months of intake of water extract of *Althaea officinalis* flowers.

* Significant difference with respect to the control. Bars denote mean ± SEM (n=8)

3.5. The effect of water extract of the flowers of *Althaea officinalis* on weight

The curve plotted for the weight changes monitored on weekly bases showed that after week 4, animals of group II showed a less increase in weight gain compared with all other groups. The trend of weight gain in group I and III and the control was similar (Figure 3.6).

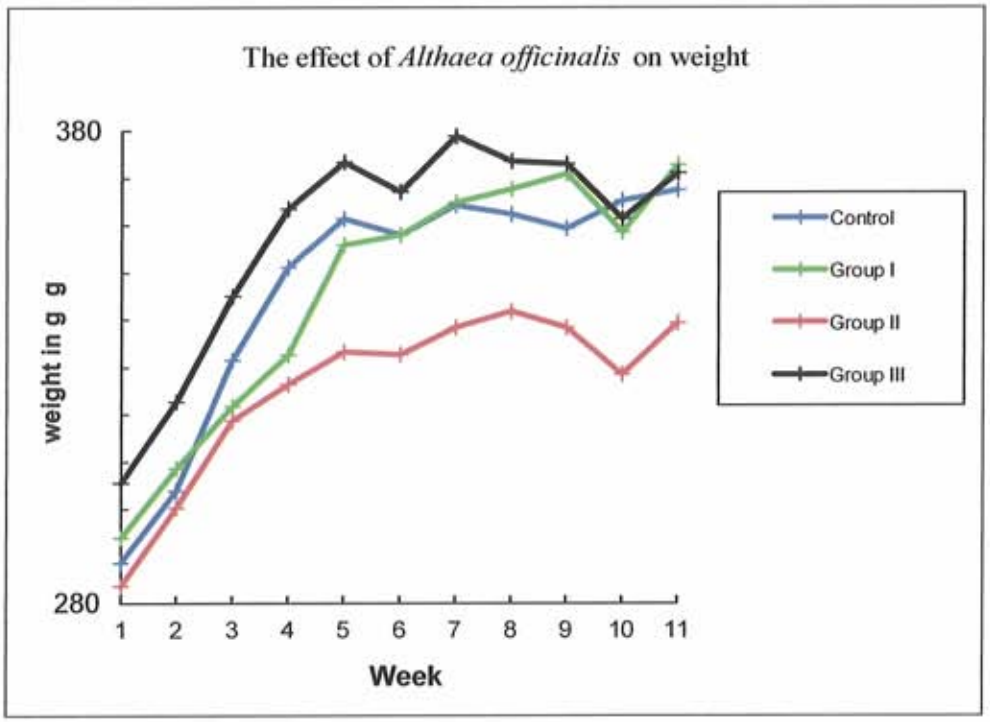


Figure 3.6 All rats were given an isocaloric diet that consisted of rat chow to which 5% coconut oil was added. During the first 3 weeks, animals received 6.5 g of food per 100 g body weight. For the remaining 8 weeks, food was reduced to 5 g / 100 g body weight since some food was not consumed completely in all the groups. Animals of groups I, II and III received *Althaea officinalis* water extract in drinking water as 59, 236 and 590 mg / kg body weight respectively.

3.6. Antiinflammatory activity

The water extract of *Althaea officinalis* flowers exhibited potential anitiinflammatory activities in both acute and chronic induced inflammation. In the acute inflammation model, the 250 mg/ kg body weight dose appeared to be the optimum dose. In the chronic inflammation model, the maximum antiinflammatory activity was also observed with the 250 mg/ kg body weight dose but this antiinflammatory activity was very close to that observe with that of the 100 mg/ kg body weight dose. Increasing the dose to 500 mg/ kg body weight reduced the antiinflammatory activity in both acute and chronic inflammation models. In both models, diclofenac, the reference drug, showed the highest antiinflammatory activities which were not far from that of group II (250 mg / kg body weight dose) (Table 3.4).

3.7. Gastroprotective effect of *Althaea officinalis* against ethanol-induced gastric ulcer

Ethanol-induced gastric damage was characterized by the presence of elongated and petechial lesions found in the glandular region. Animals receiving the extract showed a dose-dependant protection against ethanol induced ulcer. With respect to the control group, animals receiving 100 and 250 mg /kg body weight showed significant protection. The 250 mg/ kg body weight dose showed even a better protection than Cimetril, the reference drug (Table 3.5).

Table 3.4 Effect of water extract of *Althaea officinalis* flowers on carrageenan induced acute and formalin induced chronic inflammation

		Carrageenan		Formalin	
Treatment	Dose (mg/kg)	Increase in paw thickness after 3 h	Inhibition (%)	Increase in paw thickness after 6 days	Inhibition (%)
Control		1.097 ± 0.24	—	1.073 ± 0.21	—
A. officinalis extract	100	0.758 ± 0.13	41 [b]	0.502 ± 0.11[a]	60
	250	0.317 ± 0.12[a]	78	0.422 ± 0.06[a]	68
	500	0.445 ± 0.10[a]	69	0.593 ± 0.22[a]	52 [b]
Diclofenac	10	0.238 ± 0.08[a]	88	0.290 ± 0.02[a]	80

Values are represented as mean ± SEM (n=6), [a] Significant difference with respect to the control ($p < 0.05$)

[b] Significant difference with respect to the diclofenac ($p < 0.05$)

Table 3.5 Effect of water extract of *Althaea officinalis* flowers on ethanol-induced gastric damage in rats

Treatment groups	Nb of animals	Ulcer index (mm)	Protective ratio (%)
Reference (Group I)	6	0	0
Control (Group II)	6	68.4 ± 11.8	0
Extract (50 mg/kg) (Group III)	6	47.7 ± 4.2	30.26 [b]
Extract (100 mg/kg) (Group IV)	6	33.5 ± 6.1 [a]	51.02
Extract (250 mg/kg) (Group V)	6	15.8 ± 3.2 [a]	76.90
Cimetril (11.5 mg/kg) (Group VI)	5	27.4 ± 5.8 [a]	59.94

Nb: number of animals

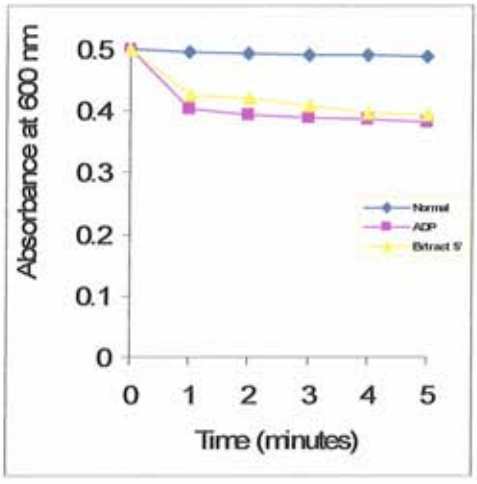
Values are represented as mean ± SEM.

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

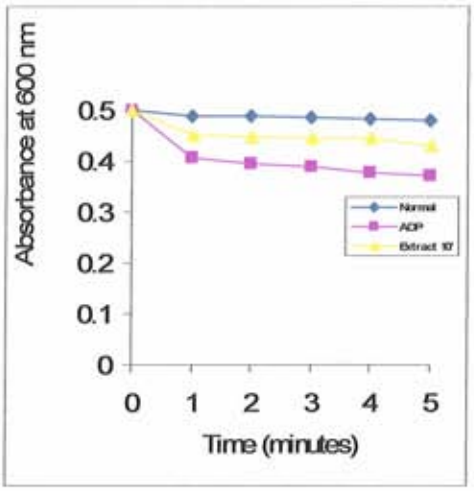
[b] Significant difference with respect to the diclofenac ($p < 0.05$)

3.8. Platelet aggregation activity

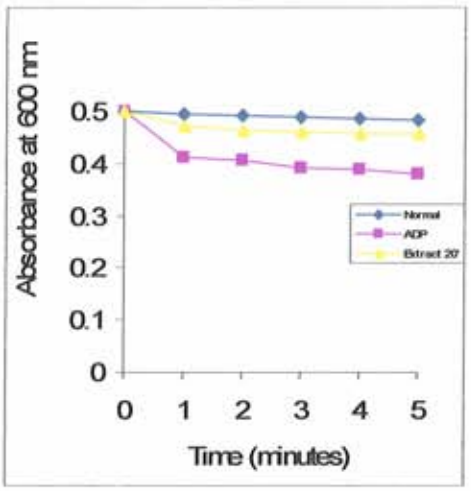
Addition of adenosine 5'-diphosphate (ADP) to washed human platelet is manifested by a marked decrease in OD at 600 nm as ADP is an inducer of aggregation. Preincubation of the platelets with the water extract of the flowers of *Althaea officinalis* (500 µg/ ml) caused an increase in O.D indicating an inhibition of platelet aggregation induced by ADP. The inhibitory effect was time-dependant (Figure 3.7 and table 3.6).



a



b



c

Figure 3.7 a. Platelet aggregation inhibiting activity of water extract of *A. officinalis* when incubated with washed human platelets for 5 minutes. **b.** Platelet aggregation inhibiting activity of water extract of *A. officinalis* when incubated with washed human platelets for 10 minutes. **c.** Platelet aggregation inhibiting activity of water extract of *A. officinalis* when incubated with washed human platelets for 20 minutes. ADP (1mM) was used as the inducer of aggregation.

Table 3.6 Effect of water extract of *Althaea officinalis* flowers (500 µg) on platelet aggregation induced by ADP (1mM) when incubated with washed human platelets for 5, 10 and 20 minutes.

Treatment Groups	Preincubation time (min)	Aggregation (%)	Inhibition (%)
ADP	_	100	0
Water extract of *Althaea officinalis* flowers	5	82.05	17.95
	10	42.21	57.79
	20	28.05	71.95

3.9. The antibacterial activity of the water extracts of *Althaea officinalis* flowers

The water extract of *Althaea officinalis* flowers was tested against 11 bacterial hospital isolates and showed no antibacterial activities against any of the bacterial strains. The zones of inhibition produced by the antibiotics were measured and the diameters are listed in table 3.7. Figure 3.11 illustrates two Muller-Hinton plates on which *Pseudomonas aeruginosa* was grown. The inhibition zones produced by Cefepime and Imipenim in the center of the plates appeared clearly unlike the water extracts of the *Althaea officinalis*

Table 3.7 Antibacterial activity of water extract of *Althaea officinalis* flowers based on disc diffusion method.

Bacteria tested	Zone of inhibition * (mm)	Reference drug	Zone of inhibition (mm)
Citrobacter freundii	0	Cefepime/ Imipenim	25/25
Enterobacter cloacae	0	Cefepime/ Imipenim	35/26
Escherichia coli	0	Cefepime/ Imipenim	35/30
Serratia marcesens	0	Cefepime / Imipenim	37/26
Klebsiella pneumoniae	0	Cefepime/ Imipenim	38/26
Proteus mirabilis	0	Cefepime/ Imipenim	37/23
Pseudomonas aeruginosa	0	Cefepime/ Imipenim	34/25
Salmonella typhi	0	Bactrim	29
MRSA	0	Vancomycin	20
MSSA	0	Vancomycin	18
Ewingella americana	0	Cefepime/ Imipenim	25/30

* There were no zones of inhibition due to the extract for any of the concentrations used.

a  b 

Figure 3.8 a. Diameter of the inhibition zone produced by Imipenim (10 μg)

b. Diameter of the inhibition zone produced by Cefepime (30 μg)

These two plates correspond to *Pseudomonas aeruginosa.*

The antibiotics discs are the one in the center of the plates while the remaining discs, with no zones of inhibition, correspond to discs containing either different concentrations of *Althaea officinalis* water extracts or simply water (control).

DISCUSSION AND CONCLUSIONS

Althaea officinalis is known for its roots more than for its flowers. Many studies were done on its mucilage-rich roots and these were found to be active against periodontopathic facultative aerobic and anaerobic bacteria including *P.gingivalis*, *Prevotella spp.* and *Actinomyces spp.* (Iauk et al., 2003), to have antitussive action (Nosal'ova et al., 1992), to have anti-inflammatory and immunostimulant activity (Scheffer and Konig, 1991), to increase the phagocytic activity of macrophages (Wagner and Proksch, 1985), to have hypoglycaemic activity (Tomoda et al., 1987), to help in the management of anti-inflammatory dermatological conditions along with steroids (Beaume and Balea, 1966; Huriez and Fagez, 1968), to inhibit the pigmentation and to serve as an ingredient for a whitening agent (Kobayashi et al., 2002). A search of the literature on *Althaea officinalis* revealed that no studies were done on its flowers. In Lebanon, the flowers rather than the roots are known and consumed as a common herbal tea. This study was undertaken to evaluate the medicinal effects of the water extract of *Althaea officinalis* flowers. The investigation included the effects of the flower water extract upon blood lipid profile, liver enzyme activity, glycemia, inflammation and gastric ulcer *in vivo*. The study also covered the potential role of the water extract *in vitro* as a bactericidal and anti-aggregant factor for platelets.

The intake of *Althaea officinalis* for a period of four weeks seems not to have a major effect on the water content of stools and consequently on constipation or diarrhea. Only when the highest dose is used, a mild decrease in the water content was observed. However, such a high dose is never commonly used in the community and this explains why it has never been claimed as a plant that causes mild constipation.

Since high levels of triglycerides and cholesterol in the blood are known to be dangerous and risky for cardiovascular diseases and heart shock, their absorption capacity by the intestinal tract was assessed by studying their amounts in the stools. The present study showed that consumption of *Althaea officinalis* for a period of four

weeks had no effect on the absorption efficiency of cholesterol and triglyceride by the digestive system. This indicates that any change to be observed in the blood lipid profile, it should be attributed to lipid synthesis and metabolism rather than to a direct effect of the plant water extract upon the normal absorptive property of the digestive system.

Lipid and lipoprotein abnormalities are associated with development and progression of coronary heart disease (CHD) (Skoumas et al., 2003). To understand the effect of water extract of *Althaea officinalis* flowers on blood lipid profile, five parameters were assessed including the total cholesterol, plasma triglycerides, HDL-cholesterol, LDL-cholesterol and cholesterol/HDL and LDL/HDL ratios. These parameters were measured after one and two months of *Althaea officinalis* flowers water extract intake in drinking water. The present investigation revealed drastic changes in the blood lipid profile of animals receiving the plant extract. A significant increase in serum total cholesterol was observed in all groups receiving *A. officinalis* flowers water extract regardless of the duration of the study. This increase in the total cholesterol levels that sounds to be dangerous for the consumers was found to be related to a drastic increase in HDL-Cholesterol rather than in LDL-cholesterol thereby conferring some cardioprotective effect of *Althaea officinalis*. It is also very important to mention that the plant was efficient in decreasing significantly the LDL- cholesterol level in all groups. These results are of importance as HDL-cholesterol or "Good cholesterol" is the type of lipoprotein that removes cholesterol from the peripheral tissues and the low-density lipoprotein known as "bad cholesterol" is an atherogenic type of lipoproteins since its high level is commonly associated with high risk for cardiovascular diseases and shock (Kingsbury and Bondy, 2003). The increase in HDL-cholesterol and decrease in LDL- cholesterol concentrations observed are probably due to the presence of pectin in the flowers of the *Althaea officinalis*. This result is consistent with that reported by Bobek and Chorvathova (1984) who monitored the effects of pectin on cholesterol distribution in the lipoproteins in rats fed cholesterol diet. They showed that HDL-cholesterol levels were significantly higher in rats fed pectin and LDL-Cholesterol and VLDL- cholesterol were lower. The results obtained in this study were higher than that obtained by Bobek and Chorvathova (1984) and suggest that the extraction of pectin and some other possible active compounds from *Althaea officinalis* plant and flowers in specific is very

precious and important to provide an inexpensive natural constituent that could be used for treating CHD through increasing HDL- cholesterol and reducing LDL-cholesterol.

In the two experiments, it was observed that the triglycerides levels increased significantly in all treatment groups. However, it is very well known that the allowable serum triglyceride range in the blood is wide; for instance in humans it is 40-160 mg/dL in men and 35-135 mg/dl in women (Bucolo and David, 1973) and in rats it is 97.2-168.5 mg/dl (Dubey et al., 2004). Therefore, the observed increase in triglyceride in the treatment groups may not be serious enough to predispose the consumer to high risks of cardiovascular diseases even though elevated serum triglyceride concentration was found to be significantly correlated to coronary heart disease risk (Grundy and Denke, 1990).

If we take into consideration the benefits observed in HDL and LDL-cholesterols with extract intake and knowing that HDL and LDL-cholesterols are better markers for cardiovascular diseases, we should not be afraid of recommending *Althaea officinalis* extract as a remedy for improving blood cholesterol profile. It is also very important to calculate the ratios of total-cholesterol / HDL-cholesterol and LDL-cholesterol /HDL-cholesterol as an additional understanding of the effect of *Althaea officinalis* on the distribution of lipoproteins. It can be noted that the ratios in both experiments decreased significantly with respect to the control. The observed decrease in the ratios is primarily attributed to high increases in serum HDL-cholesterol and secondly to large decreases in serum LDL-cholesterol levels. Indeed, the consistent drastic increase in HDL-cholesterol and decrease in LDL-cholesterol is highly important since it is very rare to observe such drastic changes in normolipidemic rats. In other words, the extract seems to be a competent inexpensive and natural therapy for patients with bad blood cholesterol profile. Finally, one must note that in this study, the LDL-cholesterol was decreased up to 50 % and HDL-cholesterol increased up to 80 %. Studies done on people with high cholesterol showed that lipitor, a commercial drug of active ingredient atorvastatin, at doses of 10 mg to 80 mg lowered LDL "bad" cholesterol levels by 39% to 60% and increased HDL "good" cholesterol levels by 5% to 9% (Schaefer et al., 2002). The drastic increase of HDL-cholesterol

even better than the commercial drugs proves more and more the competitive characteristic of *Althaea officinalis* in treating bad blood cholesterol.

It is popularly believed that herbal products are the best remedies that can be used and the safest, especially in developing countries. Because the herbal remedies are effective and inexpensive, consumers are becoming more proactive in self-treating (Pak et al, 2004). The liver is an essential organ in the body and the major one for metabolism of drugs and exogenous toxins. For this reason, reports of hepatotoxicity must be included in any study conducted on medicinal herbs (Grunhage et al., 2003). Although herbal drugs are generally perceived as harmless, reports of hepatotoxicity are more and more appearing, pointing more on the urgent need to be more precautious in consuming herbal remedies. To test the hepatoprotective activity or the hepatotoxicity of the water extract of *Althaea officinalis* flowers, the activities of transaminases (SGOT and SGPT), lactase dehydrogenase (LDH) and alkaline phosphatase (ALP) in serum of rats were measured after one month of plant extract intake. The water extract of *Althaea officinalis* flowers didn't increase the activity of SGOT and SGPT in serum but on the contrary it decreased them indicating that this extract shows no toxicity on liver cells. In addition, the ratios of SGOT/SGPT were all less than 2 meaning that there is no fear of liver disease and that the extract is safe to be consumed (Cohen and Kaplan, 1979). Similarly the ALP activity in the treatment groups was less or similar to that of the control group. If there is cholestasis, ALP levels in plasma will rise (Pratt and Kaplan, 2001). Consequently, these results suggest that *Althaea officinalis* doesn't increase the risk of cholestasis. However, the level of LDH activity was significantly increased in the extract group I and II with respect to the control but it went back to normal in group III. Looking carefully at the wide allowable range of LDH, in humans, 160- 460 U/L (Bergmeyer, 1975) and in rats the average is 458.9 U/L (Celik et al., 2002); the observed increase may not be of significant importance. This enzyme is present in almost all body cells and it is often used in medicine as a marker of tissue breakdown. The cells broken down are not hepatocytes since all the previous tests didn't show anything suspicious and diagnostic of hepatic disorder. Further studies can be done in order to trigger the cells broken down and more importance can be given to the fact that LDH is abundant in red blood cells and can function as a marker for hemolysis (Pratt and Kaplan, 2001). Since group III receiving the highest dose is expected to cause the highest toxicity effect but

showed an LDH level similar to that of the control, one should underestimate the results observed in groups I and II.

The effect of water extract of *Althaea officinalis* flowers on the glycemic profile in the fasted state was studied. In this study, the extract was found to increase significantly blood glucose level after 2 months of administration in drinking water. This increase in blood glucose level was accompanied with an increase in serum insulin concentrations. The concomitant increase of both serum insulin and glucose levels may raise the possibility of insulin resistance syndrome. If these results are compared with previous work done on this plant, some remarks are important to be made. In a previous study, the mucilage has demonstrated considerable hypoglycaemic activity in non-diabetic mice, at doses of 10 mg/ kg, 30 mg / kg and 100 mg / kg body weight (Tomoda et al., 1987). It is also known that pectin is responsible for the hypoglycemic effect of the plant since its combination with protein and fat significantly lowers the postprandial glycemic as well as insulinemic response to orally administered glucose (Siddhu et al., 1989). This effect was attributed to the fact that pectin may inhibit sugar digestion thereby delaying absorption and consequently lowering postprandial glycemia (Siddhu et al., 1989). However, it is very well known today that pectin is a type of soluble fiber that delays gastric emptying and therefore results in lower postprandial glucose level (Di Lorenzo et al., 1988). Another explanation for the contradiction in the previous results and the results of this study is that in the flowers, the amount of mucilage and pectin might not be enough to lower the glucose level. It must not be forgotten that we have used the crude extract of the flower and the concentrations of the active ingredients used are much different from that of the purified active ingredients used alone where nothing interferes with their activity and modifies it. At the same time, compounds in the crude extract may also have an inhibitory effect on insulin sensitivity when taken chronically (2 months) leading to hyperglycemia and hypereinsulinemia. Further experiments are needed in order to evaluate the effect of the extract upon insulin secretion and insulin resistance. The extract may have a stimulating effect upon pancreatic insulin secretion but at the same time it may be competing or inhibiting insulin from binding to its receptor resulting consequently in hyperglycemia and hyperinsulinemia. It is more important to purify the extract into its active components and remove those behind hyperglycemia.

It is well known that the roots of *Althaea officinalis* have been largely used for treating inflammatory diseases. In order to test any potential effects of the water extract of the flowers, the anti-inflammatory activity of water extract of *Althaea officinalis* flowers was investigated using *in vivo* models of acute and chronic inflammation induced by carrageenan and formalin respectively. The chronic inflammation was included because no previous *in vivo* studies were conducted on any part of the plant. For the acute inflammation induced by carrageenan, the extracts of doses 250 and 500mg/kg showed high percentages of inhibition of the inflammation (78 and 69 %, respectively) and no significant difference was observed compared with the reference drug diclofenac (88 %). For the chronic inflammation induced by formalin, the extracts of doses 100 and 250 mg/kg showed high percentages of inhibition of the inflammation (60 and 68 %, respectively) and no significant difference was observed compared with the reference drug diclofenac (79 %). The inhibition of inflammation was computed from the reduction in the edema caused by the inflammation induced in hind-paw of male Sprague-Dawley rats. In both types of inflammation, the 250 mg/kg dose appeared to be the optimum dose that can be used and increasing the dose beyond 250 mg/kg is no more useful to reduce further the inflammation. Further studies using doses of the extract between 250 mg and 500 mg must be performed in order to locate better the appropriate dose. It is important to note that the antiinflammatory effect of the 250 mg dose extract in both experiments was not significantly different from that of the reference drug diclofenac. The decrease in the antiinflammatory activity observed in the 500 mg dose with respect to the 250 mg dose may be attributed to the presence of other compounds that when present at high concentrations, reduce the potential antiinflammatory effect of the active ingredient. It is worth mentioning that a biological screening of Italian medicinal plants for anti-inflammatory activity revealed no activity for the ethanol extract of the roots of marshmallow in the carrageenan induced rat paw edema test (Mascolo et al., 1987). Other studies were done *in vitro* and showed that the polysaccharides extracted from the roots of *Althaea officinalis* have anti-inflammatory activity. Aqueous extracts stimulated phagocytosis and induced release of oxygen radicals, cytokines, interleukins 6 and tumor necrosis factors form human monocytes in vitro thus exhibiting anti-inflammatory and immunostimulant activity (Scheffer and Konig, 1991). Another study showed that the mucilage polysaccharides of *Althaea officinalis* administered to mice revealed a 2.2 fold increase in the phagocytic activity

of macrophages (Wagner and Proksch, 1985). All these in vitro experiments results represent a base for a promising future and for further studies that can be done on inflammation particularly where one can isolate the active compound from the plant whether the mucilage or the flavonoids that were shown to have antiinflammatory effects (Gudej, 1991; Scheffer and Konig, 1991). This compound can be used in sufficient amounts in many studies both in vitro and in vivo to be sure that no side reactions occurs in vivo and that nothing in vivo diminishes all its phagocytic induction. Taken into consideration previous studies and this present study, it seems that the active ingredient behind the antiinflammatory effect is water soluble rather than alcohol soluble.

Many studies done on plant extracts proved that they represent an attractive source for new treatments especially for gastric ulcer (Alkofahi and Atta, 1999; Borrelli and Izzo, 2000; Zayachkivska et al., 2005). In an attempt to investigate the possible anti-ulcerogenic and gastroprotective effect of the water extract of *Althaea officinalis* flowers, an ethanol-induced gastric damage was carried out. This can be evaluated easily since ethanol (50%) is a corrosive substance for the gastric mucosa that induces long and petechial lesions in a relatively short time. A pre-treatment via oral administration of water extract of *Althaea officinalis* flowers resulted in a dose-dependant protection against 50% ethanol induced gastric lesions. The protection was by 30% (dose of 50 mg/kg), 51% (dose of 100 mg/kg) and 77% (dose of 250 mg/kg) compared with the group that have received Cimetril as a reference drug and which produced 60% protection. The highest dose showed a high protective effect against gastric ulcer more than the reference drug itself. This may be explained by the fact that Cimetril is an antisecretory agent that lowers gastric acid secretion unlike the extracts that may have other protective effects such as enhancing endogenous prostaglandins and mucus synthesis. Previous studies were done on plants and showed that mucilage and flavonoids have the property of covering and protecting the mucosa and treating gastric ulcer (Izzo et al., 1944; Capasso and Grandolini, 1999). The results of this work can't be compared to previous studies done on *Althaea officinalis* since none were done either on roots or on flowers. The gastroprotective effect produced by the flowers of Althaea officinalis is most probably due to the active compounds found in the extract including flavonoids and mucilage polysaccharides.

Other active compounds must be isolated and their mechanism of protection must be revealed.

The effect of water extract of *Althaea officinalis* flowers on platelets aggregation was studied. According to the results obtained, the extract produced a time-dependant inhibition of aggregation of washed rat platelets in the presence of ADP, a potent activator of platelets aggregation (Jose et al., 2004). The 20 minutes of incubation revealed a high inhibition of the aggregation indicating that a certain compound in this extract is inhibiting the aggregation process. A recent study showed that flavonoids inhibit platelet function through binding to the thromboxane A2 receptor (Guerrero et al., 2005). Therefore, flavonoids present in the flowers of *Althaea officinalis* may be responsible for the inhibition of platelet aggregation observed and supports the use of flavonoids in folk medicine in the protection from thrombosis and heart shock.

Medicinal plants from various parts in the world can provide a rich source of antibacterial activities and many plant species have been used widely to cure infectious diseases. In the present study, water extract of *Althaea officinalis* flowers was screened against 11 hospital isolates of different bacterial strains. The extract didn't have any potential antibacterial effect against the bacterial species studied. Previous reports showed that *Althaea officinalis* roots had an antibacterial activity against periodontopathic facultative aerobic and anaerobic bacteria including *P.gingivalis*, *Prevotella spp.* and *Actinomyces spp.* The water extracts were less active than methanol extracts because the active constituents are more soluble in methanol (Iauk et al., 2003). This might be an explanation for the results of this current study since a water extract was used and not a methanolic extract. Further studies must be done on purified compounds of *Althaea officinalis* for possible antibacterial activity.

Further experiments are needed in order to evaluate the glycemic effect of the water extract of *Althaea officinalis* flowers on humans and check if it has the same effect as it had on rats. In addition to that, it is more important to purify the extract into its active components and make sure to remove the ones that are responsible for the hyperglycemic and hyperinsulinemic effects of the extract if it appears to affect humans.

Barnes, J., Anderson, L.A., & Philipson, J.D. (2002). *Herbal medicines: A guide for healthcare professionals* (2nd ed., pp. 331-332). London: Pharmaceutical Press.

Basch, E., Ulbricht, C., Hammerness, P., & Vora, M. (2003). Marshmallow (Althaea officinalis L.): Monograph. *Journal of Herbal Pharmacotherapy, 3*, 71-81.

Basri, D. F., & Fan, S. H. (2005). The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian Journal of Pharmacology, 37*, 26- 29.

Beaume, A., & Balea, T. (1966). Anti-inflammatory experimental properties of marshmallow: Its potentiating action on the local effects of corticoids. *Therapie, 21*, 341-347.

Bergmeyer, H.U. (1975). New values for the molar extinction coefficients of NADH and NADPH for the use in routine laboratories. *Journal of Clinical Chemical Biochemistry, 13,* 507-508.

Berneis, K. K., & Krauss, R. M. (2002). Metabolic origins and clinical significance of LDL heterogeneity. *Journal of Lipid Research, 43*, 1363-1379.

Blaschek, W., & Franz, G. (1986). A convenient method for the quantitative determination of mucilage polysaccharides in Althaea radix. *Planta Medica, 52*, 537.

Bobek, P. & Chorvathova, V. (1984). Effect of pectin on cholesterol distribution in the lipoproteins of streptozotocin-diabetic rats fed cholesterol diet. *Physiol Bohemoslov, 33*,230-236.

Borrelli, F., & Izzo, A. A. (2000). The plant kingdom as a source of anti-ulcer remedies. *Phytotherapy Research, 14*, 581–591.

Fielding, P.E., & Fielding, C.J. (2002). Dynamics of lipoprotein transport in the human circulatory system. In D. E. Vance & J. E. Vance (Eds.), *Biochemistry of lipids, lipoproteins and membranes* (4th ed., Vol. 36). Paris: Elsevier Science.

Flynn, M.A., Nolph, G. B., Flynn, T. C., Kahns, R., & Krause, G. (1979). Effect of dietary egg on human serum cholesterol and triglyceride. *Am. J. Clin. Nutr., 32*, 1050-1057.

Franz, G., & Chladek, M. (1973). Comparative studies on the composition of crude mucus from crossbred descendants of Althaea officinalis L. and Althaea armeniaca Ten. *Pharmazie, 28*, 128-129.

Genest, J., Jr. (1990). *Physician's guide to the management of lipoprotein disorders.* Montreal, Canada: QUE.

Ger Van, D.V., & Bittar, E. (2004). *Advances in molecular and cell biology: lipobiology* (1st ed). Paris: Elsevier Science.

Ghany M., & Hoofnagle, J.H. (2001). Approach to the patient with liver disease. In T. R. Harrison & A. S. Fauci, *Harrison's principles of internal medicine (*15th ed., pp.1707-1711). New York: McGraw-Hill.

Gharzouli, K., Khennouf, S., Amira, S., & Gharzouli, A. (1999). Effects of aqueous extracts of *Quercus ilex* (L.) root bark, *Punica granatum* (L.) fruit peel and Artemisia-alba Asso leaves on ethanol- induced gastric damage in rats. *Phytotherapy Research, 13*, 42-5.

Grundy, S. M., & Denke, M. A. (1990). Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research, 31*, 1149-1172.

Grunhage, F., Fischer, H. P., Sauerbruch, T., & Reichel, C. (2003). Drug- and toxin-induced hepatotoxicity. *Zeitschrift fur Gastroenterologie, 41*, 565-78.

Gudej, J. (1991). Flavonoids, phenolic acids and coumarins from the roots of *Althaea officinalis*. *Planta Med, 57*, 284-285.

Guerrero, J.A., Lozano, M.L., Castillo, J., Benavente-Garcia, O., Vicente, V., & Rivera, J. (2005). Flavonoids inhibit platelet function through binding to the thromboxane A2 receptor. *Journal of Thrombosis and Haemostasis, 3*,369-76.

Guevara, J. M., Chumpitaz, J. C., & Valencia, E. (1994). The in vitro action of plants on Vibrio cholerae. *Revista de Gastroenterologia del Peru, 14*, 27-31.

Gurr, M. I., & Harwood, J. L. (1991). *Lipid biochemistry: An introduction* (4th ed.). London: Chapman & Hall Inc.

Hocking, G. Mc. (1997). *A dictionary of natural product* (pp. 28-38). Medford, NJ: Plexus Publishing Inc.

Horton, H.R., Moran, L.A., Ochs, R.S., Rawn, J.D., & Scrimgeour, K.G. (2002). *Principles of biochemistry* (3rd ed.). Upper Saddle River: Prentice Hall Inc.

Huriez, C., & Fagez, C. (1968). An association of marshmallow-dexamethasone: The pommade Dexalta. *Lille Med, 13*, 121-123.

Iauk, L., Lo Bue, A.M., Millazo, I., Rapisarda, A., & Blandino, G. (2003). Antibacterial Activity of Medicinal Plant Extracts against Periodontopathic bacteria. *Phytotherapy Research, 17*, 599-604.

Iwaki, M., Kamachi, K., Heveker, N., & Konda, T. (1999). Suppression of platelet aggregation by *Bordetella pertussis* Adenylate Cyclase Toxin. *Infection and Immunity, 67*, 2763-2768.

Izzo, A.A., Di Carlo, G., Mascolo, N., Autore, G., Capasso, F. (1944). Antiulcer effect of flavonoids. Role of endogenous PAF. *Phytotherapy Research, 29*, 87-93.

Jose, N., Ajith, T. A., & Janardhanan, K. K. (2004). Methanol extract of the oyster mushroom, *Pleurotus florida*, inhibits inflammation and platelet aggregation. *Phytotherapy Research, 18*, 43-46.

Juneidi, M.J. (1999). *Wild plants of Jordan.* Amman: [n.p.].

Kingsbury, K. J., & Bondy, G. (2003). Understanding the essentials of blood lipid metabolism. *Progress in Cardiovascular Nursing, 18*, 8-13.

Kinsell, L.W., Partridge, W.J., Boling, L., Margen, S., & Michaels, G. (1952). Dietary modification of serum cholesterol and phospholipids levels. *J. Clin. Endocrinol, 122*, 909-913.

Kobayashi, A., Hachiya, A., Ohuchi, A., Kitahara, T., & Takema, Y. (2002). Inhibitory Mechanism of an Extract of *Althaea officinalis* L. on Endothelin-1-Induced Melanocyte Activation. *Biological and Pharmaceutical Bulletin, 25*, 229-234.

Krauss, R.M. (1987). Relationship of intermediate and low-density lipoprotein subspecies to risk of coronary artery disease. *Am. Heart., 113*, 578-582.

Krauss, R. M, & Burke, D. (1982). Identification of multiple subclasses of plasma lipoproteins in normal humans. *Journal of Lipid Research, 29*, 97-104.

Lauque, D., Prevost, M. C, Carles, P., & Chap, H. (1991). Signal transducing mechanisms in human platelets stimulated by cotton bract tannin. *American Journal of Respiratory Cell and Molecular Biology, 4*, 65-71.

Mabberley, D.J. (2000). *The plant-book: A portable dictionary of the vascular plants* (2nd ed., pp. 28). Cambridge Eng.: Cambridge University Press.

Mascolo, N., Autore, G., Capasso, G., Menghini, A., & Fasulo, M.P. (1987). Biological screening of Italian medicinal plants for anti-inflammatory activity. *Phytotherapy Research, 1*, 28-31.

Medical Economics Company. (1998). *PDR for herbal medicines* (1st ed., pp. 635-636). Montvale, N.J.: Medical Economics Company.

Mouterde, P. (1970). *Nouvelle flore du Liban et de la Syrie*. Beirut: Dar El-Mashreq.

Nosal'ova, G., Strapkova, A., Kardosova, A., Capek, P., Zatureck, L., & Bukovska, E. (1992). Antitussive efficacy of the complex extract and the polysaccharide of marshmallow (*Althaea officinalis* L. var. *Robusta*). *Pharmazie, 47,* 224–226.

Pak, E., Esrason, K. T., & Wu, V. H. (2004). Hepatotoxicity of herbal remedies: An emerging dilemma. *Progress in Transplantation, 14,* 91-6.

Papiez, M., Gancarczyk, M., & Bilinska, B. (2002). The compounds from the hollyhock extract (Althaea rosea Cav. Var. nigra) affect the aromatization in rat testicular cells in vivo and in vitro. *Folia Histochem cytobiol, 40,* 353-359.

Popov, S.V., Popova, G. Y., Ovodova, R.G., & Ovodov, Y.S. (2005). Antiinflammatory activity of the pectic polysaccharide from Comarum palustre. *Fitoterapia, 76,* 281-287.

Porter, M. W., Yamanaka, W., Carlson, S. D., & Flynn, M. A. (1977). Effects of dietary egg on serum cholesterol and triglycerides in human males. *Am. J. Clin. Nutr., 30,* 490-495

Pratt, D.S., & Kaplan, M.M. (2001). Evaluation of liver function. In T. R. Harrison & A. S. Fauci, *Harrison's principles of internal medicine* (15th ed., pp.1711-1714). New York: McGraw-Hill.

Quattrocchi, U. (2000). *World dictionary of plant names* (Vol. 1, pp.105-106). Boca Raton, Fla.: CRC press.

Recio, M.C., Rios, J.L., & Villar, A. (1989). Antimicrobial activity of selected plants employed in the Spanish Mediterranean area: Part II. *Phytotherapy Research, 3,* 77-80.

Rifai, N., Warnick, G. R., & Dominiczak, M. H. (1997). *Handbook of lipoprotein testing.* Washington, DC: AACC Press.

Rios, J.L., Recio, M.C, & Villar, A. (1987). Antimicrobial activity of selected plants employed in the Spanish Mediterranean Area. *Journal of Ethnopharmacology, 21*, 139-152.

Sagdullaev, B.T., Shakhidoyatov, R.K., Khodzhaeva, M.A., Chernenko, T.V., Turakhozhaev, M.T., & Abduazimova, M.A. (2001). Lipids and carbohydrates from *Althaea nudiflora* and *Althaea armeniaca* roots. *Chemistry of Natural Compounds, 37*, 208-212.

Salvemini, D., Wang, Z. Q., Wyatt, P. S., Bourdon, D. M., Marino, M. H., & Manning, P.T. (1996). Nitric oxide: A key mediator in the early and late phase of carrageenan-induced rat paw inflammation. *British Journal of Pharmacology, 118*, 829-838.

Schaefer, E. J., Eisenberg, S., & Levy, R. I. (1978). Lipoprotein apoprotein metabolism. *Journal of Lipid Research, 19*, 667 – 687.

Schaefer, E.J., McNamara, J.R., Tayler, T., Daly, J.A., Gleason, J.A., Seman, L.J., Ferrari, A.,& Rubenstein, J.J.(2002). Effects of atorvastatin on fasting and postprandial lipoprotein subclasses in coronary heart disease patients versus control subjects. *American Journal of Cardiology, 90,* 689-96.

Scheffer, J., & Konig, W., (1991). Radix althaeae und Flores chamomillae Extrakte auf Entzündungsreaktionen humaner neutrophiler Granulozyten, Monozyten und Rattenmastzellen. *Abstracts of the Third Phytotherapy Congress, 9.*

Schulz, H., & Albroscheit, G. (1988). High-performance liquid chromatographic characterization of some medical plant extracts used in cosmetic formulas. *Journal of Chromatography, 422*, 353-361.

Siddhu, A., Sud, S., Bijlani, R.L., Karmarkar, M.G., & Nayar, U. (1989). Modulation of postprandial glycemia and insulinemia by pectin in mixed nutrient combinations. *Indian Journal of Physiology and Pharmacology, 33,* 77-83.

Skoumas, J., Pitsavos, C., Panagiotakos, D.B., Chrysohoou, C., Zeimbekis, A., Papaioannou, I., et al. (2003). Physical activity, high density lipoprotein cholesterol and other lipids levels, in men and women from the ATTICA study. *Lipids in Health and Disease, 2,* 3-9.

Slater, G., Mead, J., Dhopeshwarkar, G., Robinson, S., & Alfin-Slater, R.B. (1976). Plasma cholesterol and triglycerides in men with added eggs in the diet. *Nutr. Rep. Int., 14,* 249-260

Steiner, R. P. (1986) *Folk medicine: The art and the science* (pp. 1-3, 28, 104). Washington, D.C.: American Chemical Society.

Tepperman, B.L., Jacobson, E.D. (1994). Circulatory factors in gastric mucosal defense and repair. In L.R. Johnson. *Physiology of the gastrointestinal tract* (pp. 1331-1352). New York: Raven Press.

Tomoda, M., Shimizu, N., Oshima, Y., Takahashi, M., Murakami, M., & Hikino, H. (1987). Hypoglycemic activity of twenty plant mucilages and three modified products. *Plant Med, 53,* 8-12.

Tomoda, M., Shimada, K., Shimizu, N., & Takasu, T. (1980). The structural features of *Althaea* - mucilage representative mucous polysaccharide from the roots of *Althaea officinalis. Chemical and Pharmaceutical Bulletin, 28,* 824-830.

Tortora, G.J., & Grabowski, S.R. (2003). *Principles of anatomy and physiology* (10th ed.). New York: John Wiley & Sons, Inc.

Voravuthikunchai, S. P. & Kitpipit, L. (2005). Activity of medicinal plant extract against hospital isolates of methicillin-resistant *Staphylococcus aureus. Clinical Microbiology and Infection, 11,* 510-512.

Wagner, H., & Proksch, A. (1985). Immunostimulatory drugs of fungi and higher plants. *Economic and Medicinal Plant Research, 1*, 111-153.

Wang, D. Q-H., Paigen, B., & Carey, M.C. (2001). Genetic factors at the enterocyte level account for variations in intestinal cholesterol absorption efficiency among inbred strains of mice. *Journal of Lipid Research, 42*, 1820-1830

Wang, D. F., Shang, J. Y., & Yu, Q.H. (1989). Analgesic and anti-inflammatory effects of the flower of Althaea rosea (L.) Cav. *Zhongguo Zhong Yao Za Zhi, 14*, 46-48.

Xu, C. D., Gan, R. B., Chen, S. N., Jiang, S. H., & Xu J. Y. (1998). Protection of gastric mucosa from ethanol induced injury by recombinant epidermal growth factor in rats. *Western Journal of Communication, 4*, 437-438.

Zayachkivska, O.S., Konturek, S.J., Drozdowicz, D., Konturek, P.C., Brzozowski, T., & Ghegotsky, M.R. (2005). Gastroprotective effects of flavonoids in plant extracts. *Journal of Physiology and Pharmacology, 56*, 219-231.

Appendix I

Classification and Common names
of
Althaea officinalis L.

I-Classification of *Althaea officinalis*

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Dilleniidae –
Order	Malvales –
Family	Malvaceae – Mallow family
Genus	*Althaea* L. – marshmallow
Species	*Althaea officinalis* L. –common marshmallow

II-Common names of *Althaea officinalis*

English: Marshmallow

French: Guimauve

Spanish: Malvavisco

German: Eibisch

Arabic: Khitmi خطمي

Appendix II

Illustrations of *Althaea officinalis*

(Cook, 1869)