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***Nasturtium officinalis* water extract: A potential anti-inflammatory,  
anti-ulcerogenic, and antioxidant remedy.**

by

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

Master of Science

Molecular Biology

Lebanese American University

May, 2009

**Under the supervision of**

**Dr. Constantine Daher**

648291



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### Thesis approval Form (Annex III)

Student Name: Najwan Fares I.D. #: 200600210

Thesis Title : *Nasturtium officinalis* water extract: A potential anti-inflammatory, anti-ulcerogenic, and antioxidant remedy.

Program : M.S. in Molecular Biology

Division/Dept : Natural Sciences Department

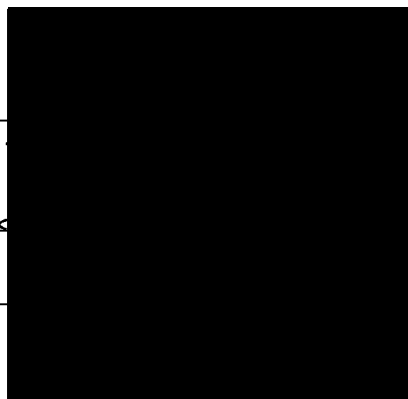
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ABSTRACT

***Nasturtium officinalis* water extract: A potential anti-inflammatory, anti-ulcerogenic, and antioxidant remedy.**

By Najwan Milad Fares

*Nasturtium officinalis* plants, commonly known as Watercress, or Habb Ar Rashad in Arabic, possess a wide range of biological activities. Although not many studies were conducted on Watercress, research is still in progress about this plant because of its well known medicinal value in folk medicine throughout the world and especially in the Arab countries. The present study explores the effects of the water extract of *Nasturtium officinalis* upon rat blood lipid profile, glycemia, liver enzymes, gastric ulcer, inflammation and antioxidant activity.

After one month of chronic extract intake of *N.officinalis* water extract (100, 250 and 500 mg/kg body weight) via drinking water, rats showed no significant changes in the serum total cholesterol, HDL cholesterol and triglyceride levels. Similarly, the glucose level was not significantly different from the control group. Extract intake did not appear to affect stool triglyceride and water content. However a significant decrease in stool cholesterol was observed with the lowest dose. The 100 and 250 mg/kg body weight doses showed a decrease in body weight. Liver enzyme activities (ALT, ALP, AST) were not negatively affected thus assuring that the extract has no hepatotoxic effects over the study period. Extract at 100, 250, and 500mg/kg body weight exhibited substantial anti-inflammatory effects in cases of acute and chronic inflammation induced by carrageenan and formalin respectively. Pre-treatment of fasted rats with the extract (100 and 250mg/kg body weight) also demonstrated significant protection

against ethanol-induced gastric ulcer. *N. officinalis* also exhibited a strong scavenging activity against DPPH radicals (55 %) and the ferric reducing antioxidant power (FRAP) value was 1085 $\mu$ mol/g. The content of phenolic compound in the extract assessed by Folin-Ciocalteu reagent was 820 mg galic acid equivalent/ g and gave a high phenolic compound content. In conclusion, these investigations suggest that the water extract of *Nasturtium officinalis* is an easy, inexpensive remedy used to treat various conditions that affect the human well-being.

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## **GLOSSARY**

**ADP:** Adenosine-5-Diphosphate

**ALT:** Alanine Aminotransferase

**ALP:** Alkaline Phosphatase

**4-AP:** 4-Aminophenazone

**AST:** Aspartate Aminotransferase

**ATPase:** Adenine Triphosphatase

**CM:** Chylomicron

**DAP:** Dihydroxyacetone phosphate

**DPPH:** 2, 2-Diphenyl-1-picrylhydrazyl

**FRAP:** Ferric reducing antioxidant power

**GAE:** Gallic Acid Equivalent

**GOD:** Glucose Oxidase

**GOT:** Glutamate Oxaloacetate

**GPO:** Glycerol-3-Phosphate Oxidase

**G3P:** Glycerol-3-Diphosphate

**GPT:** Glutamate Pyruvate- Transaminase

**HDL:** High Density Lipoprotein

**H<sub>2</sub>O<sub>2</sub>:** Hydrogen Peroxide

**IDDM:** Insulin Dependant Diabetes Mellitus

**LFT:** Liver Function Tests

**LDH:** Lactate Dehydrogenase

**LPL:** Lipoprotein Lipase

**MDH:** Malate Dehydrogenase

**NADH:** Nicotinamide Adenine Dinucleotide

**NIDDM:** Non-insulin Dependant Diabetes Mellitus

**NSAID:** Non-Steroidal Anti-inflammatory Drugs

**OD:** Optical Density

**PEITC:** Phenyl Isothiocyanate

**POD:** Peroxidase

**PMN:** Polymorphonuclear Neutrophilic Leukocyte

**SGOT:** Serum- Glutamic Pyruvic- Transaminase

**SGPT:** Serum- Glutamic oxaloacetic- Transaminase

**TAG:** Triglyceride

**VLDL:** Very Low Density Lipoprotein

## **ACKNOWLEDGMENT**

I would like to express my gratitude and appreciation to my advisor, Dr. Constantine Daher for his support, encouragement and assistance throughout my Master years at LAU. He was also a person to lean on, and talk to anytime; He was a friend. Without his help, this work would not have been possible. Over and over again, Thank you. I would also like to thank the members of the committee, Dr. George Baroody and Dr. Mohammad Mroueh for their constant concern about my work progress and for their valuable comments. Special thanks to Miss Helena Bou Farah for her assistance and support in the lab, and to Mr. Jean Karam that was my aid always in the feeding process and in maintaining the animals purposed for this research.

To my best friends; Mariam Al Ali Dabbousi, Jinane Al Agha, and Zeina Nasr, thank you for being a part of my life and sharing with me the best and the worst days ever. I would also like to thank my colleagues and friends Maya Farah, Wael Bahnan, and Nina Najjar for being with me always, bringing laughter to my world and being my lab “amigos” all the way. Rana Andraos, Jihad Andari, Nahla Issa, Jalil Daher, Sonia Youhanna, Nizar Tabet, Sleiman El Banna, Wissam Khalil, Angelique Salloum, Mazen Rizk, and Samer Younes, thank you all for your constant moral support.

Last but not least, I would like to thank my parents, brother and sister, and my loved one, for being by my side in each and every step, always supporting me mentally and morally. Thank you for your extreme love and patience. You always believed in me and wanted me to be where I am now, so this work is dedicated to you. I also dedicate my work to my late Grandparents.

## *Chapter 1*

### **INTRODUCTION**

Medicinal plants and plant-derived medicines are extensively used in conventional cultures all over the world and they are becoming very popular in modern societies as natural alternatives to synthetic chemicals (Van Wyk and Wink, 2004). Traditional medicine has been a remedy since decades. There are around 70,000 plants (including the lower plants) that are used in medicine and according to the World Health Organization (WHO), about 80% of the world population depends on herbal-based alternative systems of medicine (Daniel, 2006). Defined as "beliefs, knowledge, manual techniques and health practices involving plant, mineral, and animal based medicines, exercises, and spiritual therapies", traditional medicine can be used solely or in a mixture for the diagnosis, treatment and prevention of many illnesses (Gruenwald et al., 2004). Nowadays, traditional medicine has been referred to as "Complementary" or "Alternative" medicine. According to the National Institute of Health (NIH) "complementary and alternative medicine (CAM) is a various group of medical and health care systems, and products that are not presently believed to be part of conventional medicine" (National Center for Complementary and Alternative Medicine, 2009). Crude forms of the plants were used at the beginning as food additives. Later on, they were considered as a remedy for a variety of diseases ranging from very mild to serious illness like high blood pressure, heart insufficiency, cardiac rhythm disturbance, cerebral disorders (Dementia, Dizziness, Ringing in the ear), psychological and psychosomatic disturbances (Depression, Anxiety, Insomnia, Stress, Travel sickness, etc.), disorders of the digestive tract (Spasms, lack of appetite, Dyspepsia, Gastritis, Ulcer, Colitis, Diarrhea, Constipation, etc.), disorders of the liver and bile system,



kidneys and urinary tracts, asthma, cancer and others (Daniel, 2006). People are shifting to herbal drugs due to some factors: low cost, safeness, effectiveness, curiosity of being involved in the decision making, and its rare toxic reactions (Saad et al., 2005). Some people who use traditional remedies may not understand the scientific rationale behind their use, but they know that some medicinal plants can be effective if taken properly and according to a specific dose (Van Wyk and Wink, 2004). Although various herbs have considerable values in treating side effects of some conventional treatments, many can hamper conventional medicine. Thus, it is not always safe to use herbal medicine because of possible toxicity or side effects on the human health. Therefore, it is recommended to seek a qualified practitioner whenever they are used.

The Middle East is known as the cradle of civilization and many of the plants we grow as crops today were cultivated in this region (Van Wyk and Wink, 2004). In the Mediterranean region traditions, medicinal plants are used to treat a variety of ailments. Recent surveys reported the presence of more than 2600 plant species the Middle Eastern region of which more than 700 are recorded for their use as botanical pesticides or as medicinal herbs (Saad et al., 2005).

### 1.1. Plant Distribution and Taxonomy

*Nasturtium officinalis*, known in Arabic as “Habb Ar Rashad” or “Huruf Al May”, belongs to the family “Cruciferae”. Its common name is “Watercress” (Tabal, 1994). Watercress is a perennial herb having pungent leaves and young stems which are widely used for salads and garnishing (**Figure 1.1**). It has hermaphrodite flowers (have both female and male organs) that are pollinated by bees and flies (Stephans, 1994). Indigenous to the Mediterranean border, it is recently cultivated all around the world especially in Africa, Britain, Dominican Republic, France, Haiti, Hawaii, Iraq, Italy, Mexico, Spain, Turkey, and Venezuela .



**Figure 1.1:** *Nasturtium officinale* (<http://www.passionbassin.com/plante-aquatique/Nasturtium-officinalis.php>).

#### **1.1.1. Cultivation and Harvest:**

*N. officinale* is in flower from May to October, and the seeds ripen from July to October. It is a type of plant that prefers all types of soil and easily grows when given the correct conditions of slowly flowing clean water. A preference is growing in water about 5cm deep with an optimum pH of 7.2 and it is often cultivated for its edible leaves only (Tabal, 1994).

#### **1.1.2. Plant History, Traditional and Medicinal Use:**

*N. officinale* has been used for many decades in many cultures for many uses and is normally consumed as a vegetable in salads, soups, and other various recipes. According to Yazdanparast et al. (2008), when *N. officinale* hydroalcoholic extract (500mg/kg body weight per day) was given to hypercholesterolemic rats in vivo for 30 days, their triglyceride, blood total

cholesterol, and low density lipoprotein cholesterol decreased respectively by 44, 37, and 48%, while the blood high density lipoprotein cholesterol levels increased by 16%. Its leaves are also widely used as a home remedy against diuretic, depurative, hypoglycemic, expectorant, and odontalgic conditions (Yazdanparast et al., 2008). The diuretic effect may be due to the mustard oil content (Gruenwald et.al, 2004). When watercress is given in diet, it alters the blood antioxidant status in healthy adults and reduces lymphocyte DNA damage (Gill et al., 2007). Moreover, *N. officinalis* extract modulates antioxidant enzyme activities, suppresses lipid peroxidation and increases glutathione (GSH) levels of the liver (Yazdanparast et al., 2008). Studies conducted by Bahramikia and Yazdanparast (2008) proved that the hydroalcoholic extract of watercress is also a cardioprotective agent. In the medieval ages, *N. officinalis* was used as a remedy in treating toothaches that did not relate to humoral imbalances (Anderson, 2004). Because it is rich in vitamin C and A, it is considered as an excellent remedy against scurvy (Naegele, 1970). Acting as an amaroid drug, it stimulates appetite and digestion (Naegele, 1970).

Watercress is an expectorant that is able to clean the bronchi and ease coughing (Tabal, 1994). In vitro studies have shown that it has a high antimicrobial activity (100µg/ml) against drug-sensitive and resistant *Mycobacterium tuberculosis* (Camacho-Corona Mdel et al., 2008). Used internally and externally, the fresh juice or extract of *N.officinalis* has been used to treat inflammations of the chest and skin, in addition to kidney problems, and chronic irritations (Tabal, 1994). It is well known for being an effective hair boost, helping in the endorsement of the growth of thick hair (Tabal, 1994). Upon chewing *N. officinalis*, phenethyl isothiocyanate (PEITC) is released and it is a chemopreventive agent against lung cancer (Hecht et al., 2005).

### 1.1.3. Precautions:

*N. officinalis* like any other herb should be taken carefully and under the supervision of a practitioner in the field of botanical therapy since certain active substances of herbs can intermingle with other supplements or medications and result in side effects in patients. Watercress is safe when taken in recommended doses, but if used excessively, in salads, it can lead to stomach upsets due to the mucous membrane-irritating effect of the mustard oil (Gruenwald et al., 2004). Watercress may also be a host of enteroparasites, the deadly liver fluke; therefore it is recommended to eat those only grown commercially in watercress beds (Oliveira and Germano, 1992). Studies also proved that *Fasciola hepatica*, a parasite that invades a water snail host, lives on *N. officinalis* and causes symptoms in humans including irregular fever, abdominal tenderness, epigastric pain, jaundice and eosinophilea (60%) of patients. For this reason researchers advise to boil fresh watercress for a few seconds or just dry them (Carrada-Bravo, 2003).

### 1.2. Gastric Ulcer

Gastric mucosal layers act as a hurdle preventing the internal or external exposure of the gastric mucosal cells to different irritants and luminal, injurious agents. The microbiological, physical or chemical agents that act from the gastric lumen attack the mucosal surface epithelium and play important roles in several diseases including peptic ulcer, gastritis, or gastric cancer (Zayachivska et al., 2005). Ulcer is a disease that is caused by disruptions of the mucosal integrity of the duodenum or stomach. Due to active inflammation, it leads to a local defect and in severe cases, cancer and hemorrhage (Braunwald et al., 2001). Affecting a great number of the world population, gastric and duodenal ulcers are provoked by many factors: genetic

susceptibility, drugs, alcohol, stress, smoking, nutritional deficiencies, bile salts, and an excessive secretion of acid and pepsin. The presence of *Helicobacter pylori*, a gram -negative bacteria that is found in the stomachs of most peptic ulcer patients, remains the main factor inducing gastric ulcer (Widmaier et al., 2004; Belaiche et al., 2002). In addition to other factors such as non-steroidal anti-inflammatory drugs (NSAID) that are very well known to induce gastric mucosal damage (Braunwald et al., 2001).

Inhibition of acid secretion is the basis of ulcer treatment, and this is achieved by two drug classes. Class one (example cimetidine) blocks specific histamine receptors ( $H_2$ ) that are found on parietal cells and thus stimulates acid secretion. Class two (example omeprazole) inhibits the  $H^+/K^+$ -ATPase pump in parietal cells, which pumps proton into gastric lumen (Widmaier et al., 2004). Gastrointestinal disorders have been treated by several plants and herbs in traditional medicine, and have shown promising results in the treatment of gastric ulcer (Borelli and Izzo, 2000; Toma et al., 2003). Although anti-histamines and anti-acids are two major medications for the treatment of gastric ulcer, they have been shown to have side effects including gynecomastia, impotence, and hematopoietic changes (Ariypshi et al., 1986). Therefore, there is more demand for anti-ulcer agents that have a higher efficacy and lower toxicity (Alkofahi and Atta, 1999). Due to the widely utilization of several plant and herb extracts in treating gastric ulcer in traditional medicine, more effort is required in this respect to prove the safety and effectiveness of herbal supplements when used as a remedy for treating gastric diseases (Kushima et al., 2005).

### **1.3. Inflammation**

Inflammation a complex biological response to any infection that is essential and common. It starts with an infection and is manifested by pain, redness, heat and swelling of the damaged or inflamed tissue (Tortora and Grabowski, 2003). The cellular progression of an inflammatory response eventually results in the healing or scarring of the lesion (Sell and Max, 2001). There are two types of inflammation: acute and chronic.

Acute inflammation is a type of inflammation that is characterized by vasopermeability, vasodilation, and infiltration of polymorphonuclear neutrophilic leukocyte (PMNs) into the injured area (Sell and Max, 2001). On the other hand, chronic inflammation is characterized by infiltration of macrophages and lymphocytes (T & B) into the injured area, and then restoration of normal structure or scarring and is known to complete the healing process and be more defensive against persistent infections(Sell and Max, 2001). Inflammatory diseases are linked with the release of reactive oxygen, cytokines and all nitrogen species (Conner and Grisham, 1996). Many natural and synthetic antioxidants derived from plant extracts have been considered as potential therapeutic mediators due to the significance of reactive oxygen species in the inflammatory process (Sell and Max, 2001).

### **1.4. Lipid Profile**

#### **1.4.1. Overview :**

Lipids are lipophilic biological molecules that are soluble in organic solvents and insoluble in aqueous solutions and are of great physiological importance for humans. They have four major functions that include serving as structural components of biological membranes and providing energy reserves mainly in the form of triacylglycerols (Vance and Vance, 2004). Some

lipids work as pigments (carotene), cofactors (vitamin K), hormones (sex hormones), transporters (eicosanoids), and detergents (bile salts). The main component of both triacylglycerols (triglycerides) and phospholipids is the fatty acids that are a major class of biologically important lipids (Nelson and Cox, 2004). Cholesterol and its derivatives represent the second class of lipids that play a major role in biological system (Ger, 2004).

#### **1.4.2. Cholesterol:**

Cholesterol is an important lipid component of biological membranes influencing membrane fluidity and acting as a precursor for bile formations and steroid hormones. It is a structural component in cell membranes and plasma lipoproteins, and a crucial nutrient that forms vitamin D. Absorbed from the diet and synthesized in the liver and other body tissues, it is then metabolized to steroid hormones including mineralocorticoids and glucocorticoids (Ger and Van der Vusse, 2004).

#### **1.4.3. Triglycerides:**

Triglycerides, the main storage form of lipids, constitute about 95% of fatty tissues. They are made of one molecule of glycerol bonded to three molecules of fatty acids (Loeb, 1994). The serum levels of triglycerides are determined by two factors: the ability to hydrolyze circulating triglyceride and the rates of hepatic secretion of VLDL triglyceride. Hypertriglyceridemia is a condition caused by hepatic overproduction of VLDL triglycerides or defective lipolysis of triglyceride-rich lipoproteins (Grundy and Denke, 1990).

#### **1.4.4. Lipoproteins:**

Triglycerides, cholesterol, and phospholipids are compounds that are insoluble in water and are carried in the blood plasma from one tissue to another as plasma lipoproteins which are macromolecular complexes called apolipoproteins (specific carrier proteins)(Nelson&Cox,2004). The major lipoproteins are classified according to their density. Lipoproteins that have a high ratio of lipid to protein have a lower density than a lower ratio of lipid to protein since lipids have a lower boyant force than proteins. Chylomicrons (CM) are large lipoproteins that are synthesized in the small intestine to transport dietary triacylglycerols to various tissues. Very low density lipoproteins (VLDL) are types that are synthesized in the liver for the export of endogenous triacylglycerols, while low density lipoproteins (LDL) arise from the metabolic transformation of VLDL in circulation. LDLs deliver cholesteryl esters to peripheral tissues and liver. High density lipoproteins (HDL) are another type of lipoproteins that are made and assembled in the intestine and liver or are formed from metabolic transformations of other lipoproteins in circulation. They form cellular lipids at the cell membranes. Excess cholesterol is usually removed from cells by HDL and transported to the steroidogenic tissue and the liver for normal metabolism and excretion (Vance, 2004). Since HDLs prevent the accumulation of cholesterol in the blood, a high level of HDL is associated with a lower risk of coronary artery disease (Tortora and Grabawski, 2003).

#### **1.5. Liver assessment**

Biotransformation of all drugs and xenobiotics and synthesizing a range of body proteins are important to the liver (Lee, 1993). A number of disease conditions are analyzed by some enzyme activities in blood serum. These tests are mainly called the liver function tests or the



“LFTs “. Two important enzymes in the diagnosis of liver damage are: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Nelson and Cox, 2004). A single metabolome, which is the sum total of primary and secondary metabolites of a plant, contains about 4,000 - 10,000 compounds. All these compounds are used by plants for various purposes such as reproduction, nutrition, maintenance, healing, offense, defense, etc. Secondary metabolites or natural product, are low molecular weight compounds that do not play any role in the primary plant metabolism. They just constitute the active ingredient of the medicinal plant (Van Wyk and Wink, 2004). The liver can detoxify consumed plants and the secondary metabolites released might damage the liver and raise the liver enzymes (Grunhage et al., 2003; Pak et al., 2004). Therefore, all herbs and herbal supplements should be tested before being consumed for their possible liver toxicity.

## **1.6. Glycemia**

Diabetes Mellitus is a chronic metabolic disease that is increasing rapidly worldwide and affecting more and more people in countries all over the world. Diabetes mellitus is defined as a metabolic disorder and is characterized by persistent hyperglycemia with disorder of carbohydrate , fat, and protein metabolism that result from defects in insulin secretion, insulin action, or both (Andrade-Cetto and Heinrich, 2005). There are two types of diabetes: *type 1 diabetes* (insulin dependent diabetes mellitus or IDDM) that is due to an autoimmune mediated destruction of pancreatic  $\beta$  cell islets, and that is treated with consecutive insulin injections depending on the patient's blood glucose level, and *type 2 diabetes* (non-insulin dependent diabetes mellitus or NIDDM) that is caused by low insulin secretion from the pancreas and low insulin action, and that, is treated with several anti-diabetic medicines that improves insulin resistance (Boyle et al., 2001 and Inzucchi, 2002).  $\beta$ -cells of the islets of Langerhans secrete

insulin that lower the blood glucose level by enhancing the uptake of glucose into the cells where it is stored as glycogen or used as an energy substrate for the synthesis of fats or proteins (Hadley, 2000). Studies conducted on plants were to examine their insulin- release stimulatory and hypoglycemic effect (Ivorra et al., 1989). Many studies on hypoglycemic plants have been conducted and a great diversity of compounds have been isolated (alkaloids, glycosides terpenes, flavonoids, etc.) but the main concern is knowing how to use them wisely to develop clinically useful medicine and/or phytomedicine to help patients directly (Andrade-Cetto and Heinrich, 2005).

### **1.7. Phenolic Contents**

Phenols or phenolics are a class of chemical compounds possessing a hydroxyl group attached to an aromatic hydrocarbon group (Daniel, 2006). Phenols are found in the natural world especially in the plant kingdom where they exhibit great potential for developing antioxidants or anticancer drugs, and thus are phytochemicals (Zhong et al., 2007). Plant tissues may contain several grams per kilogram. External stimuli such as ultraviolet radiation, microbial infections, and chemical stressors induce the synthesis of these phenolic compounds (Waterman and Mole, 1994). Several thousands of phenolic compounds have been described in plant foods and can be grouped into different classes according to their basic chemical structure (such as type and number of phenol rings), and into different subclasses, according to specific substitutions in the basic structure, association with carbohydrates and polymerized forms (Manach et al., 2004). In the recent years, there is a growing interest in phenolic compounds (Karakaya, 2004). Most of these compounds have received considerable attention as potentially protective factors against human chronic degenerative diseases (macular degeneration, cataracts,

neurodegenerative diseases, and diabetes mellitus), cardiovascular disease and cancer (Scalbert et al., 2005).

### **1.8. Antioxidant Activity**

Antioxidants prevent oxygen radical and hydrogen peroxide induced cytotoxicity and tissue damage in various human diseases and this concept is increasingly acknowledged (Couladis et al., 2002). They are chemo-preventive agents in which they lower cholesterol and limit cell damage. Antioxidants interact in synergistic ways and may protect one another against oxidative destruction (Karou et al., 2005). Free radicals are important in that they have many severe effects on the cardiovascular system, either through vasoconstriction or lipid peroxidation (Zakaria et al., 2007). Scientists, food manufacturers, and consumers are being more interested these days with the antioxidant constituents of plants due to their importance in maintaining health and protecting from diabetes, coronary heart disease, atherosclerosis, Alzheimer's disease and cancer (Ka"hko"nen et al., 1999 and Khalaf et al., 2008). A large number of medicinal plants have shown beneficial therapeutic potentials in which various herbs have been reported to exhibit antioxidant activity (Khalaf et al., 2008). Plant secondary metabolites are involved in various adaptable functions that include the protection against excess oxidation caused by chemical oxidants, UV irradiation, pathogen attack or other kinds of stress and scavenging of free radicals (Matkowski and Wo"niak , 2005).

## 1.9. Purpose of the Project

The purpose of this project is to examine the potential medicinal effect of the aqueous extract of *N. officinalis* leaves in lipemia, glycemia, gastric ulcer, inflammation, and as an antioxidant. It also evaluates the safety of plant extract intake by assessing liver toxicity. The study included the following:

1. Assessment of blood lipid and glycemic profiles and liver enzyme activities in rats after 30 days of intake of *N. officinalis* leaves water extract:
  - Plasma total cholesterol.
  - Plasma HDL- cholesterol.
  - Plasma LDL- cholesterol.
  - Plasma Triglycerides.
  - Serum glucose.
  - Serum Enzymes ALT , ALP and AST
2. Assessment of the anti-ulcerogenic effects of aqueous extract of *N. officinalis* leaves in Ethanol induced gastric ulcer.
3. Assessment of the anti-inflammatory effects of aqueous extract of *N. officinalis* leaves in acute and chronic inflammation models induced by carrageenan and formalin respectively
4. Assessment of the antioxidant effects of aqueous extract of *N. officinalis* leaves by two different *in vitro* assays on plant extract :

**FRAP**

**DPPH**
5. Assessment of the total phenolic contents on plant extract.

## *Chapter 2*

### **MATERIALS AND METHODS**

#### **2.1. Plant Collection and water extract preparation:**

*N. officinalis* leaves were collected from Akkar valley and recognition was done by characterization described in the botanic and plant taxonomy book (Duke et al., 2002). Left in the shade until they became dry, all collected leaves being used in the different experiments were weighed and the dry weight of the leaves' water extract per g dry leaves was determined by soaking 5g of dry leaves in 200 ml of pre-boiled water for 20 minutes, filtering the solution using Whattman filter paper and evaporating the filtrate first on a mild flame until a small volume is left and then in an oven at 45<sup>0</sup>C overnight to assure total evaporation of water. Data have shown that every 1g of plant leaves yields 0.298 g of powdered water extract.

#### **2.2. Animals on lipid diet:**

Male Sprague rats (n=40) (Lebanese American University stock) were divided into four groups (10 rats/group) with an average weight of 235 g. A temperature of 20-22<sup>0</sup>C was maintained and all tested animals of all groups received rat chow diet (Hawa chicken stock). The first three groups were considered as the experimental groups and received the plant water extract in drinking water at doses 100 (group I), 250 (group II) and 500 (group III) mg/kg body weight, while the fourth group was considered as the control group. After one month of excessive intake of the plant extract, fasted animals (12hr) were sacrificed using diethyl ether and approximately 6 ml of blood were collected from the inferior vena cava.

### **2.2.1. Serum Analysis:**

#### **2.2.1.1. Samples preparation:**

The collected venous blood (6 ml) was put into glass tubes and allowed to clot at room temperature for 30 - 45 minutes. Centrifugation for 20 minutes at 3000g and 10<sup>0</sup>C of the collected blood was then performed and then the serum (supernatant) samples were aliquoted into different Eppendorf tubes and kept on ice . Liver enzyme (AST, ALP and ALT) activities were directly measured using fresh samples while the remaining samples were stored at -20<sup>0</sup>C for later use in the assessment of lipid (TAG, total-cholesterol, and HDL-cholesterol) and glycemic(glucose) profiles.

#### **2.2.1.2. Determination of Triglyceride:**

Triglycerides (TAG) concentration was determined using SPINREACT kit. Lipoprotein lipase (LPL) , an enzyme, rapidly hydrolyzes TAG to glycerol and free fatty acids. Glycerol is then converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. G3P is then converted by Glycerol dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Finally H<sub>2</sub>O<sub>2</sub> reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) enzyme to give a red colored dye .

The concentration of TAG indicates the color intensity of the sample formed. This can be measured by determining the optical density (OD) by the spectrophotometer at wavelength of  $\lambda=505$  nm.

- **Calculation of TAG:**

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

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

The conversion factor from mg/dl to mmol/L is  $\text{mg/dl} \times 0.0113 = \text{mmol/L}$ .

#### **2.2.1.3. Determination of the Total Cholesterol:**

Cholesterol concentration was determined following the SPINREACT kit protocol. Cholesterol esterase hydrolyzes the cholesterol ester form and releases free cholesterol and fatty acids. Then  $\text{H}_2\text{O}_2$  is formed by the enzymatic oxidation of cholesterol by cholesterol-oxidase. The hydrogen peroxide produced reacts with phenol and 4-aminophenazone (4-AP) in the presence of peroxidase (POD) and forms a red dye quinonimine.

The concentration of cholesterol indicates the color intensity of the sample formed. This can be measured by determining the optical density (OD) by the spectrophotometer at wavelength of  $\lambda=505 \text{ nm}$ .

- ***Calculation of Cholesterol:***

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

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

The conversion factor from mg/dl to mmol/L is  $\text{mg/dl} \times 0.0258 = \text{mmol/L}$ .

#### **2.2.1.4. Determination of HDL-Cholesterol:**

Phosphotungstic acid and magnesium ions precipitate low density lipoproteins (LDL) and very low density proteins (VLDL) that are then removed by centrifugation while HDL remains in the supernatant.

HDL-Cholesterol concentration was determined following the SPINREACT kit protocol in which clear supernatant was used.

- ***Calculation of HDL- Cholesterol:***

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

#### **2.2.1.5. Liver enzymes:**

##### **2.2.1.5.1. Aspartate aminotransferase (AST) :**

Formely called glutamate oxaloacetate (GOT) ,aspartate aminotransferase (AST) catalyzes the reversibale transfer of an amino group from aspartate to  $\alpha$ -ketoglutarate forming glutamate and oxaloacetate. Produced oxaloacetate is then reduced by malate dehydrogenase (MDH) and NADH to malate. When measured photometrically (340nm) , the rate of decrease in concentration of NADH is proportional to the catalytic concentration of AST present in the sample (SPINREACT Kit) .

- ***Calculation of AST:***

$\Delta A/\text{min} \times 1750 = U/L \text{ AST}$
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##### **2.2.1.5.2. Alanine aminotransferase (ALT) :**

The reversible transfer of an amino group from alanine to  $\alpha$ -ketoglutarate forms pyruvate and glutamate that are then catalyzed by ALT, an enzyme that is formerly called glutamate pyruvate transaminase (GPT). Produced pyruvate is then reduced to lactate by lactate



dehydrogenase (LDH) and NADH. The rate of decrease in concentration of NADH, measured photometrically (340nm) is proportional to the catalytic concentration of AST present in the sample (SPINREACT Kit).

• **Calculation of ALT:**

$$\Delta A/\text{min} \times 1750 = U/L \text{ ALT}$$

**2.2.1.5.3. Alkaline phosphatase (ALP):**

The hydrolysis of p-nitrophenyl phosphate is catalyzed by alkaline phosphatase (ALP) at pH= 10.4, producing phosphate and p-nitrophenol. The rate of decrease photometrically (405nm) is proportional to the catalytic concentration of ALP present in the sample (SPINREACT Kit).

• **Calculation of ALP :**

$$\Delta A/\text{min} \times 3300 = U/L \text{ ALP}$$

**2.2.1.6. Glycemia:**

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid.  $\text{H}_2\text{O}_2$  is produced and is detected in the presence of peroxidase (POD), by phenol-aminophenazone, a chromogenic oxygen acceptor. The intensity of the color formed is proportional to the glucose concentration in the sample measured by spectrophotometer at 505 nm (SPINREACT).

**Calculation of glucose concentration:**

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

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

### 2.2.2. Determination of TAG, Cholesterol and Water content in Feces:

Stool samples from each rat were collected before sacrifice, and then weighed immediately and placed overnight in an oven at 70°C. Another weighing was done the next day and the percentage water content was calculated. Stools were then crushed into powder using a mortar and pestle. 0.2g of the powdered stools was weighed, placed in a glass tube and 2 ml of n-hexane (hydrophobic solvent) was added. The sample mixtures were stirred frequently for 2 hours by a glass rod and put in a water bath at 40°C. Another centrifugation (4000 g) for 10-15 minutes of the samples was done and aliquots (300µl) of the supernatant were taken for triglyceride and cholesterol assays. Before the assessment, evaporation of n-hexane was done by placing the aliquot samples in a water bath at 70°C. The lipids left in the tubes were then tested for TAG and cholesterol using the SPINREACT kit.

- **Calculation of TAG and Cholesterol :**

$$(\text{Abs. Sample} / \text{Abs. Standard}) \times 200$$

- **Calculation of % water content in feces:**

$$(\text{Weight of fresh feces} - \text{Weight of dried feces}) / \text{weight of fresh feces} \times 100$$

### 2.2.3. Body weight change:

All animals were subjected to body weight measurement at day 1 and day 30 of the experiment in order to study the effect of the water extract of *N. officinalis* leaves on body weight changes. Rats were given 10g of food per 100 g body weight and their eating behavior

was monitored. The percentage increase in body weight was calculated according to the formula below:

$$\% \text{ change in weight} = \text{change in body weight} / \text{initial body weight} \times 100$$

### **2.3. Ethanol-induced gastric ulcer :**

The effect of *N. officinalis* on induced gastric ulcer was conducted on male rats weighing between 235 and 300 g. These rats were kept in a controlled environment (20-22 °C) and were fed regular chow diet and had open access to water. To ensure an empty stomach, rats were fasted for 48 hours before ulcer induction and to avoid excessive dehydration during the starvation period, they were given 8% sucrose (w/v) solution in 0.2 % NaCl (w/v). 1 hour before the treating with either plant extract or drug, the saline solution was removed. The rats were divided into 6 groups of 8 rats each. Group I received 5 ml of 100 mg/kg body weight of the plant extract. Groups II and III received 5ml of 250 and 500 mg/kg body weight of the plant extract respectively. Group IV received 10mg/kg of the reference drug cimitril. Group V received 3mg of omeprazole (Known as Gastrimute) intraperitoneally every 12 hours. Group VI served as the control group and received only 10ml/kg body weight of distilled water. Stomach ulceration was induced by 10 ml/kg body weight of ethanol 50% (v/v) in distilled water. It was given orally to all groups before sacrifice by an over-dosage of chloroform. The stomachs were then removed, opened along the greater curvature and gently washed under tap water (Kushima et al., 2005). An illuminated magnifying microscope was used to determine the ulcerative lesions in the stomach. Long lesions were counted and measured using a ruler, while each five dotted

lesions were considered as 1 mm of ulcer (Gurbuz et al., 2005). The curative ratio was determined using the formula:

$$\text{Curative Ratio} = \frac{(\text{Control Ulcer index} - \text{Test Ulcer index})}{\text{Control Ulcer index}} \times 100$$

### **2.3. Inflammation:**

250 and 300 g male rats were used. Acute and chronic anti-inflammatory effects were appraised using carrageenan and formalin induced inflammation respectively in rat hind paw (Jose et al., 2004). All rats were fed regular chow diet and had open access to water. Before intraperitoneal injection, the water extract of *N. officinalis* was sterilized using syringe filtration.

#### **2.3.1. Acute Inflammation:**

The experiment was initiated by measuring the thickness of the right hind paw of all animals by a vernier caliper. Rats were then randomly divided into five groups each of 8 rats. Three groups I, II, and III received *N. officinalis* water extract through intraperitoneal injection at doses of 100, 250, and 500 mg/kg body weight respectively. The remaining two groups served as control groups where one group was injected with Diclofenac (10mg/kg) intraperitoneally and considered as positive control while the other group was injected with only carrageenan and served as negative control. After 30 minutes of water extract and Diclofinac injection, rats of all groups were subjected to subplanter injection with 0.02 ml of 1% carrageenan in the right hind paw to induce edema. Paw thickness was re-measured after 3 hours post-edema induction (Jose et al., 2004).

#### **2.3.2. Chronic inflammation:**

The experiment was initiated by measuring the thickness of the right hind paw of all animals by a vernier caliper. Rats were then randomly divided into five groups each of 8 rats.

Three groups I, II, and III received *N. officinalis* water extract through intraperitoneal injection at doses of 100, 250, and 500 mg/kg body weight respectively. The remaining two groups served as control groups where one group was injected with Diclofenac (10mg/kg) intraperitoneally and considered as positive control while the other group was injected with formalin and served as negative control. Subplanter injection of rats of all groups with 0.02 ml of 2% formalin in the right hind paw was performed to induce edema after 30 minutes of water extract and Diclofenac injection. There was a continuation of extracts and reference drug intake for 6 consecutive days and once per day. The paw thickness was measured using vernier calipers the 6<sup>th</sup> day after 3 hours of injection (Jose et al., 2004).

### 2.3.3. Paw Thickness Calculations:

The paw thickness increase in acute and chronic inflammation was calculated based on the following formula :  $P_t - P_o$ .

$P_o$ : the paw thickness at time zero (pre-inflammation induction)

$P_t$ : the paw thickness at time t (post-inflammation induction)

C : the increase in paw thickness of the control group

T: the increase in paw thickness of the treatment group

$$\text{percentage of inhibition} = \frac{(C - T)}{C} \times 100$$

#### **2.4. Detection of Phenol Compounds in plant extract :**

The total phenolic compounds of *N. officinalis* were determined according to the method described by Kruawan and Kangsadalampai and there was a slight modification of the concentrations . 100 µl of water extract was added to 1.6ml of distilled water . After mixing the contents, 100 µl of Folin-Ciocalteu reagent and 200 µl of saturated sodium carbonate solution were added and mixed well and then incubated for 30 min and the absorbance was read at 750nm. The total phenolic contents were calculated as a gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions (25,50,100,200 and 800 mg/ml ), and expressed as mg of gallic acid per gram of dry sample. All samples were done in duplicates.

#### **2.5. Detection of Antioxidant Activity in plant extract :**

The free radical scavenging activity of *N. officinalis* was determined by the 2,2-diphenyl-1-picrylhydrazyl ( DPPH ) assay described by Kruawan and Kangsadalampai(2008) but there was a small modification in which the DPPH sample was placed in darkness. 22 µl of plant extract was added to 200 µl of freshly prepared DPPH + 80 % methanol . The absorbance of the solution was read at 520nm after incubation for 20 mins at 37 degrees. The radical scavenging activity was calculated as a percentage of DPPH scavenging activity by the following formula :

$\% \text{ scavenging activity} = 100 * ( A_D - A_E ) / A_D$
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$A_D$  = absorbance of the DPPH solution

$A_E$  = absorbance of the DPPH solution with the extract

The ferric reducing antioxidant power of *N. officinalis* was measured by the FRAP assay also described by Kruawan and Kangsadalampai. The FRAP reagent was prepared by adding 20  $\mu$ l of extract to 150  $\mu$ l of freshly prepared and warmed FRAP reagent. The change in absorbance at 595 nm from the initial blank was read after 8 mins and compared to that of the standard solution. From a calibration curve, the FRAP values of the extract were measured at different concentrations ( 62.5, 125, 250, 500, and 1000  $\mu$ M ). And this value is expressed as  $\mu$ M of ferric ion reduced per gram of dried sample. Increased absorbance at 595 nm indicates a stronger reducing power.

## **2.6. Statistical analysis:**

The data was expressed as mean values  $\pm$  S.E.M. The *t* test was used to determine the significant difference between the treated groups and the control. All values were considered significant when  $p < 0.05$ .

## Chapter 3

### RESULTS

#### 3.1. Serum lipid profile:

##### 3.1.1. Total Cholesterol, HDL, and TAG

When all animal groups received the water extract of *N. officinalis* leaves (100, 250 and 500mg/kg body weight) results of the 3 groups showed no significant changes in the serum concentration of total cholesterol, HDL- cholesterol, and TAG with respect to the control. Results are shown in table 3.1.

**Table 3.1.** Serum total cholesterol, HDL-cholesterol and TAG concentrations in control and experimental groups I, II and III after one month of *N. officinalis* water extract administration (100, 250, and 500mg/kg body weight respectively).

Parameters	Control	Treatment groups		
		Group I	Group II	Group III
Total Cholesterol	52.23 $\pm$ 2.99	51.63 $\pm$ 3.42	64.38 $\pm$ 2.04*	50.39 $\pm$ 3.35
HDL-Cholesterol	44.16 $\pm$ 3.6	50.9 $\pm$ 3.81	49.9 $\pm$ 3.3	42.8 $\pm$ 3.82
Triglycerides	41.35 $\pm$ 8.31	35.87 $\pm$ 3.00	36.87 $\pm$ 3.14	36.6 $\pm$ 4.91

Values are expressed as mean  $\pm$  S.E.M. (n=10)

\*Represents  $p < 0.05$



### 3.1.2. Liver function tests:

The activities of the liver enzymes ALT, AST and ALP were determined in control and experimental groups I, II, and III after one month of *N. officinalis* water extract administration (100, 250, and 500 mg/kg body weight respectively). Results shown in Table 3.2., did not show any significant difference between the control and the experimental groups with all enzymes studied.

**Table 3.2:** Determination of serum hepatic enzymes: ALT, AST and ALP levels (U/L) in Control and experimental groups I, II, and III after one month of *N. officinalis* water extract administration (100, 250, and 500 mg/kg body weight respectively).

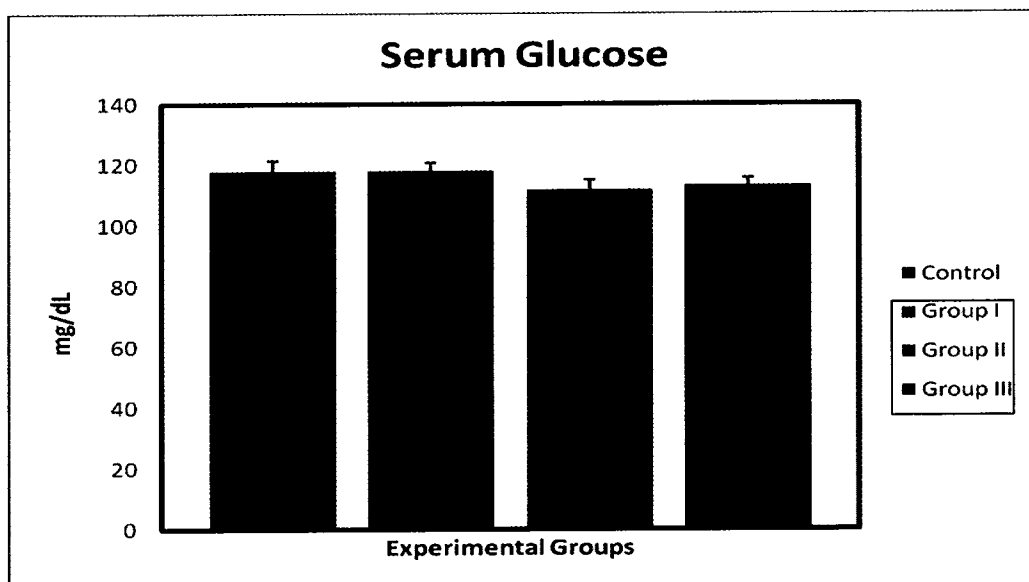
Parameters	Control	Treatment groups		
		Group I	Group II	Group III
ALT(SGPT)	44.43 ± 4.57	42.18 ± 6.25	43.88 ± 2.57	40.99 ± 1.13
AST(SGOT)	43.38 ± 2.89	39.50 ± 5.30	40.50 ± 4.45	35.12 ± 5.35
ALP	138.24 ± 9.94	141.77 ± 7.42	149.89 ± 12.52	135.5 ± 6.02

Values are expressed as mean ± S.E.M. (n=10/group)

\*Represents p<0.05

### 3.1.3. Glycemia:

Glucose concentrations were measured in the blood of the control and experimental groups I, II, and III after one month of *N. officinalis* water extract administration (100, 250, and 500 mg/kg body weight respectively). No significant changes were detected among the different groups. Results are shown in Figure 3.1.

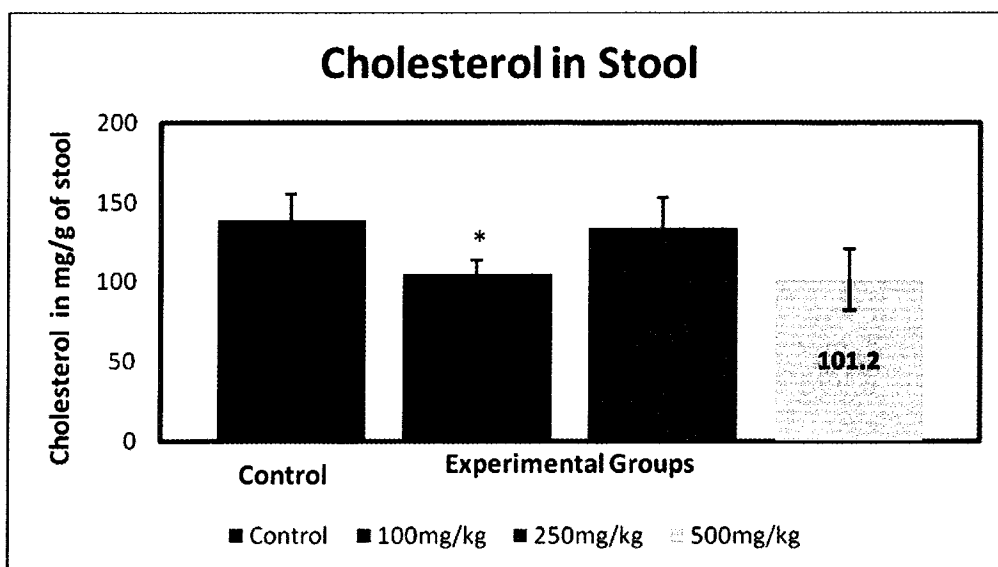


**Figure 3.1:** Serum glucose concentration (mg/dl) after 1 month of intake of *N. officinalis* water extract in the control and experimental groups. Bars represent means  $\pm$  S.E.M. (n=10)

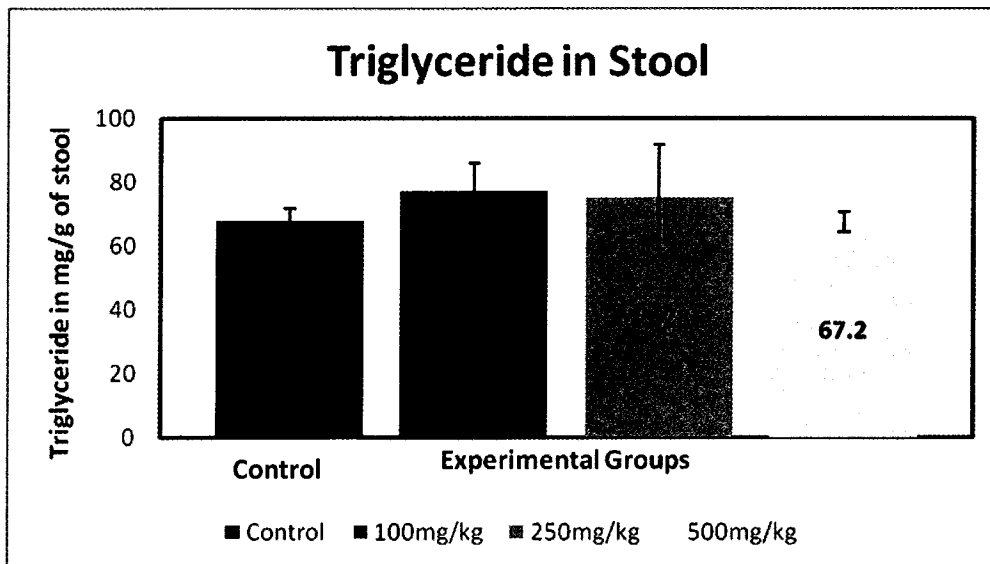
## 3.2. Stool Analysis:

### 3.3.1. Water, cholesterol and TAG content in Stools

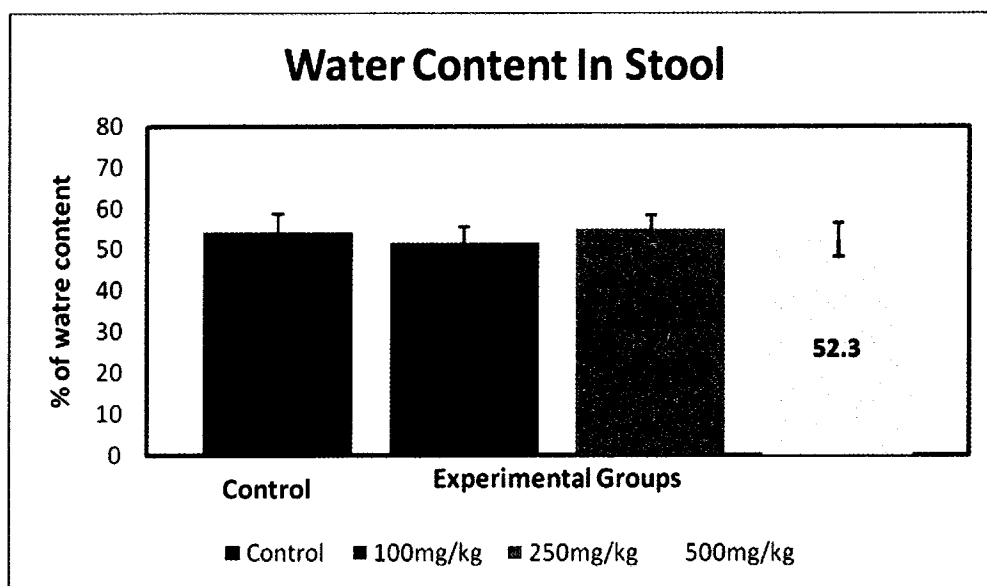
The effect of the water extract of *N. officinalis* leaves on water, cholesterol and triglyceride content in stools was evaluated. With respect to the control group, water extract intake showed no significant changes in the concentration of cholesterol (Figure 3.2), triglyceride (Figure 3.3), and water content in the stools of the experimental groups with respect to the control (Figure 3.4).



**Figure 3.2:** Cholesterol content of stools in control and experimental groups I, II, and III after one month of *N. officinalis* water extract administration (100, 250, and 500 mg/kg body weight). \* Represents  $p < 0.05$ .



**Figure 3.3:** Triglyceride content of stools in control and experimental groups I, II, and III after one month of *N. officinalis* water extract administration (100, 250, and 500 mg/kg body weight).



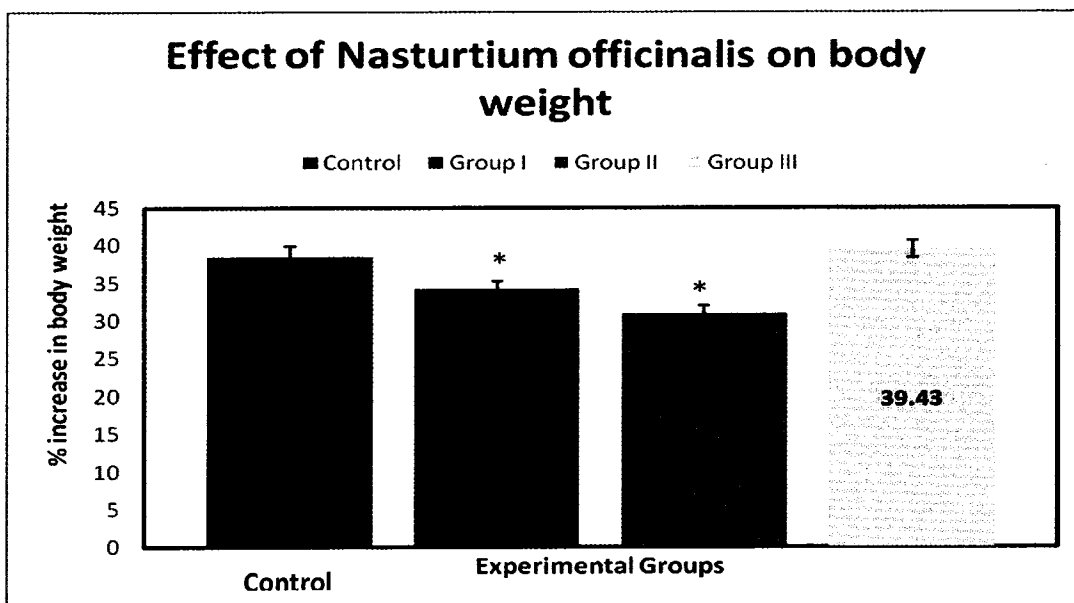
**Figure 3.4:** The % of water content of stools in control and experimental groups I, II, and III after one month of *N. officinalis* water extract administration (100, 250, and 500 mg/kg body weight).

### 3.3. Effect on Body weight:

*N. officinalis* water extracts 100 and 250 mg/kg doses decreased significantly the body weight of rats after one month of intake of the extract, while the 500 mg/kg dose did not (Figure 3.5).

### 3.4. Gastro protective effect against ethanol-induced gastric ulcer:

The effect of aqueous extract of *N. officinalis* on ethanol induced gastric ulcer was identified by measuring both long and small lesions in the glandular region of the stomach. Protection was significant in all groups and was shown in Table 3.3.



**Figure 3.5:** The change in body weight of animals after 1 month of intake of *N. officinalis* water extract in the control and experimental groups (100, 250 and 500mg/kg body weight).

\* Represents  $p < 0.05$

**Table 3.3:** Effect of water extract of *N. officinalis* dry leaves on ethanol-induced gastric ulcer

Treatment Groups	Ulcer Index (mm)	Curative Ratio ( % )
Cimetidine (10mg/kg)	2.04 ± 0.8	50.24 %
Omeprazole (3mg/kg)	2.14 ± 0.72	47.81%
Group I (100mg/kg)	2 ± 0.98	51.22 %
Group II (250mg/kg)	2.78 ± 0.84	32.2 %
Group III(500mg/kg)	5.63 ± 1.15	-37.3 %
Control	4.1 ± 1.21	

### 3.5. Anti-inflammatory activity:

In acute inflammation, water extracts of *N. officinalis* of all the doses showed slight inhibition. On the other hand, all doses of the treatment groups showed significant effects in chronic inflammation. Data are shown in Table 3.4.

**Table 3.4. The effect of *N. officinalis* water extract on edemas on Carrageenan induced acute and formalin induced chronic inflammation**

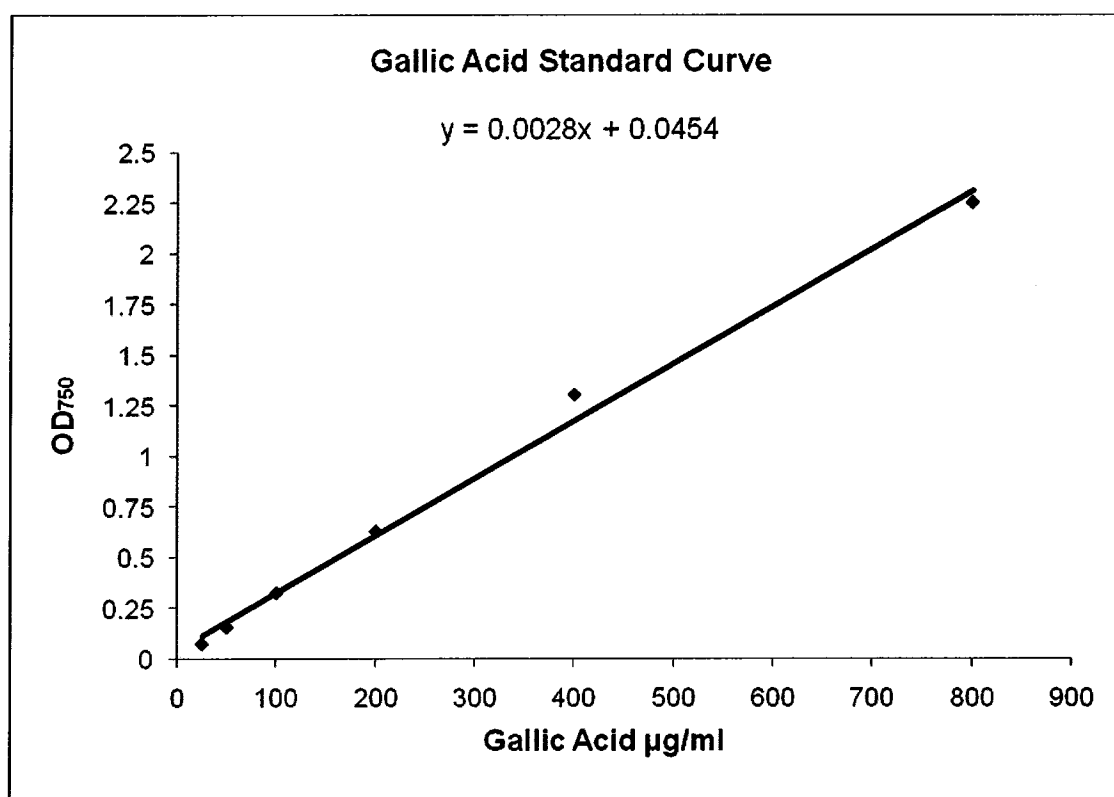
		Carrageenan		Formalin	
Treatment	Dose (mg/kg)	Increase in paw thickness after 3 h	Inhibition (%)	Increase in paw thickness after 6 days	Inhibition (%)
Control		0.9 ± 0.13	-	1.69 ± 0.32	-
<i>N. officinalis</i> Extract	100	0.36 ± 0.11	60	1.61 ± 0.14	4.7
	250	0.44 ± 0.06	51.1	0.82 ± 0.23*	51.5
	500	0.485 ± 0.11	46.11	0.725 ± 0.08 *	57.1
Diclofenac	10	0.24 ± 0.03	73.33	0.6 ± 0.07*	64.5

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

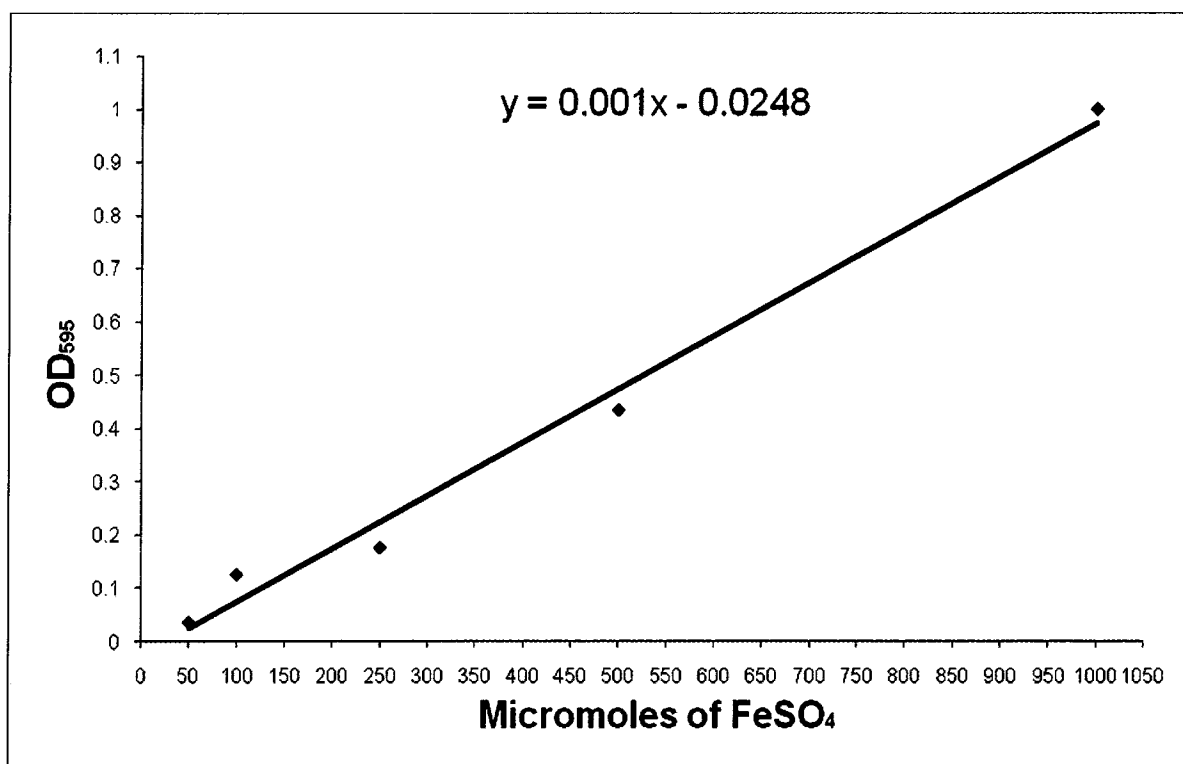
Values are represented as mean ± S.E.M. (n=6)

### 3.6. Antioxidant Activity Assay:

Total phenol content of each gram of dry extract of *N. officinalis* was estimated to be equal to (820 mg of Gallic acid equivalent( GAE)/g) and this was calculated from a Gallic Acid standard curve (Figure 3.6). The *N. officinalis* extract demonstrated a concentration dependant scavenging activity and was compared with Ascorbic acid as a positive control standard. The % DPPH scavenging activity by *N. officinalis* gave a value of 55%. In the FRAP method, the crude extract showed an ability to reduce  $\text{Fe}^{+3}$  to  $\text{Fe}^{+2}$  by a value of (1085  $\mu\text{mol/ g}$ ) dried extract and this value was compared with the ferrous sulfate standard curve( Figure 3.7) . Data is shown in table 3.5.



**Figure 3.6:** Gallic acid standard curve ranging from 25 to 800  $\mu\text{g/ ml}$ .



**Figure 3.7:** Ferrous sulfate standard curve.

**Table 3.5:** Antioxidant activity and total phenolic content of 1g of dried *N. officinalis* extract:

Extract	Total Phenolic content (mg of GAE/g)	Antioxidant activity	
		FRAP value( $\mu$ mol/g)	DPPH (%scavenging effect)
<i>Nasturtium officinalis</i>	820	1085	55 %
Ascorbic Acid( 1g/100ml)		1324	



## ***Chapter 4***

### **DISCUSSION AND CONCLUSION**

*N. officinalis* leaves have been used for years as a traditional medicine in stimulating appetite, treating scurvy and inflammations of the skin, chest and kidney conditions (Tabal, 1994; Yazdanparast et al., 2008). It is reported to have hypoglycemic, expectorant, diuretic, odontalgic, and depurative actions (Naegele, 1970). A literature review on *N. officinalis* revealed that most studies dealt with the pharmacological actions of its hydroalcoholic extracts (Bahramikia and Yazdanparast, 2008). The present study investigates the medicinal role of *N. officinalis* water extract in lipemia, glycemia, ulcer, and inflammation in rats. It also tackles the antioxidant activity.

The plant should be harvested from the same area in order to reduce variations due to geographic factors (Ellof, 1999). The overall quality of the extract is also affected by the time of harvest and the condition of the leaves (Tewari and Virmani, 1987). In the present study, all dried leaves used were collected only from Akkar valley area in Lebanon during the month of July in order to avoid both geographic and harvest time effect on the plant constituent variation.

Many researchers proved that a high cholesterol and lipid diet in human and animals can cause a very serious condition called hyperlipidemia (Blazovics et al., 1993). The development of coronary heart disease (CHD) is linked to lipid and lipoprotein abnormalities (Skoumas et al., 2003). Serum total cholesterol, HDL-cholesterol, and triglycerides were assessed after one month of excessive plant extract intake in drinking water at three different

doses to understand the effect of water extract of *N. officinalis* dry leaves on blood lipid profile. Data have shown that the extract did not show any significant effect on total cholesterol, HDL-cholesterol, and triglycerides although studies conducted previously on the hydroalcoholic extract of *N. officinalis* proved the cardioprotective role of the extract. *N. officinalis* leaves are famous in having phenols and flavonoids (Barnes et al., 2002). Previous investigations revealed the importance of flavonoids in the LDL oxidation (Hertog et al., 1993). In the present study, although the plant water extract has a neutral effect on lipidemia, it may have a beneficial effect on LDL oxidation because of the flavonoids and phenols present in the extract. Many studies have pointed to the fact that the antioxidant activity of most plants is due to their phenolic compounds (Pourmorad et al., 2006). Flavonoids are a class of secondary plant phenolics or polyphenolic compounds which act as pharmacologically active ingredient in many medicinal plants and that exhibit a powerful antioxidant property including free radical scavenging, anti-inflammatory actions and inhibition of hydrolytic and oxidative enzymes (Yazdanparast et al., 2008; Pourmorad et al., 2006). Their mechanism of action is through scavenging or chelating metal ions. In the present study, the total phenolic content of *N. officinalis* water extract using the Folin Ciocalteu reagent was 820 µg /g in terms of galic acid equivalent. High contents of these phenols are behind their high radical scavenging activity (Pourmorad et al., 2006). Among the various methods used to evaluate the total antioxidant activity of the water extract of *N. officinalis*, the DPPH radical scavenging and FRAP assay were methods used in the present study. *N. officinalis* water extract showed a high DPPH radical scavenging activity (55%). This may be attributed to the hydrogen donating ability of the plant extract (Pourmorad et al., 2006). In the FRAP method, the reducing capacity of *N. officinalis* was measured.

Based on the results obtained, *N. officinalis* water extract showed a significant reducing power (1085  $\mu\text{mol/g}$ ). Previous studies done by Yazdanparast et al., 2008 have proved that *N. officinalis* hydroalcoholic extract contains mainly flavonoids and  $\beta$ -carotene and this might be responsible for enhancing antioxidant capacity and suppressing lipid peroxidation in the liver of rats fed a high-fat diet.

The demand on the use of medicinal plants for the treatment of several disorders and diseases has increased in the past decade (Bolkent et al., 2005). Since the liver is responsible for the metabolism of exogenous toxins, drugs or herbal medicine can be harmful to it and therefore, liver toxicity assessments must be well investigated to prove the safety of the plant extract used (Grunhage et al., 2003; Pak et al., 2004). To test the hepatoprotective activity or hepatotoxicity of the water extracts of *N. officinalis* leaves, the activity of the liver enzymes alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in serum of rats were measured after one month of plant extract intake. Liver cellular damage in the diagnosis and study of acute hepatic disorders can be indicated by the serum activity levels of ALT and AST. A rise in transaminases' concentrations indicates a cellular damage in the liver (Sallie et al., 1991). However, these enzymes are not only located in the liver but also in the extrahepatic tissues and any change in the serum activity levels may reflect cell damage in the heart and muscle (He and Aoyama, 2003; Harold et al., 2002). Since there was no increase in serum activity levels of ALT, AST, and ALP after 30 days of *N. officinalis* water extract diet in rats, the intake of this water extract appeared to be safe on hepatic cells in all the 3 doses given and thus safe for the integrity of the liver. So, *N. officinalis* seems to be a safe medicinal plant for the treatment of diseases without causing any harm to the liver.

Type II Diabetes mellitus, which is the most common endocrine disorder, is associated with many serious complications that are mostly correlated with a chronic increase in glucose levels (Sabu et al., 2002; Andrade-Cetto and Heinrich, 2005). According to the World Health Organization (WHO), diabetes mellitus affects more than 176 million people world wide (Andrade-Cetto and Heinrich , 2005). Thus, insulin, dietary modifications and oral hypoglycemics are used to treat such conditions (Vats et al., 2002). For this reason, medicinal plants are widely used nowadays to substitute the unfavorable effect of the therapeutic medicines mentioned above. In the present study, the administration of the water extract of *N. officinalis* leaves showed no significant change in the serum glucose level and thus this extract is of no value in such conditions.

To investigate the effect of *N. officinalis* water extract on the absorptive capacity of nutrients by the gastrointestinal tract, an assessment of the triglyceride and cholesterol absorption was made. The effect of the extract on fecal triglyceride has shown that the water extract had no impact on this parameter compared to the fecal cholesterol in which it showed significance in the 100mg/kg body weight dose, concluding that the *N. officinalis* water extract does not interfere with gastrointestinal lipid absorption/or digestion. Although the fecal concentration of cholesterol was somehow reduced (without reaching significance) in rats that received 500mg/kg body weight of *N. officinalis* water extract, no similar trend was observed in the serum cholesterol concentration. Also, the intake the water extract does not cause neither constipation nor diarrhea since it showed no effect on the water content in stool when given for 1 month. Similarly, the water extract did not show any significant effect on body weight changes when

given in a 500 mg/kg body weight dose. But a decrease in body weight changes during the experiment was observed in rats given the 100 and 250 mg/kg body weight concentration. However, such a change is not considered of importance when considering the total body weight changes.

Excessive ethanol instillation intragastrically results in gastric mucosal injury characterized by mucosal edema, cellular exfoliation, inflammatory cell infiltration, and subepithelial hemorrhages (Hiraishi et al., 1998). Ethanol reduces the defensive factors in the stomach such as the reduction of bicarbonate secretion and mucus production and this leads to disturbance of mucosal microcirculation, ischaemia, appearance of free-radicals (ROS), inhibition of prostaglandin production and degranulation of mast cells (Samonina et al., 2004). A pre-treatment by oral administration of *N. officinalis* water extract have resulted in a dose-dependent protection against ethanol induced gastric ulcer. The anti-ulcer effect of this water extract was investigated using an *in vivo* model of ethanol induced gastric ulcer (rats). The protection was 51.22% (dose of 100mg/kg) and 32.2% (dose of 250mg/kg) compared with the positive control group. The anti-ulcer drugs Cimetidine (dose 10mg/kg) and Omeprazole (3mg/kg) showed 50.24% and 47.81 % protection respectively. This showed that the lowest dose of the plant extract gave a similar protection to the reference drugs used in this study. Oral administration of the highest dose (500 mg/kg) showed no protection (-37.2 %), but instead, caused gastric mucosal injury when given to rats and thus produced many small and long lesions. Cimetidine (Cimetril) and Omeprazole (Gastrimute) are known to act by blocking the histamine receptors on parietal cells and in turn prevent acid secretion and thus mediate protection. The protection against gastric ulcer by these two anti-ulcerogenic drugs in this study was consistent

with other studies (Kushima et al., 2005). But the gastro protective mechanism of the *N. officinalis* water extract is not known. It is most probably due to the presence of active compounds in the water extracts including flavonoids and alkaloids that might be behind this effect. Previous studies done on these compounds have shown that alkaloids show antispasmodic and antisecretory effects in the treatment of gastroenteritis and peptic ulcer and thus have gastroprotective and antiulcer activities (Sousa Falcão et al., 2008). Flavonoids have also been shown to have an anti-ulcer activity by protecting the mucosa of the stomach (Izzo et al., 1944). However, further studies should be conducted on this plant extract to know exactly what is behind the actual antiulcer effect and the mechanism involved.

Another aim of this study was to investigate the anti-inflammatory property of *N. officinalis* water extract using an *in vivo* model of acute and chronic inflammation induced by carrageenan and formalin respectively. Carrageenan is able to produce edema with plasma exudation and intestinal epithelial cell inflammation, while formalin can be used to provoke chronic inflammations (Henriques et al., 1987). Reduction in the edema seen on the hind-paws of male Sprague-Dawley rats indicates an inhibition of inflammation. In the acute inflammation model, all 3 water extract doses 100, 250 and 500 mg/kg showed somehow high percentages of anti-inflammatory effects (60, 51.1 and 46.11% respectively) compared to that of the reference drug, Diclofenac (73.33%). For the chronic inflammation models, the extract of dose 100mg/kg showed no inhibition of the inflammation (4.7%) whereas in the doses 250 and 500mg/kg, significant inhibition of inflammation was observed (51.5 and 57.1 % respectively) compared to the control. In acute inflammation, the 100 and 250 mg/kg doses appeared to be optimum doses that can be used. The decrease in the anti-inflammation effect after the 100mg/kg body weight

may be attributed to the presence of compounds in the water extract that if present in high concentrations, makes a negative effect on the inflammatory presence. In the chronic inflammation models, as the dose of the water extract increases, the anti-inflammatory effect increases. Since *N. officinalis* contains many important constituents such as flavonoids, the anti-inflammatory activity might be attributed to this agent. Flavonoids are known to inhibit eicosanoid biosynthesis, producing phospholipase A2, lipoxygenases, and cyclooxygenases thereby leading to a reduction in the concentrations of prostanoids and leukotrienes and in turn an anti-inflammatory response (Kuo et al., 1995; Kim et al., 2004). Further studies are required in order to specify the exact flavonoid responsible for this anti-inflammatory effect in the *N. officinalis* leaves and whether other ingredients are involved. In addition, more studies should be performed to know the exact appropriate extract dose.

In conclusion, the present study reveals the safety and importance of the aqueous extract of *N. officinalis* as an inexpensive cure for several important diseases that affect human beings. After one month period of persistent consumption of *N. officinalis* water extracts in the rat model, the extract appeared not to have any significant effect on blood lipemia, and thus is not protective against the number one fatal disease, the cardiovascular disease. Similarly the *N. officinalis* water extract at the different doses used did not affect the absorptive property of the intestine and the integrity of the liver cells. Furthermore, it had a substantial anti-inflammatory activity in both acute and chronic types of inflammation. At low doses (100mg/kg body weight) a potent protective effect against gastric ulcer was seen. The water extract showed a high antioxidant activity and a high phenolic content, which in turn means that this plant is protective against free radicals and oxidation.

## Chapter 5

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