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Urtica Dioica Seed Extracts: A Possible Medicinal role in Lipemia, Glycemia, Inflammation and Gastric Ulcer

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Urtica Dioica Seed Extracts: A Possible Medicinal role in Lipemia, Glycemia, Inflammation and Gastric Ulcer

By

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A thesis submitted in partial fulfillment of the

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Under the supervision of Dr. Costantine Daher

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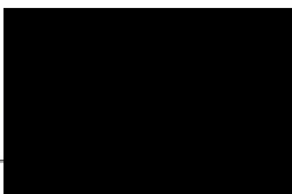

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Abstract

The importance of herbal medicine has grown tremendously over the last few decades. In the present study, aqueous and polyphenol extracts of *Urtica Dioica* seeds were investigated for their potential role in lipemia, glycemia, inflammation and gastric ulcer using the rat as a model. After one month of intake of a high fat diet rich in saturated fatty acids, aqueous but not polyphenol extract was able to significantly improve serum triglycerides, HDL cholesterol and LDL/HDL ratio. However, both extract were able to reduce in a dose dependent manner serum glucose levels. Significance was reached with the highest dose used (250 mg/kg body weight). Serum insulin concentrations were not significantly affected although a slight general reduction in insulin levels was observed. Data suggest that the drop in serum glucose is attributed to an improved cellular sensitivity to insulin. Liver enzymes activities (SGPT, SGOT) were not affected by any of the extract intake at the end of the study period, indicating safety of the extracts. In addition, both extracts exhibited a significant dose dependent protection against ethanol induced gastric ulcer. *U. Dioica* seed water extract showed significant anti-inflammatory effects with both models of acute and chronic inflammation induced by carrageenan and formalin respectively. However, polyphenol extract showed some potential anti-inflammatory activity only at the lowest dose used, and as the extract dose increases the extract turned to be an inducer of inflammation rather than an inhibitor in both acute and chronic types of inflammation. In Conclusion, the aqueous extracts of *Urtica Dioica* seeds has promising effects in improving lipidemia and glycemia while demonstrating an anti-inflammatory potential and gastroprotective role against ethanol-induced ulcer. The use of polyphenol extract proved to be less valuable medicinally since it only reduced blood glucose level and showed some protection against ethanol-induced ulcer.

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Glossary

ACAT: Acetyl-CoA-cholesterol Acetyltransferase

ADP: Adenosine Diphosphate

AST: Aspartate Aminotransferase

CAD: Coronary Artery Disease

CE: Cholesteryl Esters

CETP: Cholesterol Ester Transfer Protein

CHE: Cholesterol Esterase

CHOD: Cholesterol Oxidase

CM: Chylomicron

ELISA: Enzyme Linked Immuno-sorbant Assay

FA: Fatty Acids

FFA: Free Fatty Acids

GK: Glucose Kinase

GOD: Glucose Oxidase

GPO: Glycerol-3-Phosphate Oxidase

HDL: High Density Lipoprotein

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

IDL: Intermediate Density Lipoprotein

LDH: Lactate Dehydrogenase

LDL: Low Density Lipoprotein

LPL: Lipoprotein Lipase

MDH: Malate Dehydrogenase

NADH: Nicotinamide adenine dinucleotide

POD: Peroxidase

SGPT: Serum – Glutamic oxaloacetic- Transaminase

SGOT: Serum – Glutamic pyrovic- Transaminase

TAG: Triglyceride

VLDL: Very low density lipoprotein

Chapter I

INTRODUCTION AND LITERATURE REVIEW

It was estimated by the World Health Organization that more than 75 percent of the world's population still depends mainly on herbal medicine for health care, especially in developing countries. Thousands of years ago people around the world have used the herbs growing in their yards as part of their primary health care. Indeed, archaeologists have found the remains of plants used as medicine at archaeological dig sites in many areas world wide and dating back to 8000 B.C. At least 1,300 plant species in the northwestern Amazon only, are used to create "wilderness drugs" for the primary health care needs in the area today; many of these have never been subjected to any type of scientific research yet (WHO, 1999).

Imitative uses of medicinal plants can be very important, especially to drug companies and researchers. If a plant has been used in a specific way for a specific objective for many years and in many different geographical areas, there might be a reason for it. This rapidly growing industry (termed ethnobotany) that helps scientists target which plants to subject their research first and what to study them for. Originally people discovered the medicinal uses of three-quarters of drugs derived from plants in use these days. Complementary medicine as part of botanicals is developing great interest in the United States of America nowadays. Particularly consumers and physicians are developing their awareness for the use of herbs as natives; commercially botanicals are being used as dietary elements in the United States. Though, these supplements correspond to a small variety of plant species of the vascular classes (Bruneton, 1995).

In spite of the significant advancements, medicinal herbs are taken conventionally. In rural regions cultural practices believe in self-care and home remedies. Due to their faith in spiritual therapists Arabian cultures have acquired these therapeutic methods. The Mediterranean region has rich customs in the use of medical plants for treating different ailments. According to last surveys, people in the Middle East have more than 2500 plant species of which more than 800 are used as botanical pesticides or as medicinal herbs. Nowadays, fewer than 200 to 250 plant species are in use as medicine by Arabs for treating various diseases (Saad et al., 2005).

1.1. Plant taxonomy and distribution

Urtica Dioica is commonly known as Nettle. Nettle means to sting, to whip with or to irritate. It is classified as follows (Bradly et al., 1994). :

- Kingdom: *Plantae*
- Sub-kingdom: *Tracheobionta*
- Superdivision: *Spermatophyta*
- Division: *Magnoliophyta*
- Class: *Magnoliopsida*
- Subclass: *Hamamelidae*
- Order: *Urticales*
- Family: *Urticaceae*
- Genus: *Urtica*
- Species: *Urtica Dioica*

Other names are: big string nettle, stinging nettle, common nettle, isirgan, gerrais, kazink, ortiga, nabat al nar, grande ortie, urtiga, ortie, chichicaste, racine d'ortie, gross d'ortie, brennessel, and korais (in Lebanon).

Urtica Dioica is indigenous to western Asia and Africa, but is now found in all temperate regions of the world in Asia, South and North America, Africa, Europe and Australia (Bruneton, 1995). This species is widely distributed all around the world due to the abundancy of its habitat (Capasso, 2003).

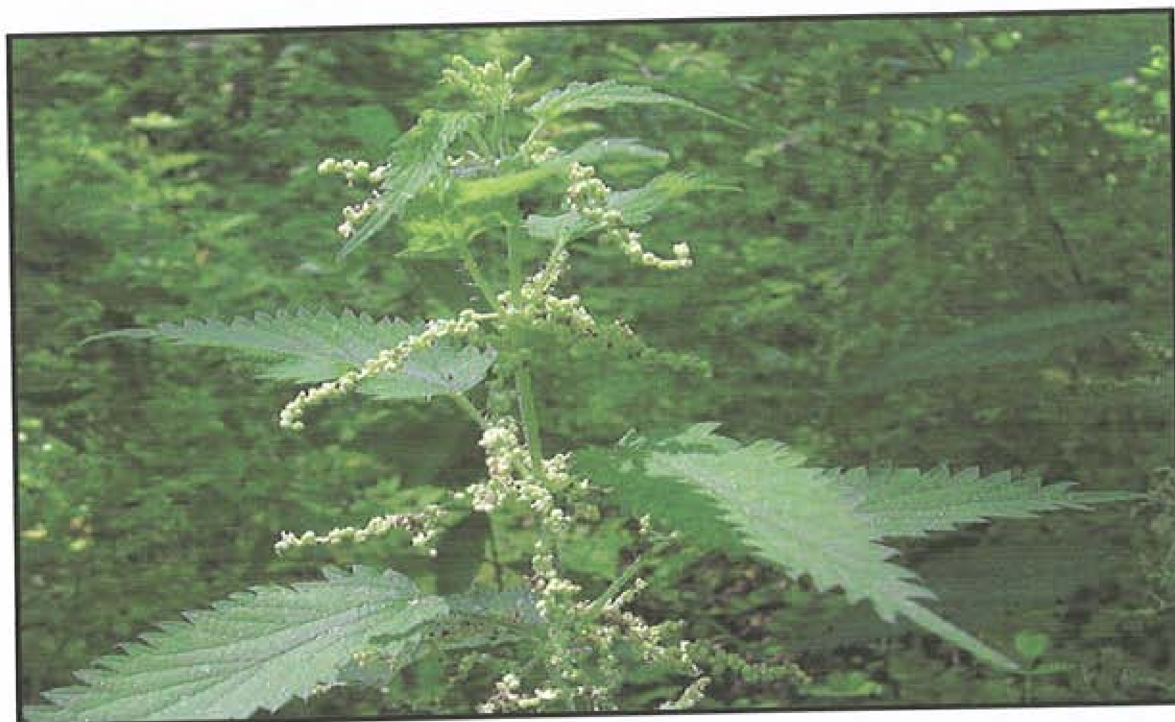


Fig 1.1 "*Urtica Dioica*" (White Clay Creek State Park, 2002)

After the fall of the Roman Empire for many centuries, Arabic cultures were the center of medical and scientific knowledge. Texts from Rome and Greece were translated into Arabic language and studied by Islamic researchers (Bown, 1995). They refined and developed Hippocrate's theories and Islamic physicians used the diet regulations, as exercise and the prescription of medicinal herbs in treating of the patients. Arabs in the Iraqi areas were the first in history to separate pharmacological

science from medicine. The separation and differentiation of two scientific disciplines, pharmacy and medicine, started since the eighth century (Pengelly, 1996).

1.2. Plant description and constituents

Stinging nettle is a perennial herb, growing up 100-200 centimeters tall in summer and dying down to ground in winter. Its green leaves are 3-15 cm long, with strong serrated margins, an acuminate tip and a chordate base (Grieve, 1984). Both leaves and stems are covered with bristle, which are hollow, characterized by silky hairs that were thought to have chemicals like formic acid as a defensive tool against grazing animals; but recently it was discovered that the sting is caused by three chemicals histamine to irritate the skin, hydroxytryptamine that activates the other two chemicals, and acetylcholine to bring on a burning sensation. The dark green, dull leaves grow opposite to each others on the stem. U. Dioica reproduces through creeping rootstocks and by seeds. They produce seeds abundantly (Bassett, 1977). Plants growing in sunlight produce 10,000 to 20,000 seeds per shoot, and shade growing plants produce up to 5,000 seeds per shoot. Seeds remain on the plant all the season before they fall to the ground. They do not show dormancy but they have the ability to germinate 5 to 10 days after maturity. After flowering in the summer, they reveal tiny greenish-white flowers that hang in clusters just above where the leaves are inserted into the stem (Guarrera, 2004).

1.2.1. *Urtica Dioica* constituents

Urtica Dioica plant contains a pool of minerals, acids, fatty components, vitamins and many other components. Tables 1.1, 1.2, 1.3 and 1.4 summarize some of the major constituent of *U. Dioica*.

Table 1.1: Minerals found in the whole plant of *U. Dioica* (Duke, 2002; Newall et al., 1996).

Minerals		
Aluminium	Fluorine	Manganese
Arsenic	Iron	Magnesium
Boron	Lead	Mercury
Bromine	Zinc	Molybdenum
Cademium	Selenium	Nickle
Calcium	Silicon	Nitrogen
Chlorine	Sodium	Phosphorus
Chromium	Sulfur	Potassium
Cobalt	Tin	
Copper	Rubidium	

Table 1.2: Some Acidic and fatty Components found in the whole plant of *U. Dioica* (Duke, 2002)

Some Acidic and fatty Components		
Acetic acid	Formic acid	Linoleic acid
Butyric acid	Glycerol	Linolenic acid
Caffeic acid	Palmitic acid	Oleic acid
Ferulic acid	Pantothenic acid	P-coumaric acid

Table 1.3: Some other Components found in the whole plant of *U. Dioica* (Duke, 2002)

Other Components		
2-Methylhepten-(2)-on(6)	Folacin	Protoprphyrin
5-Hydroxytryptamine	Histamine	Scopoletin
Acetophenone	Betaine	Serotonin
Acetyl-choline	Strach	Sitosterol
Beta-Carotene	Lecithin	Koproporphyrin
Cellulose	Lycopene	Sitosterol-glucoside
Chlorophyll	Mucilage	Violaxanthin
Choline	Protein	Xanthophyll-Epoxide

et al., 1996)

Alpha Tocopherol	Niacin
Riboflavin	Thiamin

In addition a large number of compounds of various polarity and belonging to different chemical classes, including phenylpropanes, coumarins, triterpenes, terpenes, lignans, ceramides, lectins and sterols, have been isolated from *Urtica Dioica* (Leung, 1996).

1.3. Various Plant Uses and toxicity

1.3.1. Edible Uses

Young *U. Dioica* leaves are usually cooked as a pot herb and taken as tea (Facciola, 1990). They can also be dried for later uses. This plant is very valuable in regular healthy diet (Phillips and Foy, 1990), it is very nutritious and easily digested and is high in vitamins (especially A and C) and minerals (especially iron) (Allardice, 1993). Cooking the herb, or drying them thoroughly, neutralizes the sting, rendering it safe to eat (Phillips and Foy, 1990). Young shoots, harvested in spring time when it is 15-20cm long with the underground stem have special characteristics, for instance leaves can be laxative. The plant is harvested commercially for extraction of chlorophyll, which is used as a green coloring substance (E140) in medicines and foods (Bown, 1995). *U. Dioica* adds as well a bland flavor as a tonic to China tea (Bown, 1995). The juice of the herb can be used as a rennet substitute in curdling plant milks (Facciola, 1990). And finally, Nettle beer is extracted from the young shoots (Huxley, 1992).

1.3.2. Medicinal Uses

Nettles have a deep history of home use as herbal nutritious and remedy addition to daily diet. Traditionally tea is made from its leaves which used as a blood purifier and cleansing tonic so the plant is often used in the treatment of hay arthritis, fever, and anemia (Grieve, 1984). The whole plant is a hemostatic, antiasthmatic, diuretic, depurative, galactogogue, antidandruff, hypoglycemic and a stimulating tonic (Grieve, 1984). The plant is also used in stemming internal bleeding as well as treating excessive menstruation, haemorrhoids, and rheumatism (Grieve, 1984). Externally, the plant is used to treat hair problems and skin complaints, such as eczema (Grieve, 1984). The fresh leaves of *U. Dioica* have been beaten or rubbed onto the skin for treatment of rheumatism as well (Moerman, 1998). This process is called urtification, it usually causes severe irritations to the skin. It is thought that this procedure works in two ways. Firstly, the formic acid from the herb needle hairs is believed to have a beneficial effect on the rheumatic joints. Secondly, it acts as a counter-irritant, by collecting more blood to the damaged area to facilitate the removal of toxins that cause the disease (Bown, 1995). This plant showed also interesting results against urinary system ailments and kidney (Foster and Duke, 1990). The root has been proven to have a beneficial effect upon enlarged prostate glands (Chevallier, 1996).

1.3.3. Common Uses and effects

Strong fibers are obtained from the stems. Used for making clothes and strings (Huxley, 1992). It also makes good quality papers. They are harvested before the plant starts to die in early autumn and are soaked before the fibres are extracted (Turner, 1979). They produce fibers in less abundance than flax (*Linun usitatissimum*) and are also more arduous to extract. The plant matter left is good source of biomass

and has been used in the synthesizing of starch, sugar, ethyl alcohol and protein. Oils that are extracted from the seeds are used as illuminants (Bruce, 1977). *U. Dioica* is considered as one of the QR collection, a dried mixture of several herbs, which can be a supplement to a compost heap in order to quicken bacterial activity and thus lessen the time needed to make the compost (Philbrick and Gregg, 1979), they also can be soaked for 7 to 21 days in water to make an excellent nutritious liquid for plants. This liquid is also considered as insect repellent (Hatfield, 1977). It is also used as a tonic in antidandruff treatment (Allardice, 1993).

1.3.4. Side Effects/Toxicology

There are no major side effects. *Urtica Dioica* can cause a stinging sensation (urticaria). Orally taken Nettle root tea can lead to mild gastrointestinal complaints, burning sensation of the skin, gastric irritation, oliguria (decreased urine output ratio to fluid intake), and edema. Some allergic reactions to leaf preparations have been observed in rare cases (Allardice, 1993).

1.4. Gastric Ulcer

Mucosa of the stomach constitutes the main barrier that prevents the free exposure of the gastric mucosal cells especially to different luminal, injurious agents and irritants whether from external or internal sources. Mucosal surface epithelial cells are usually assaulted by physical, chemical or microbiological agents that act via the gastric lumen, which are implicated with various diseases, such as gastritis, peptic ulcer, or gastric cancer (Zayachivska et al., 2005). Ulcer is known as disruptions of the mucosal integrity of the duodenum or stomach leading to a local defect due to massive inflammation and may cause cancer and hemorrhage in sever cases

(Braunwald et al., 2001). Around Four million individuals in the United States are affected by acid peptic disorders (Braunwald et al., 2001). Stomach Ulcers are areas of mucosal ulceration in the wall of the stomach beyond (Hodgson et al., 2000). The majority of Gastric ulcer can be attributed to either Nonsteroidal anti-inflammatory Drugs induced mucosal damage or the bacterium *Helicobacter pylori* (Braunwald et al., 2001). Gastric ulcer can also be due to many factors such as smoking, stress and other nutritional deficiencies (Belaiche et al, 2002). Furthermore, an imbalance between gastric enzymes and the acidity of the stomach may cause ulcer (Baron et al., 1980). On the other hand, bile, acids, pancreatic enzymes and lysolecithine may injure the gastric mucosa; although no definite evidence for these factors has been reported in the gastric acid pathology (Braunwald et al., 2001). In spite of the existence of several medications for gastric ulcer treatment such as anti-acids and antihistamines, most of these medical methods have side effects including gynecomestia, hematopoeisis and impotency to do activities (Arapaho et al., 1986). Hence, there is a demand for decreasing the toxicity of anti-ulcer agents (Alkofahi and Atta, 1999). In traditional medicine, several herbal extracts were used widely in treating gastric ulceration. Therefore, there was a need to investigate scientifically these herbal extracts for their potential role as gastric ulcer remedies.

1.5. Lipids

1.5.1. Introduction

Lipids are organic molecules that are soluble in organic solvents only. Lipids play important physiological role in all living organisms. They serve as the main structural components of cell membranes. They provide energy reserves in the form of triglycerides. They are used also as precursors to hormones, vitamin synthesis

(vitamin D) and lipophilic bile acids. Lipids are devided into: glycerides such as triglycerides, fatty acids (unsaturated and saturated), complex lipids such as glycolipids and lipoprotein, waxes, steroids and nonglycerides such as sphingolipids (Vance and Vance, 2004).

1.5.2. Triglycerides

Triglycerides constitute about 95% of fatty tissues. They are considered as the main stored form of body lipids. In principle triglycerides consist of three molecules of fatty acids bound to one molecule of glycerol (Loeb, 1994). They normally fluctuate between 40 to 160 mg/dl in adult men bloodstream and between 35 to 135 mg/dl in adult women. In rats the mean triglyceride concentration is 80 mg/dl. Elevated triglycerides levels (is often associated with high cholesterol) have been attributed to cardiovascular diseases, the hidden factor that causes heart stroke. It was observed that high lipid level is the consequence of enzymatic troubles of carbohydrates and lipids, like in obese people, in heavily alcoholic people and diabetic patients of type II (Loeb, 1994).

1.5.3. Cholesterol

Cholesterol is a main structural element in biological membranes and plasmatic lipoproteins. It is taken from fatty diet and mostly produced in the liver. Steroid hormones including glucocorticoids and mineral corticoids produced in the adrenal cortex and sex steroid hormones synthesized in the female and male gonads are cholesterol derivatives: Cholesterol is also a precursor for the synthesis of bile acids in the gallbladder. It is also contributes to the formation of vitamin D (Vusse, 2004).

High serum cholesterol levels in most of the cases are the corner stone of cardiovascular diseases (Loeb, 1994).

1.5.4. Lipoproteins

Lipoproteins are the core of most hydrophobic lipids (cholesteryl esters and triglycerides) within protein membranes (apoprotein) associated with phospholipids and free cholesterol. So, lipoproteins are soluble complexes of lipids and proteins that transport lipids in the body. They are produced in the liver, intestine, assembled at the cell membranes from cellular lipids and exogenous apolipoprotiens and lipoproteins, or arise from metabolism of primary lipoproteins. In blood circulation, lipoproteins undergo conformational changes in response to variations, and they are responsible for transferring soluble Apolipoproteins. Lipoproteins are taken up and metabolized in peripheral tissues, liver and kidneys (Vance, 2004).

The majority of lipoproteins are classified into five categories as follows:

Chylomicrons (CM): CM, consisting mostly of triglycerides and much smaller amounts of cholesterol, phospholipids and proteins, has the lowest density among all lipoproteins. Its main function is to deliver exogenous dietary lipids absorbed from the intestine to the blood then to the liver. When CM passes in muscle and adipose tissue cells, lipoprotein lipase enzyme attached to the capillary endothelium hydrolyzes most of the triglyceride core of the chylomicron molecules rendering it a CM remnant particle (much smaller in size) that can be cleared by the liver (Loeb, 1994; Cooper, 1997). Dietary cholesterol is supplied to the liver by the interaction of CM remnant with chylomicron remnant receptor. Apolipoproteins that predominate before they

enter the circulation are: apoB-48 and apoA-I, apoA-II and apoA-IV (Vance and Vance, 2004).

Very Low Density Lipoprotein (VLDL): VLDL consists mainly of triglycerides, and smaller amounts of protein, cholesterol, and phospholipids (Loeb, 1994). Triglycerides are condensed into VLDLs and secreted in the circulation to be delivered to various tissues like fatty and muscle tissues for storage and energy production. Thus, VLDLs are complexes that transport endogenous triglycerides from hepatic tissues. Moreover, VLDLs contain cholesteryl esters and cholesterol and the apoproteins, apoC-I, apoC-II, apoC-III, apoB-100 and apoE. Newly released VLDLs acquire apoE and apoCs from HDLs in circulation. The fatty acid portion of VLDLs is released to muscle and adipose tissues through the action of lipoprotein lipase (Vance, 2004).

Intermediate Density Lipoprotein (IDL): are short-lived complexes and contain equal amounts of triglycerides and cholesterol, and smaller amounts of proteins and phospholipids. IDLs are synthesized as triglycerides are eliminated from VLDLs. IDLs are either converted to LDLs or directly up taken by the liver by receptor mediated endocytosis. They are converted to LDL upon further action of the lipoprotein lipase enzyme (Loeb, 1994). The liver takes up IDLs after they have bound to the LDL receptor to form molecular complexes, which are absorbed by the hepatocytes. The recognition of LDL receptors to IDLs requires the presence of both apoB-100 and apoE (Ger and Van der Vusse, 2004).

Low Density Lipoprotein (LDL): known as "bad cholesterol" is about half cholesterol and half protein, triglycerides and phospholipids (Loeb, 1994). LDLs are the main carriers of cholesterol in the blood. ApoB-100 is considered as the only apolipoprotein

of LDLs. Usually LDLs enter cells through LDL receptor-mediated endocytosis. Tissues that mostly uptaking LDLs are liver (75%) adipose and adrenal tissues. The binding of LDLs on their receptors requires apoB-100 (Vance, 2004; Ger and Van der Vusse, 2004).

High Density Lipoprotein (HDL): HDL known as good cholesterol, are about half phospholipids, triglycerides and cholesterol and half protein (Loeb, 1994). HDLs are produced in the small intestine and liver. The newly synthesised HDLs are devoid of any cholesteryl esters and cholesterol. The primary apoproteins of HDLs are apoC-I, apoA-I , apoE and apoC-II. The main role of HDLs is to act as circulating stores of apoC-I and apoE. HDLs are converted into spherical lipoprotein particles by accumulating cholesteryl esters. This accumulation transforms nude HDLs to HDL_2 and HDL_3. Cholesterol-rich HDLs go to the liver, where they are re-endocytosed (reverse cholesterol transport). Macrophages also internalize HDLs via apoA-I receptor interaction (Vance and Vance, 2004).

1.6. Glycemia

Diabetes Mellitus is considered as a chronic metabolic disorder. There are two types of diabetes mellitus: type one diabetes mellitus (IDDM; insulin dependent diabetes mellitus) which is due to an organ specific autoimmune disease that destroys pancreatic β cells producing insulin, and type two diabetes mellitus (NIDDM; non-insulin dependent diabetes mellitus) that is caused by weak insulin interaction action on target cells. Type 1 diabetes can be lethal if not treated with exogenous insulin via injections in order to replace the missing hormone. It is very well known that there is

no preventative measure that can be taken to avoid against type 1 diabetes. The majority of people affected by type 1 diabetes are otherwise healthy with a normal body weight when onset occurs. Type 1 diabetes cannot be reversed or prevented. Type 2 Diabetes mellitus may be managed by dietary modification and exercise engagement. The incidence of Type 2 diabetes is rapidly increasing in developed countries, and there is some evidence that the rest of the world in coming years will be following the same trend (WHO, 1999). There are more than 143 million people world wide with diabetes and this number will double by the year 2030. Diabetes is associated with high rates of mortality following serious complications such as renal disease, myocardial infarction, atherosclerosis, retinopathy, and strokes (Boyle et al., 2001). The characteristic symptoms are polyuria (excessive urine production), polydipsia (thirst and increased fluid intake) and blurred vision; these symptoms may be absent if the blood sugar is only mildly elevated.

1.7. Liver Toxicity Assessment

The liver is an organ located in the right abdominal side. Its main role is glycogen storage, and manipulation of digested food proteins and fats; as well as neutralization of circulating toxins or poisons and bile production. Of course these functions require the synthesis of certain chemical substances that may affect the liver functioning leading to bad concequences. Thus there are many tests that allow detecting the actual functionality of liver. These tests which are commonly performed on a blood serum are called liver function tests LFTs' namely AST, ALT, Bilirubin, albumin and total protein (Braunwald et al., 2001).

Aspartate Aminotransferase (AST) or (GOT): is an enzymes that catalyzes the transformation of the amine group of an amino acid to a residue. It is indispensable

for energy production. AST is predominant in the cytosole and mitochondria of many cells, mainly in the kidneys, pancreas, heart, liver and traces in the RBCs (Loeb, 1994).

Alanine Aminotransferase (ALT) or (GPT): is a second enzyme that catalyzes a reversible conversion of a nitrogenous groups during the Krebs cycle. It is a specific indicator of acute hepatic damage. ALT predominates in hepatocytic cytoplasm, with low amounts in the heart, kidneys, and skeletal muscle. Therefore, ALT is release from the hepatoctes into the bloodstream, leading to high serum levels that persist from days to weeks (Loeb, 1994).

1.8. Anti-inflammatory effect

Inflammation is a nonspecific immune response to infections and many other factors. Inflammatory response begins with unusual increase of blood flow at the site of infection, followed by migration of leukocytes to the infected area. Also inflammatory signs are manifested by the secretion of cytokines in large amounts in the damaged tissue. Thus the increased blood flow causes rise in temperature and redness. In addition, the accumulation of the enormous amounts of fluids and blood cells is recognized by swelling and edema in the affected tissue. Eventually these cellular progressions of inflammations result in scarring of the lesion (Sell, 2001). Inflammation is either acute or chronic. The acute inflammation, is characterized by redness, edema, heat and pain. Elements of the acute inflammation are kinin, complement, coagulation, and mast cells. On the other hand, chronic inflammation a response that follows acute inflammation is in case when the latter is incapable to stop the infection. Its effect is more powerful. It is the cooperation of lymphocytes, macrophages and plasmocytes (Sell, 2001). Inflammatory symptoms are associated

with the release of nitrogenous elements, cytokines and reactive oxygen (Conner and Grisham, 1996). Thus, since oxygen is a reactive agent that plays important role in inflammatory process, numerous natural and synthetic antioxidants extracted from plant have been considered as potential therapeutic remedies (Sell, 2001).

1.9. Purpose of the Project

U. Dioica is considered as one of the medicinal herbs in folk medicine. It is widely spread in the Mediterranean rim and Tropical regions. Although many studies have been conducted on *U. Dioica*; however, most of these studies did not consider particularly seeds. The present study dealt specifically with the seeds of *U. Dioica* where two fractions were used; the crude water extract and the polyphenols extracts. The following parameters were investigated:

- Lipid Profile: Serum triglycerides, total cholesterol, HDL-Cholesterol and LDL-cholesterol concentrations.
- Liver toxicity by measuring the liver enzyme activities of GPT and GOT.
- Glycemic profile: Glucose and Insulin levels.
- Gastric Ulcer protection.
- Inflammation studies: both acute and chronic inflammation models.

Chapter II

Materials and Methods

2.1. Plant Material

U. Dioica plant was collected from different places in Lebanon between April and June 2006. The plants were identified in reference to the criteria inscribed in "Handbook of Medicinal Herbs" book (Duke et al., 2002). Fresh *U. Dioica* were harvested among wild bushes, then separated, and desiccated at room temperatures in shade. Finally, the dried seeds were then collected and used for the preparation of water and polyphenols extracts.

2.2.1. Determination of Water Extract Dry Weight

In order to determine the weight of dry water extract per gram of seeds, 3 g of *U. Dioica* seeds were simmered in 100 ml of boiled distilled water for 20 minutes with occasional stirring. The solution was then filtered through Whatman filter paper (size #1). The filtrate was then subjected to evaporation in an incubator of moderate temperature and the dry mass of the extract was measured. Results have shown that the dry water extract yield is 10.67 % (w/w). Thus, all experimental protocols performed in this study depended on this yield and methodology.

2.2.2. Determination of polyphenols Extract Dry Weight

In order to determine the mass of dry polyphenols extract per gram of seeds, 3 g of U. Dioica seeds were added to 22.5 ml 1:1 water-ethanol solution (1:7.5 solid to liquid ratio) and left in an agitator at 65 °C for 1.5 hours (Shi, 2003). The solution was then filtered using What-man filter paper (size # 1). The filtrate was then subjected to evaporation and the dry mass of the extract was determined. The results have shown

that the dry polyphenols extract yield is 2.2 % (w/w). Therefore, all experimental protocols performed in this study were based on this yield and methodology.

2.3. Animals Preparation

This study used male Sprague-Dawely Rats from the Lebanese American University Stock. The rats were kept in a controlled environment (23±1°C, 40-60% humidity and 30 to 40 air changes per hour with a 12-h dark: 12-h light cycle) with free access to standard rat chow food (Laboratory rodent starter diet no.1, Hawa chicken Co., Lebanon) and tap water.

2.4. Animals Treatment

Rats were divided into four groups each containing ten rats with an average weight of 270 to 300 g. They were fed with the standard rat chow diet to which 5% coconut oil was added. Coconut oil was provided to increase diet atherogenicity (Daher et al, 2003). One group was used as a control while the other three groups "the treatment groups" received the same food as the control but in addition to the *U. Dioica* seeds extract in drinking water (50mg/kg body weight, group I (GI); 100 mg/kg body weight, group II (GII); and 250 mg/kg body weight, group III (GIII)). These doses were determined according to the fact that a rat consumes 100ml of drinking water for each 100g body weight (Waynforth and Flecknell, 1992). After a period of one month of extracts intake, the animals were sacrificed with diethyl ether and blood samples were collected. Another three groups similar to the preceding ones were treated with polyphenols extract for the same period of time. Then, one month later with the same treatment, the animals were sacrificed using diethyl ether and blood samples were taken.

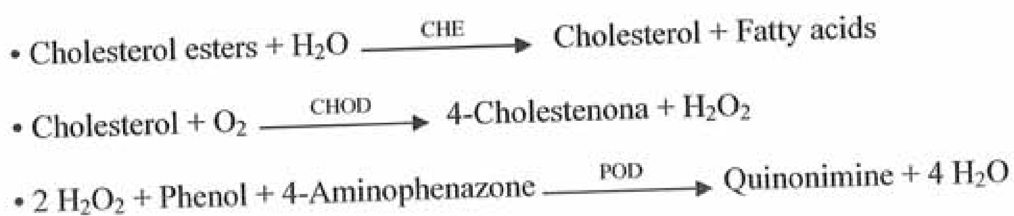
2.5. Serum Assays

2.5.1. Samples Preparation and Experimental Protocols

Rats were fasted for 18 hours before being sacrificed then 6 to 7 ml of venous blood were withdrawn from the vena cava using a 10 ml sterile syringe. Blood samples were put in glass tubes for 35 to 45 minutes at room temperature to clot then centrifuged (20 minutes at 1000xg at 10°C). Then, the sera were aspirated, divided in Eppendorf tubes and kept on ice or in freezer for later uses in assessing lipid profile (Total cholesterol, HDL cholesterol, LDL cholesterol, and Triglycerides), glycemic profile (Glucose and Insulin) and liver enzymes activities (SGPT and SGOT).

2.5.2. Determination of Total Cholesterol

Cholesterol is determined enzymatically depending on Cholesterol esterase (CHE), peroxidase (POD) and Cholesterol oxidase (CHOD) activity based on the following reactions.



Red dye is produced due to Hydrogen peroxide. The intensity of this color is proportional to the cholesterol quantity in the sample that can be identified using a spectrophotometer at a wavelength of 505 nm (SPINREACT).

2.5.3. Determination of HDL-Cholesterol

Phosphotungstate in the presence of magnesium ions precipitates very low density lipoprotein (VLDL) and low density lipoprotein (LDL) of the serum. Therefore, they can be eliminated by centrifugation while high density lipoprotein (HDL) remained in the supernatant. Thus, determination of HDL cholesterol is possible using the clear supernatant (SPINREACT).

2.5.4. Determination of LDL-Cholesterol

Direct determination of LDL-cholesterol depends on the interaction of a sugar compound and lipoproteins (VLDL and Chylomicrons) and on benefit a non-ionic detergent that selectively solubilizes LDL-cholesterol. When a detergent is introduces in an enzymatic reaction for determining cholesterol concentration (Cholesterol esterase CE, and Cholesterol oxidase CHO coupling reaction), the relative cholesterol reactivity of a lipoprotein complex decreases as follows:

LDL>VLDL>Chylomicrons>HDL. A sugar complex in presence of magnesium ions reduces the enzymatic reaction for the measurement of cholesterol in Chylomicron and VLDL. The combination of a detergent with sugar molecules permits the selective determination of LDL- cholesterol in serum.

- LDL- cholesterol esters + $H_2O \xrightarrow[\text{detergent}]{\text{CE}}$ Cholesterol + Free Fatty acids
- LDL- cholesterol + $O_2 \xrightarrow{\text{CHO}} \Delta^4$-cholesterol + H_2O_2
- $2H_2O_2$ + 4-amino-antipyrine + HSDA + H^+ + $H_2O \xrightarrow{\text{peroxidase}}$ Purple-blue

pigment + 5 H_2O

The concentration of LDL-cholesterol determined by the spectrophotometer at a wavelength of λ=505nm is proportional to the color intensity obtained (SPINREACT).

2.5.5. Determination of Triglycerides

Serum triglycerides are determined according to the following enzymatic reactions:

- Triglycerides + H_2O $\xrightarrow{LPL}$ glycerol + free fatty acids
- Glycerol + ATP $\xrightarrow{\text{glycerol kinase}}$ G3P + ADP
- G3P+O_2 $\xrightarrow{GPO}$ DAP + H_2O_2
- H_2O_2 + 4-AP + p-chlorophenol $\xrightarrow{POD}$ Quinone + H_2O

The concentration of triglycerides in a given sample is proportional to the intensity of the red dye produced at the end of the enzymatic reaction, and can be determined by spectrophotometry at wave length λ = 505nm.

* G3P: Glycerol-3-phosphate; LpL: lipoprotein lipase; 4-AP: 4-aminophenazone ADP: adenosine-5-diphosphate; DAP: dihydroxyacetone phosphate; POD: peroxidase; GPO: phosphate dehydrogenase (SPINREACT).

2.5.6. Determination of Serum GOT

Glutamate oxaloacetate (GOT) or aspartames aminotranferase (AST) is one of the liver enzymatic pools which catalyses the reversible transfer of the amine group from aspartames to α-ketoglutarate. Then the obtained oxaloacetate is reduced to malate by the action of malate dehydrogenase (MDH) and NADH as follows:

The rate the concentration decreases detected by spectrophotometery of NADH to measure at a wavelength = 340 nm. The cease in the concentration of NADH is proportional to the catalytic amount of AST found in the sample.

2.5.7. Determination of Serum GPT

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

therefore, by making use of spectrophotometer, the decrease in the concentration of NADH is registered at a wavelength = 340 nm of NADH is proportional to the catalytic concentration of ALT found in the given sample.

* LDH: lactate dehydrogenase

2.5.8. Glucose Determination

Serum glucose (glycemia) is measured based on the following catalytic reactions:

The intensity of the red-violet dye obtained at the end of the reaction is directly proportional to the concentration of glucose and is detected by spectrophotometer at a wavelength 505 nm.

* GOD: glucose oxidase; POD: peroxidase (SPINREACT).

2.6. Determination of Serum Insulin

The concentration of the rat serum insulin is measured by using the Rat-insulin ELISA (Enzyme Linked IminunoSorbent Assay) kit. The kit is a solid phase two-site enzyme immunoassay on the bases of direct sandwich ELISA technique where two monoclonal antibodies specific to separate epitopes on the insulin molecule. In the incubation period, horseradish peroxidase conjugated to anti-insulin biotinylate antibodies and anti-insulin antibodies bound to the microtiter well react with insulin. Following a washing step removes non-specifically bound enzyme labeled antibodies. The obtained conjugate complex is detected by the reaction with 3, 3', 5, 5' - tetramethylbenzidine substrate. This reaction stops after the addition of the acid to give colorimetric end products. The absorbance variations are measured by spectrophotometer on wavelength of 450 nm, corrected by the absorbance at 590 nm. Thus, the increase in the measured absorbance is proportional to the amount of bound insulin in the unknown sample, and then the latter can be derived by interpolation from a reference curve created by using reference standards of known rat insulin concentrations curve.

2.7. Effect of Urtica Dioica Seed Extracts on Ethanol Induced Gastric Ulcer

The effect of water and polyphenols extracts of *U. Dioica* seeds on ethanol induced gastric ulcer was conducted on male Sprague-Dawley rats of an average weight of

270-300 g. The rats were kept in standard well controlled conditions with free access to food and water. They were subjected to fasting for 2 days before ulcer induction to ensure an empty exposed stomach. During the fasting period, animals were consuming 8% sucrose (w/v) solution in 0.2% NaCl (w/v) to avoid severe dehydration during this period. The solution was removed one hour before treatment with either plant extracts or drug. Animals were divided into eight groups of 6 rats for each treatment. Group I received only water (10 ml/kg body weight) and served as a control group. Group II, Group III and Group IV, receiving the plant extracts in three different dosages 50 and 100 and 250 mg/kg body weight respectively, considered as the treatment groups. While Group V received 10 mg/kg body weight of the reference drug Cimetril. All Cimetril, water and polyphenols extracts were administered by intra-gastric gavages using a stainless steel intubations needle. Drug and extract doses were given in shots as follows, twice in the first day, at 08:00 h and 16:00 h. Then a third shot was given in the second day 1.5 h prior to gastric ulceration. The other three groups were similarly treated with polyphenols extracts using doses of 50, 100, 250 mg/Kg body weight.

To induce stomach ulcer, 50% ethanol (v/v) in distilled water was given orally using a syringe tube directed into the stomach to all groups in a dose of 10 ml/kg body weight (Alkofahi and Atta, 1998). One hour after ethanol administration, the rats were sacrificed by an over dose of ether. Then, stomachs were collected, opened along the greater curvature and gently washed with tap water (Kushima et al., 2005).

To determine the stomach ulcerative lesions mainly in the glandular region, a stereomicroscope was favorable. Thus, long lesions were determined, counted and measured using a ruler, where as each five petechial lesions was considered as 1 mm of ulceration (Gurbuz et al., 2005).

• Ulcer index (mm) = the sum of the total measured length of long and petechial lesions (for a group) / number of rats per group.

• The curative ratio = [(control ulcer index) - (Test ulcer index) / (control ulcer index)] x 100

2.8. Anti-Inflammatory Activity

To study the effect of the leaves of *U. Dioica* water and polyphenols seed extracts upon acute and chronic inflammation we induced it through Carrageenan and formalin respectively. Male Sprague-Dawley rats weighing between 230 to 300g were used. The rats were divided into five groups of six rats each for every extract treatment. All animals had free access to regular chow diet (6.5g per 100g body weight) and tap water.

2.8.1. Acute Anti-Inflammatory Activity

Eight groups of 6 animals each were utilized in this experiment. All animals were subjected to edema induced by injecting 0.02 ml of freshly prepared 1% Carrageenan in normal saline in the sub-plantar area of the rat right hind paw. Three groups of rats received the water extracts of *U. Dioica* seeds 30 mm away from the induced inflammation at three different concentrations which were 50, 100 and 250 mg/Kg body weight. One group was injected with Carrageenan only and was considered as the positive control group and another group received Diclofenac alone (10mg/kg body weight) and considered as a standard reference drug group. Thirty minutes before the Carrageenan injection, the paw thickness was measured and then the thickness was also measured three hours after Carrageenan injection using the vernier calipers (Ajith and Janardhanan, 2001). The same experiment was also applied to

other the three remaining groups in the same manner for the use of polyphenols extract. Both extracts were sterilized through syringe filter sterilization.

2.8.2. Chronic Anti-Inflammatory Activity

In chronic inflammation experiment, paw edema was induced by injecting 0.02 ml of 2% formalin in the rat sub-plantar right hind paw. Three groups of rats received the water extracts of *U. Dioica* seeds (50, 100 and 250 mg/Kg body weight). Another three groups received the polyphenols extracts *U. Dioica* seeds (50, 100, 250 mg/Kg body weight). One group received Diclofenac (10mg/kg body weight) as a standard drug reference, and one control group did not receive any treatment. All treatments were given intraperitoneally 30 minutes prior to formalin injection. Extracts and diclofenac treatments were continued once daily for six consecutive days. Paw thickness was determined before and six days after formalin injection by using the vernier calipers (Jose et al., 2004).

2.8.3. Paw Thickness Calculations

The increase in paw thickness in both acute and chronic inflammation was calculated according to the following formula:

$$\mathbf{P-P_o}$$

Where (P) corresponds to the paw thickness at time t (6 days after the formalin injection and 3 hours after the Carrageenan injection in chronic and acute inflammation experiments respectively) and P_o corresponds to the paw initial thickness.

The following formula was used to calculate the percentage inhibition:

$$\mathbf{(C-T/C) \times 100}$$

Where C represents the increase in paw thickness of the control group and T the increase in paw thickness of the treatment groups.

Chapter III

RESULTS

One month after high fat intake, in both control and experimental groups, animals were sacrificed and blood samples were collected and tested for their serum Total cholesterol, HDL-cholesterol, LDL-cholesterol, Triglycerides, Glucose and Insulin levels. Liver function was assessed through measuring the activity of liver enzymes in serum: SGPT and SGOT for both extracts. Different groups of rats were used to determine the effects of *U. Dioica* seeds extracts upon ethanol induced gastric ulcer, acute inflammation and chronic inflammation.

3.1. Effects of *U. Dioica* seeds on serum lipid, glycemia and liver enzymes

3.1.1. Serum total cholesterol

There was a slight increase but no significant difference between Serum Cholesterol levels of the control and that of the water seeds extract treatment groups. Data are shown in figure 3.1.

On the contrary, no significant difference between Serum Cholesterol levels between the control and that of the polyphenols seeds extract treatment groups. Data are shown in figure 3.2.

3.1.2. Serum HDL-cholesterol

There was a significant increase in the HDL serum Cholesterol levels of the experimental groups as a result of water seeds extracts. Data are shown in figure 3.3. On the contrary, no significant difference between Serum HDL levels between the control and that of the polyphenols seeds extract treatment groups, but a light increase as the concentration of the extract increases. Data are shown in figure 3.4.

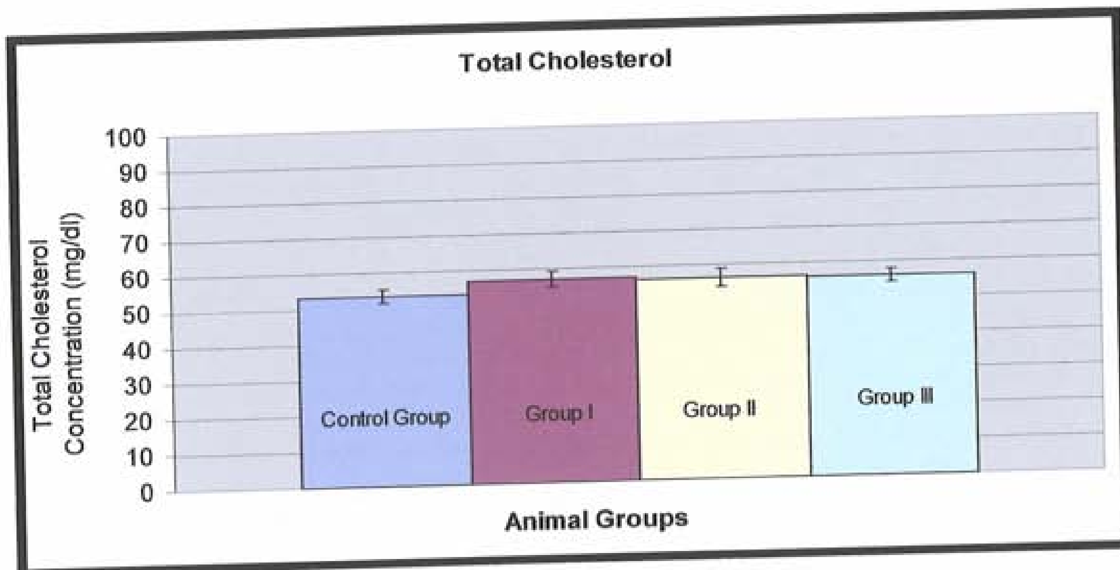


Fig. 3.1: Serum Total cholesterol levels after one month of in-vivo treatment with *U. Dioica* water seed extract in rats fed a high fat diet. Data are presented as mean ± SEM, (n=10).

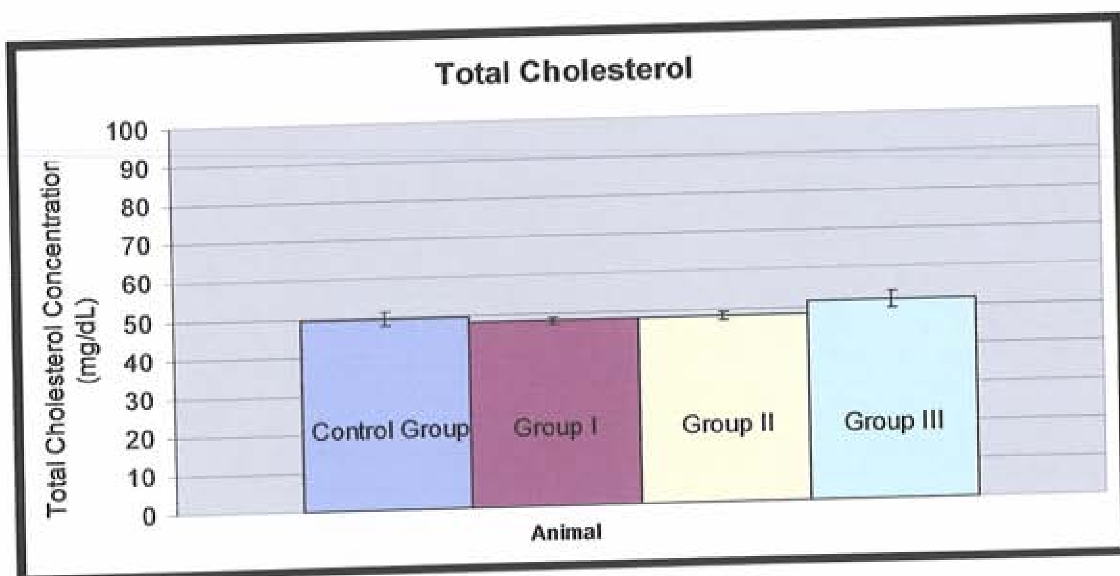


Fig. 3.2: Serum Total cholesterol levels after one month of in-vivo treatment with *U. Dioica* seed polyphenols extract in rats fed a high fat diet. Data are presented as mean ± SEM, (n=10).

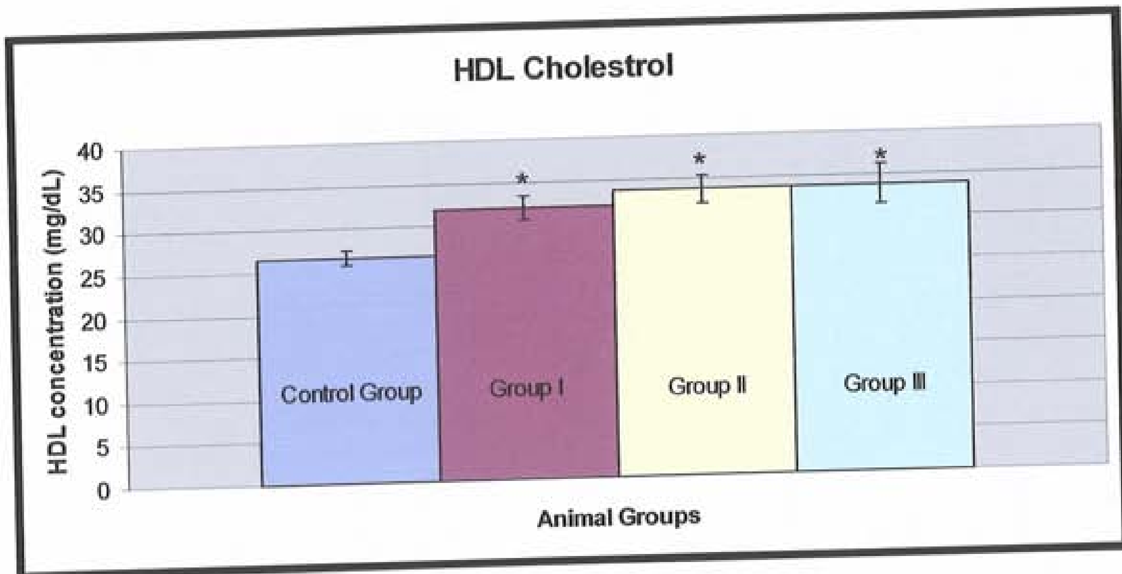


Fig. 3.3: Serum HDL cholesterol levels after one month of in-vivo treatment with *U. Dioica* water seed extract in rats fed a high fat diet. Data are presented as mean ± SEM, (n=10). * *Significant difference (<0.05) with respect to the control.*

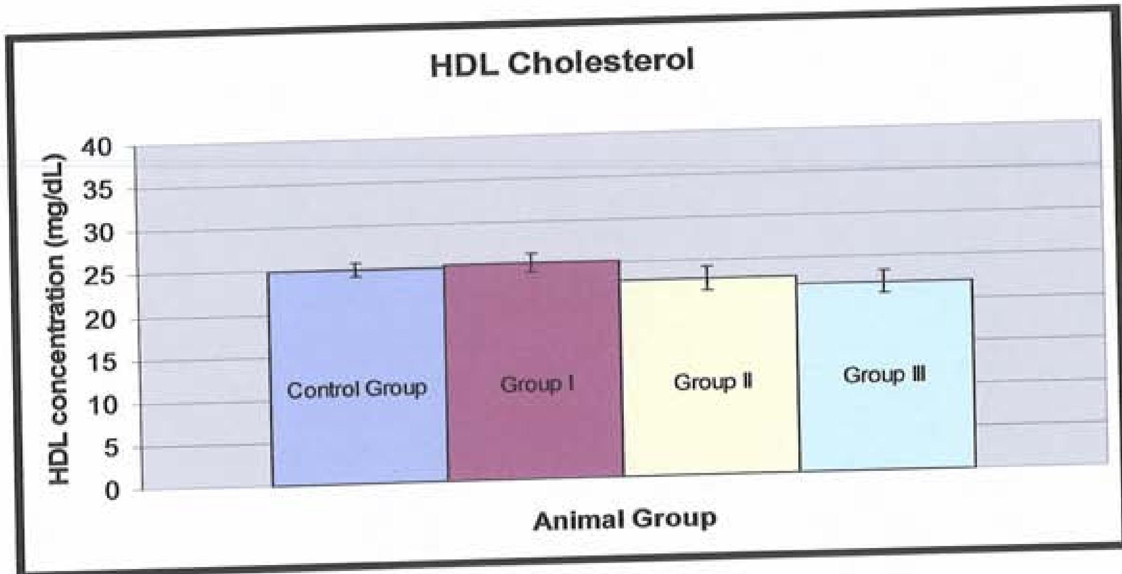


Fig. 3.4: Serum HDL cholesterol levels after one month of in-vivo treatment with *U. Dioica* seed polyphenols extract in rats fed a high fat diet. Data are presented as mean ± SEM, (n=10).

3.1.3. Serum LDL-cholesterol

There was a slight decrease but no significant difference between Serum LDL Cholesterol levels of the control and that of the water seeds extract treatment groups. Data are shown in figure 3.5. On the other hand, a decrease in the treatment groups I then the LDL levels increases in the other groups but not significantly in comparison between the control and that of the polyphenols seeds extract treatment groups. Data are shown in figure 3.6.

Calculation of the total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol of water and polyphenols extracts are shown in table 3.1 and table 3.2 respectively.

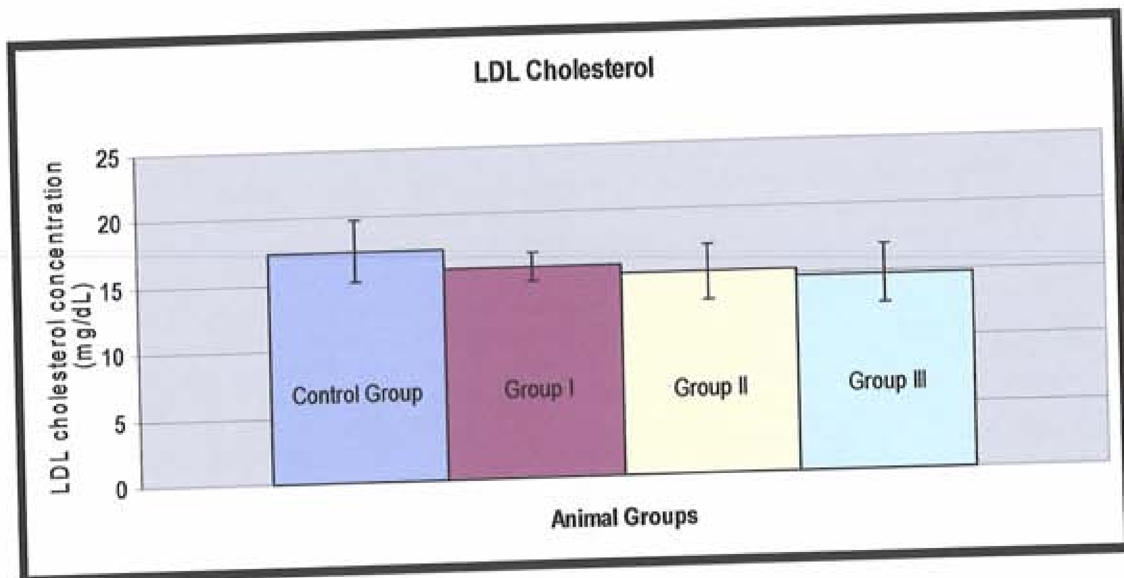


Fig. 3.5: Serum LDL cholesterol levels after one month of in-vivo treatment with *U. Dioica* water seed extracts in rats fed a high fat diet. Data are presented as mean ± SEM, (n=10).

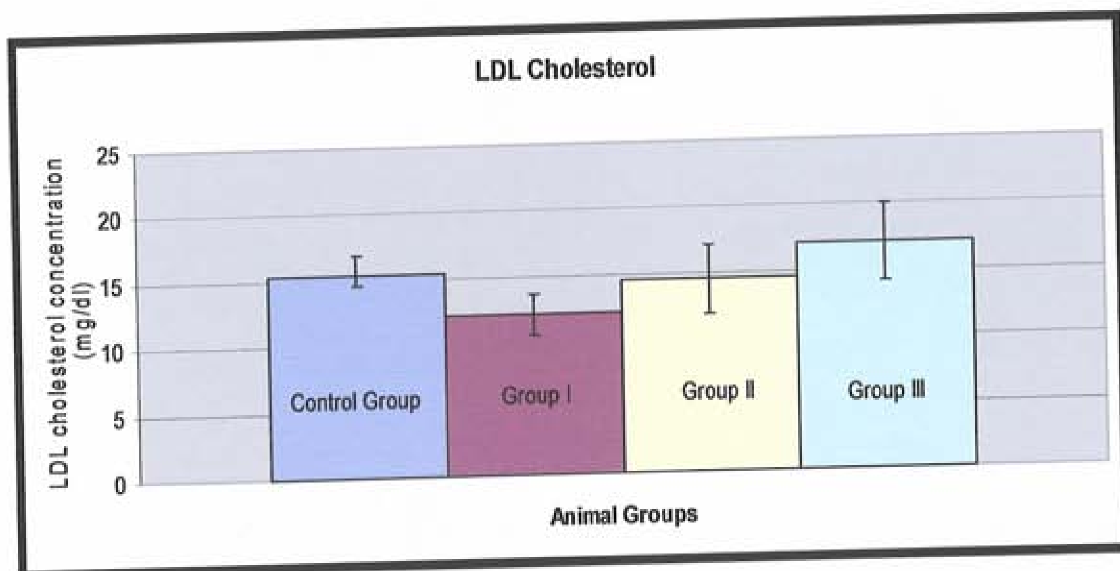


Fig. 3.6: Serum LDL cholesterol levels after one month of in-vivo treatment with ***U. Dioica*** seed polyphenols extract in rats fed a high fat diet. Data are presented as mean ± SEM, (n=10).

Table 3.1 Total cholesterol/HDL cholesterol, LDL/HDL cholesterol ratios after one month of in-vivo treatment with the seeds of *U. Dioica* water extract in rats fed on a high fat diet. Values are expressed as ±SEM, (n=10).

	Total cholesterol / HDL-cholesterol Ratio	LDL/HDL Ratio
Control Group	2.03 ± 0.11	0.68 ± 0.11
Group I	1.79 ± 0.03	0.50 ± 0.03
Group II	1.69 ± 0.08	0.46 ± 0.07
Group III	1.71 ± 0.09	0.48 ± 0.08

Table 3.2 Total cholesterol/HDL cholesterol, LDL/HDL cholesterol ratios after one month of in-vivo treatment with the seeds of *U. Dioica* polyphenols extract in rats fed on a high fat diet. Values are expressed as ±SEM, (n=10).

	Total cholesterol / HDL-cholesterol Ratio	**LDL/HDL Ratio**
Control Group	1.99 ± 0.02	0.61 ± 0.02
Group I	1.94 ± 0.09	0.50 ± 0.07
Group II	2.17 ± 0.13	0.70 ± 0.14
Group III	2.43 ± 0.19	0.86 ± 0.19

3.1.4. Serum Triglycerides

Assessment of the serum triglyceride concentration revealed that there was a significant decrease observed in the different water extract treatment groups as shown in Figure 3.7. Whereas, the serum triglyceride concentration revealed a slight increase in the different polyphenols extract treatment groups, but no significant value was recorded as shown in Figure 3.8.

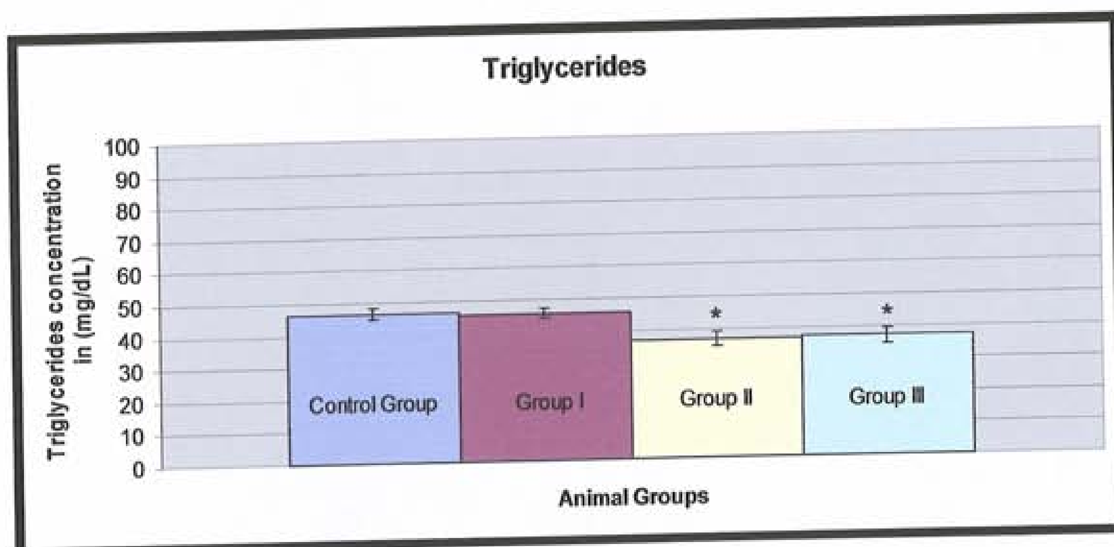


Fig. 3.7: Serum Triglycerides levels after one month of in-vivo treatment with ***U. Dioica*** seed water extract in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10). * *Significant difference (<0.05) with respect to the control.*

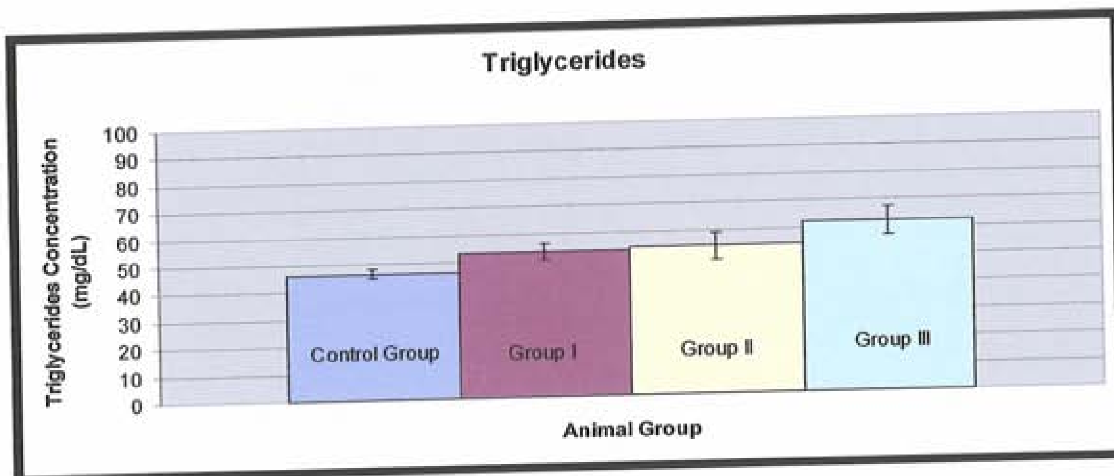


Fig. 3.8: Serum Triglycerides levels after one month of in-vivo treatment with ***U. Dioica*** seed polyphenols extract in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10).

3.1.5. Serum glucose levels

The fasting plasma glucose concentrations of the Sprague-Dawely male rats after one month of treatment with water extract of *U. Dioica* was gradually decreasing compared with the control group (Fig. 3.9). However, the decrease in the concentration reached significance only at the highest dose used.

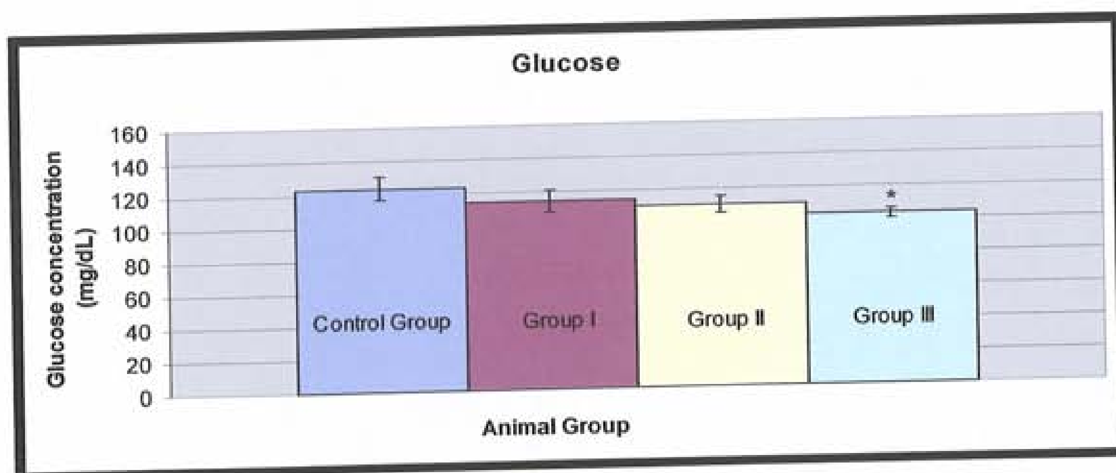


Fig. 3.9: Fasting blood glucose levels after one month of in-vivo treatment with ***U. Dioica*** seed water extract in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10). * *Significant difference (<0.05) with respect to the control.*

Similarly, the fasting plasma glucose concentrations of the Sprague-Dawely male rats after one month of treatment with polyphenols extract of *U. Dioica* approximately remained constant compared with the control group, then it decreases to a significant level with high dose (Fig. 3.10).

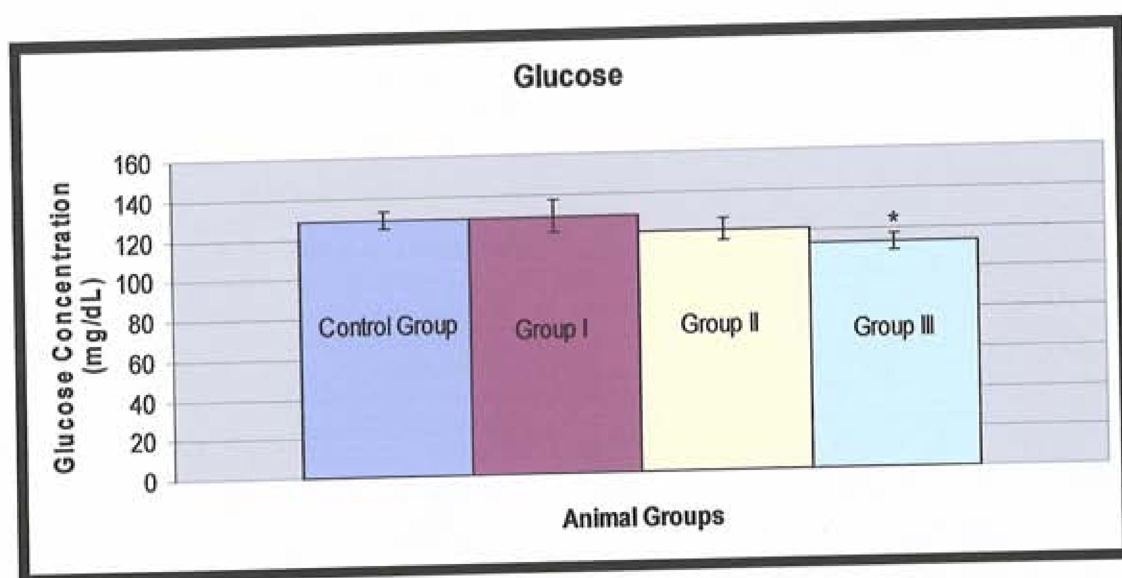


Fig. 3.10: Fasting blood glucose levels after one month of in-vivo treatment with *U. Dioica* seed polyphenols extract in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10). * *Significant difference (<0.05) with respect to the control.*

3.1.6. Insulin levels

Serum insulin levels of the different groups were measured on the basis of ELIZA method. Data presented in figure 3.11 showed that there were no significance changes among the different groups but slight decreasing values with the water extracts of ***U. Dioica*** seeds.

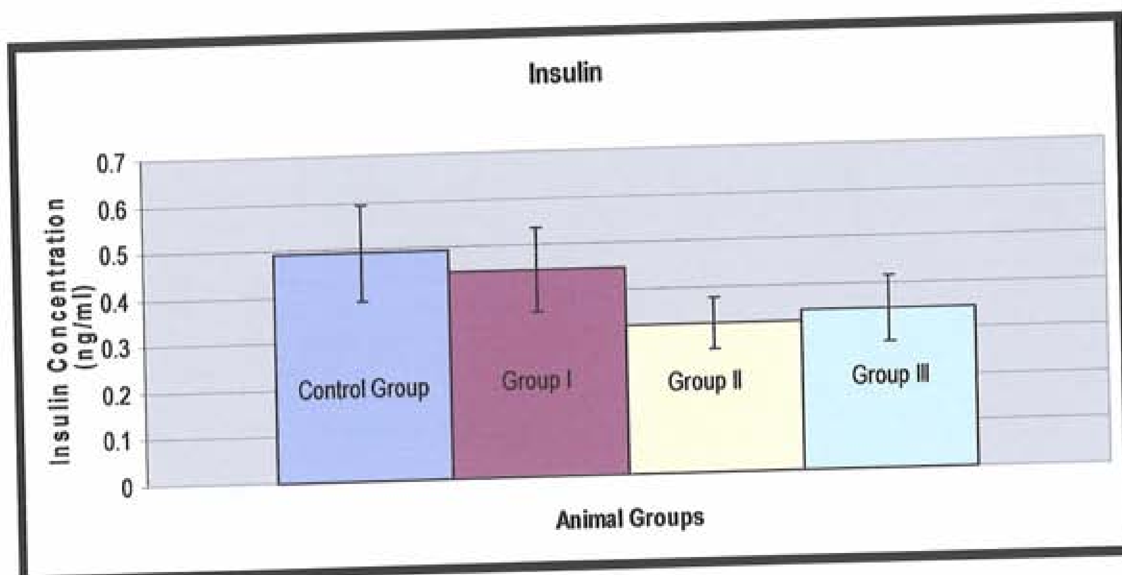


Figure 3.11: Fasting serum insulin levels after one month of in-vivo treatment with water extract of *U. Dioica* in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10).

On the other hand, Serum insulin levels of the different groups were measured on the same ELIZA technique. Data presented in figure 3.12 showed that there were no significance changes among the different groups but slight decreasing values with the water extracts of *U. Dioica* seeds.

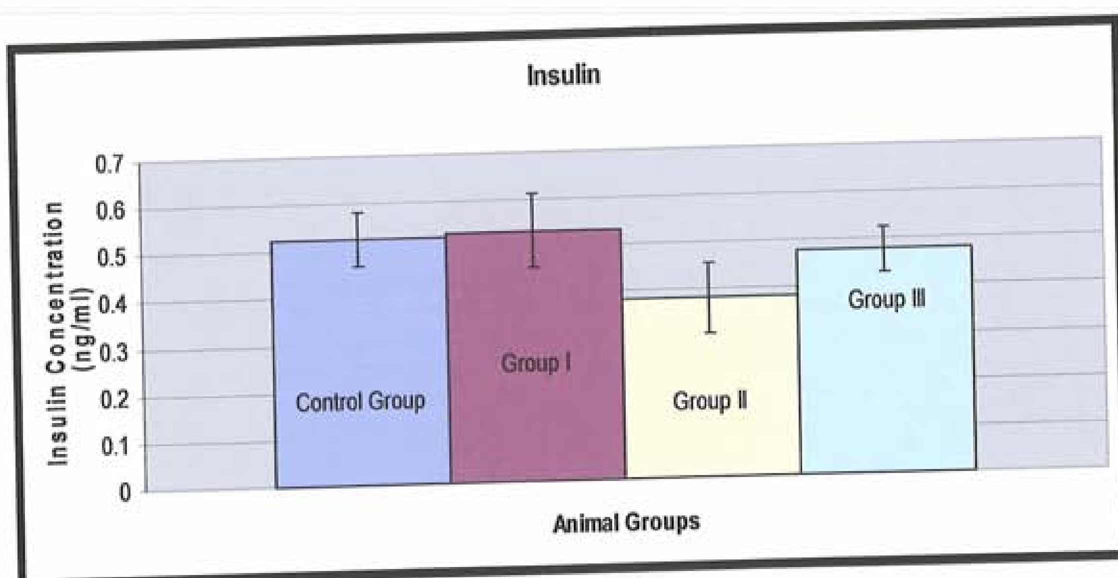


Figure 3.12: Fasting serum insulin levels after one month of in-vivo treatment with water extract of *U. Dioica* in rats fed on a high fat diet. Data are presented as mean ± SEM, (n=10).

3.1.7. Liver enzymes

Liver enzymes activities in serum were measured in all groups for both experiments. Table 3.3 illustrates the enzymes activities of SGOT, SGPT for the control and treatment groups of the water extract of *U. Dioica* seeds. The serum levels of AST and ALT in the first experimental group were similar to that of the control one. However they increase slightly in the group II treatment experiments. Thus, serum SGPT levels in Group III gradually continued to increase but not reaching a significant value. On the other hand, serum SGOT levels in Group III was significantly higher than that of the control group.

Table 3.3: SGPT and SGOT activities after one month of water extract of *U. Dioica* seeds in control and treatment groups of rats. Values are expressed as mean ± SEM (n=10).

Liver Enzymes (U/L)	Control group	Treatment Group I (50mg/kg body weight)	Treatment Group II (100mg/kg body weight)	Treatment Group III (250mg/kg body weight)
AST (SGOT)	43.5 ± 1.23	43.8 ± 4.09	48.2 ± 3.68	51.8 ± 2.76*
ALT (SGPT)	29.3 ± 2.41	29.8 ± 16.23	30.5 ± 3.33	33.2 ± 1.90

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

Where as Liver enzymes activities in serum were measured in all groups for polyphenols extracted from *U. Dioica* seeds. Table 3.4 illustrates the enzymes activities of SGOT, SGPT for the control and the treatment groups. The serum levels

of AST and ALT in all the experimental groups showed similarities to that of the control one inspite of some non significant variations.

Table 3.4: SGPT and SGOT activities after one month of polyphenols extract of *U. Dioica* seeds in control and treatment groups of rats. Values are expressed as mean ± SEM (n=10).

Liver Enzymes (U/L)	**Control group**	**Treatment Group I (50mg/kg body weight)**	**Treatment Group II (100mg/kg body weight)**	**Treatment Group III (250mg/kg body weight)**
AST (SGOT)	49.8 ± 2.05	39.5 ± 13.21	42.2 ± 3.42	49.7 ± 9.22
ALT (SGPT)	33.1 ± 6.83	26.7 ± 3.26	27.6 ± 2.80	29.2 ± 8.77

3.2. Ulcer

The effect of aqueous and polyphenols extracts of *U. Dioica* seeds on ethanol induced gastric ulcer was determined by measuring both long and small lesions in the stomach glandular region epithelium. Different doses of *U. Dioica* seeds water extract showed resulted in highly significant anti-ulcerative effect with respect to the control group and even higher than that of Cimetidine (anti-ulcer drug) where the 250 mg/kg body weight dose produced the most protective effect. Ulcer index and curative ratios are presented in Table 3.5.

Table 3.5: Effect of water extract of *U. Dioica* seeds on ethanol induced gastric damage in rats.

Treatment Groups	Ulcer Index (mm)	Curative Ratio (%)
Control Group	6.02 ± 0.80	-
Cimetidine Group	3.97 ± 0.33	34.1 %
Group I (50mg/kgb.w.)	4.47 ± 0.82	25.8 %
Group II (100mg/kgb.w.)	2.28 ± 0.40*	62.0 %
Group III (250mg/kgb.w.)	1.67 ± 0.43*	72.3 %

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

On the other hand, animals treated with doses of *U. Dioica* seeds polyphenols extract presented also significant results in anti-ulcerative effect with respect to the control group and even higher than that of Cimetidine (anti-ulcer medication) where the 250 mg/kg body weight dose produced the most protective effect. Ulcer index and curative ratios are presented in Table 3.6. Ulcerated and non-ulcerated stomachs are shown in figure 3.13

Table 3.6: Effect of polyphenols extract of *U. Dioica* seeds on ethanol induced gastric damage in rats.

Treatment Groups	Ulcer Index (mm)	Curative Ratio (%)
Control Group	6.02 ± 0.80	-
Cimetidine Group	3.97 ± 0.33	34.1 %
Group I (50mg/kgb.w.)	4.58 ± 0.54	23.8 %
Group II (100mg/kgb.w.)	2.70 ± 0.62*	55.1 %
Group III (250mg/kgb.w.)	2.37 ± 0.88*	60.7 %

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

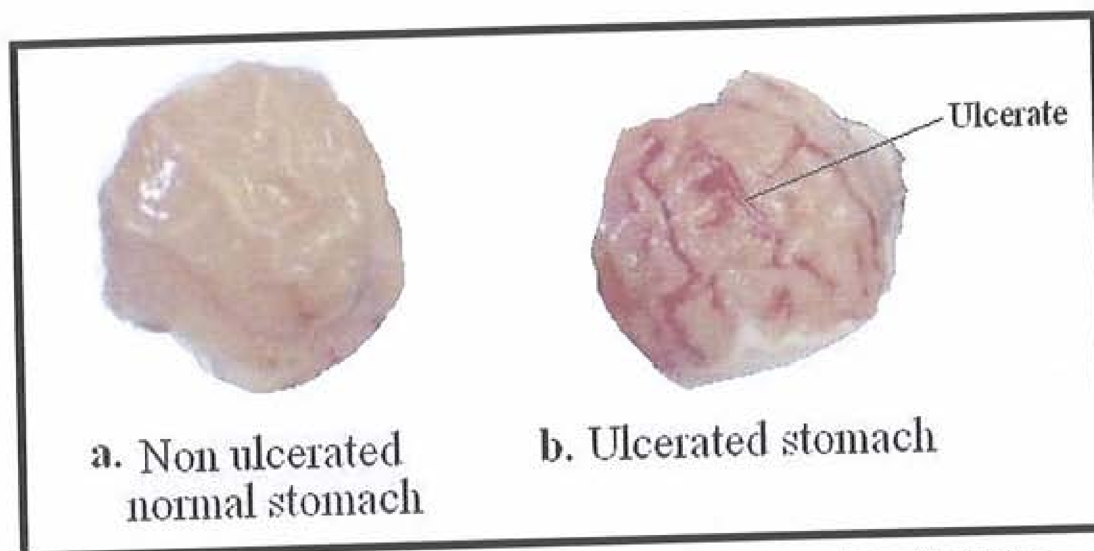


Figure 3.13: Ulcer lesions as shown in the treatment groups receiving *U. Dioica* extracts where a: non-ulcerated normal stomach, b: ulcerated stomach where ulcers were induced by 50% ethanol solution in absence of any protective treatment.

3.3. Anti-inflammatory Effect

In acute inflammation, water extract of *U. Dioica* seeds was able to significantly inhibit it. On the other hand, also the water extract was able to significantly inhibit chronic inflammation in all of the treatment groups. Results are shown in tables 3.7 and 3.8. However, in acute inflammation, polyphenols extracted from ***U. Dioica*** seeds was able to significantly inhibit it in Group I only. Whereas, the polyphenols extract was not able to inflammation in Group II and it increased it in Group III. Also the polyphenols extract was able to inhibit chronic inflammation but not significantly in Group I only. While it increased inflammation in Groups II and III. Results are shown in tables 3.9 and 3.10.

Table 3.7: Effect of water extract of *U. Dioica* seeds on carrageenan induced acute inflammation.

Treatment Groups	**Increase in paw thickness after 3 hours(mm)**	**Percentage of inhibition**
Control Group	1.66 ± 0.09	-
Diclofinac Group	0.49 ± 0.06*	70.49 %
Group I (50mg/kgb.w.)	0.53 ± 0.09*	67.77 %
Group II (100mg/kgb.w.)	0.52 ± 0.11*	68.78 %
Group III (250mg/kgb.w.)	0.43 ± 0.06*	73.82 %

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

Table 3.8: Effect of water extract of *U. Dioica* seeds on formalin induced chronic inflammation.

Treatment Groups	**Increase in paw thickness after 6 days(mm)**	**Percentage of inhibition**
Control Group	1.84 ± 0.39	-
Diclofinac Group	0.56 ± 0.06*	69.63 %
Group I (50mg/kgb.w.)	1.46 ± 0.20	20.67 %
Group II (100mg/kgb.w.)	0.72 ± 0.14*	60.83 %
Group III (250mg/kgb.w.)	0.58 ± 0.17*	68.63 %

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

Table 3.9: Effect of polyphenols extract of *U. Dioica* seeds on carrageenan induced acute inflammation.

Treatment Groups	**Increase in paw thickness after 3 hours(mm)**	**Percentage of inhibition**
Control Group	1.14 ± 0.12	-
Diclofinac Group	0.35 ± 0.05	69.25%
Group I (50mg/kgb.w.)	0.50 ± 0.13	56.37%
Group II (100mg/kgb.w.)	1.10 ± 0.11	3.37%
Group III (250mg/kgb.w.)	1.52 ± 0.18	-33.24%

Table 3.10: Effect of polyphenols extract of *U. Dioica* seeds on formalin induced chronic inflammation.

Treatment Groups	Increase in paw thickness after 6 days(mm)	Percentage of inhibition
Control Group	1.35 ± 0.35	-
Diclofinac Group	0.38 ± 0.09	71.98 %
Group I (50mg/kgb.w.)	1.10 ± 0.19	18.89 %
Group II (100mg/kgb.w.)	1.42 ± 0.28	-4.94 %
Group III (250mg/kgb.w.)	2.70 ± 0.14	-99.88 %

Chapter IV

DISCUSSION AND CONCLUSION

The present study focuses on assessing the effects of a six weeks period of consumption of carob molasses on blood lipid profile, glycemia and liver enzymes. Fecal Cholesterol, TAG, and water content were determined in order to assess possible secondary effects of the carob molasses on the digestive tract. This study was conducted in the rat model and took into consideration two major types of dietary life-styles; a regular or high-fat diet. The rat model was chosen since chronic human studies are conflicted by differences such as gender, liver function, nutritional factors, smoking, physical activity, and other confounding factors. Therefore, rats as mammals are much easier to control.

In the present student, the long term intake of carob molasses appeared to have no significant effect on the fasting blood lipid profile except for high density lipoprotein (HDL). Serum HDL cholesterol levels were significantly increased in the carob treated rats when either a regular or a high-fat diet was administered to the animals. The increase in HDL cholesterol, also known as the good cholesterol, indicates a cardioprotective role of carob molasses when taken consistently for about a month time. Luckily, the increase in HDL cholesterol was not accompanied with an increase in LDL cholesterol. The LDL/HDL ratios were lower in the carob treated groups if compared with the control groups regardless of the amount of fat in the diet. This further reinforces the benefit of carob molasses as a dietary supplement. Although carob molasses intake appeared

somehow to have a positive role in reducing fasting serum TAG only when the diet is high in fat, such an effect is limited and did not reach significance.

In the present study, carob molasses intake did not appear to affect the total plasma ApoB concentration. Hence, such a finding indicates that carob molasses has no effect on the metabolism of VLDL to LDL and the clearance of the latter molecules from the blood. Also, this reinforces our finding regarding the lack of effect on LDL cholesterol. Previous studies have shown that carob pulp has decreased significantly TAG, total cholesterol, and LDL cholesterol levels (Zunft 2003). The differences in results, if compared with the present study may be attributed to the differences in the constituent of carob molasses and carob pulp. Up to our knowledge no studies have investigated the effect of carob molasses on serum HDL cholesterol levels.

Results have shown no effect of carob molasses on fecal cholesterol in the presence of a regular or a high-fat diet. This indicates that carob molasses intake does not interfere with the absorption of cholesterol in the digestive system. However, carob molasses has increased significantly fecal TAG content when the animals were exposed to a regular fat diet but failed to show this effect when the fat content in the diet was elevated. Therefore, carob molasses seems to have a limited intervention with the absorption of TAG in the gastrointestinal tract, and such an intervention is only effective when the diet is not high in fat.

There are contradictory results on the effect of carob on fecal water content. Leob et al. (1989) and Brown (1996) mentioned that carob tannins have an astringent effect in the gastrointestinal tract making them useful for treating diarrhea. Similar findings were reported when carob powder was administered to children with diarrhea (Batlle and Tous 1997). However, Hostettler et al. (1995) showed that carob intake did not help adults with traveler's diarrhea. The present study did not investigate a pathological condition such as diarrhea, but it covered only normal physiological condition. Data of the present investigation revealed that carob molasses help in increasing the water content in the stools regardless of the fat content in the diet. Therefore, taking carob molasses may help in relieving the mild physiological constipation that many people in the community have. Also, it appeared that animals put on a high fat diet showed relatively more water content in their stools with respect to animals on a regular fat diet. However, increasing water in the stool of normal animal does not allow us to extend our finding and conclude about pathological conditions such as diarrhea since the body may respond differently to herbal medicine under normal and abnormal physiological conditions.

Liver enzymes such serum GOT, GPT, and LDH are used to detect hepato-cellular damage. The activities of these enzymes were assessed in order to determine the liver function and investigate possible hepatotoxic effect that may be exerted by the long term intake of carob molasses along with the diet. Data revealed that carob molasses had no effect on the activity of the liver enzymes (GOT, GPT, and LDH) tested when either a regular or a high-fat diets is lead by the animals. Although no previous studies have dealt

with such an effect to compare with, it appeared that carob molasses are safe to consume at the dose used and no hepato-cellular damage is observed. On the other hand, determination of the percentage fat content in the liver further confirms the safety of carob molasses intake.

In addition to the fasting blood lipid profile, the present study investigated the fasting glycemic profile in order to observe possible side effects that may appear following the long term intake of carob molasses, a sugar rich food. According to the results obtained the serum glucose concentration in both experimental groups of regular and high fat diets has slightly increased, but it was far from reaching significance. Determination of serum insulin concentration showed similar fasting serum insulin level in the different groups regardless of the fat content in the diet. Therefore, in spite of the high sugar content of carob molasses the glycemic profile was not badly affected in the normal rat tested. It is worth investigating in the future the glycemic profile when diabetic animals are used.

The postprandial lipid profile when carob molasses is taken along with a lipid load was also investigated in the present study. Data revealed that the presence of carob molasses reduced significantly the postprandial serum TAG concentration. The decrease in serum TAG is attributed to a decrease in chylomicron TAG rather than VLDL TAG. Chylomicrons are the initial form of lipoprotein generated from exogenous dietary lipids (Groff and Gropper, 2000). Consequently, carob molasses seems to affect either the rate at which TAG is absorbed by the intestinal enterocytes or it may be helping in speeding up the lipoprotein lipase activity and consequently the clearance of TAG from the blood.

Further, studies are needed to find out which mechanism is being involved. Whatever the mechanism is, the intake of carob molasses along with a lipid load appears to help avoiding tremendous increase in serum TAG postprandially. Also, carob molasses intake appeared to reduce significantly chylomicron cholesterol. Such a decrease in chylomicron cholesterol resulted only in a mild decrease in serum cholesterol. This is attributed to the fact that chylomicrons carry only a limited amount of cholesterol in the blood unlike LDL, HDL and VLDL.

In conclusion, a six week period of consumption of carob molasses in rats subjected to a regular or high-fat diet appeared to have a cardioprotective effect since a significant increase in fasting HDL-Cholesterol was. HDL-cholesterol nowadays is considered a very important risk factor in assessing cardiovascular problems since it plays a profound role in the protection against atherosclerosis and thus cardiovascular disease. Also, carob molasses helps in reducing postprandial triglyceridemia that occurs when a fat load is ingested and help in reducing the effectiveness of TAG absorption by the gastrointestinal tract. In addition, carob molasses appeared to have a laxative effect and can be used as a remedy against mild physiological constipations. No secondary harmful effects were observed in the present study. Finally, it can be interpreted that carob molasses has a positive effect on atherosclerosis, it does not cause hepatotoxicity, it has a laxative effect, and can be taken on a regular basis as a dietary supplement or with a meal to reduce high postprandial triglyceridemia.

Chapter V

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