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***Tragopogon porrifolius* water extract: a potential remedy for  
hyperlipidemia, hepatotoxicity, inflammation and gastric ulcer**

By

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### ABSTRACT

#### ***Tragopogon porrifolius* water extract: a potential remedy for hyperlipidemia, hepatotoxicity, Inflammation and Gastric ulcer**

*Tragopogon porrifolius* (Purple salsify) is commonly known as the oyster plant or vegetable oyster. It is cultivated for its florid flower, edible root and shoot, and herbal qualities. The present research project investigates the effects of the water extract of *Tragopogon porrifolius* shoot upon lipemia, glycemia, inflammation, oxidative stress, hepatotoxicity and gastric ulcer using a rat model. After one month of *Tragopogon porrifolius* water extract intake (50, 100 and 250 mg/kg body weight) via drinking water, animals exhibited a significant decrease in the levels of serum cholesterol, triglyceride, glucose and liver enzyme (ALP, ALT, LDH). Pretreatment of the rats with the extract at 50, 100 and 250 mg/kg body weight demonstrated strong anti-inflammatory effects in both acute (51, 82, 92%) and chronic (18, 59, 85%) inflammation induced by carrageenan and formalin respectively. The extract also showed effective anti-ulcerogenic property (42, 64 and 76%) against ethanol induced gastric ulcer. Consistent dose dependent protection against paracetamol induced toxicity was observed after testing the serum levels of ALT, ALP, AST and LDH. *Tragopogon porrifolius* also revealed effective anti-oxidant capacity due to its notable scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (81%). In conclusion, it can be inferred that *Tragopogon porrifolius* provides a potential source of therapeutic herbal supplements.

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## GLOSSARY

**ALT:** Alanine Aminotransferase

**ALP:** Alkaline Phosphatase

**AST:** Aspartate Aminotransferase

**LDH:** Lactate Dehydrogenase

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

**GAE:** Gallic Acid Equivalent

**HDL:** High Density Lipoprotein

**LDL:** Low Density Lipoprotein

**OD:** Optical Density

**TAG:** Triglyceride

**VLDL:** Very Low Density Lipoprotein

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

**SGPT:** Serum- Glutamic Oxaloacetic- Transaminase

**NSAID:** Non-steroidal Anti-Inflammatory Drugs

**ROS:** Reactive Oxygen Species

**FAD:** Flavin Adenine Dinucleotide

**FMN:** Flavin Mononucleotide

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To you mom I dedicate this thesis along with my past, present and future achievements...



## **Chapter 1**

### **Introduction**

The plant kingdom is known to be a powerful source of benefits to mankind in numerous aspects especially herbal therapy (Saad et al. 2005). Records of ancient civilization in all parts of the world reveal that the curative capabilities of plant species have made a substantial contribution in the foundation and evolution of modern medicine (Kala et al. 2006). Until present times, herbal-derived substances constitute the foundation of a large proportion of current synthetic medications used for the treatment of heart disease, hypertension, pain, asthma and other illnesses (Saad et al. 2005). The significant reawakening of interest in medicinal plants which was witnessed recently can be attributed to many factors, predominantly: cost of obtaining synthetic drugs, their inappropriate supplies, the undesired adverse effects caused and the traditional faith in natural remedy (Magaji et al. 2008). Furthermore, the dependence of herbal therapy has increased in under-privileged societies where people cannot afford pharmaceuticals and therefore, rely on natural supplements to treat undesirable conditions such as inflammation (Kala et al. 2006). The therapeutic potential of a plant is primarily ascribed to its curative bioactive constituents (Azaizeh et al. 2006). Substances such as alkaloids, polyphenols, and saponins are among the most important bioactive ingredients present in plants (Azaizeh et al. 2006). Accordingly, there exist a linear correlation between the medicinal effect of a certain plant and its content of potentially curative ingredients (Saad et al. 2006). For example the anti-oxidation effect of plants is accredited by the present quantities of polyphenols, implying that plants with higher concentrations of phenols are better anti-oxidants (Kala et al. 2006).

Commonly, it is well established that undesired adverse effects are properties of synthetic pharmacology much more than natural medicine; however this does not imply that herbal remedies are totally safe and healthy (Saad et al. 2006). One should be extremely careful when dealing with herbal remedies to avoid the toxicity and undesired side effects generated by the synergistic result of chemical interaction between the different active compounds present in plants (Saad et al. 2006).

Complementary and Alternative Medicine (CAM), predominantly plant therapy is popular all over the world however it has been well documented that the Mediterranean region possesses a distinguished inventory of beneficial medicinal plants which contributed efficiently to the rational development of the use of herbal drugs in this geographical area (Azaizeh et al. 2006). Based on historical and recent surveys, there exist among the rich natural inventory of the Mediterranean region, more than 700 plants with medicinal properties (Saad et al. 2005). This fact aroused the interest of ethnopharmacologists in the Mediterranean region and urged them to invest this extensive availability of medicinal herbs in the development of natural remedies against various diseases and health conditions (Saad et al. 2005).

*Tragopogon porrifolius* is a member of the family: Asteracea. It is commonly known as Purple salsify; Oyster Plant; or Vegetable Oyster (Stephens 1994). "Tragopogon" is derived from the Latin word "tragos" meaning goat and "pogon" meaning beard; "porrifolius" means 'with leaves like a leek' (Davidson 1999).

The plant's common name "Salsify" originates from the old Latin name 'solsequium' in reference to the way the flowers follow the course of the sun throughout the day (Salsify, <http://www.innvista.com>). In the 13th century, salsify was harvested from the wild in Europe; however it was

not cultivated until the early 16th century in Italian gardens (Stephens 1994). Purple Salsify is an admired biennial and wild flower, indigenous to Mediterranean regions but cultivated in different areas of the world (Davidson 1999).

Currently it has been found growing wild everywhere in America except in the extreme south-east (Salsify, <http://www.innvista.com>). Its lengthy, meager, sharp, soft, and flat leaves are 10-12 inches long. The roots are usually long, cylindrical and brown in color. Older roots contain a milky white sap. The stem is mostly unbanked (Salsify, <http://www.innvista.com>). The flowering period is from June to September, except in warmer areas where it can flower starting April (Stephens 1994). The flower head is about 5cm crossways, enclosed by green bracts which are longer than the petals. The flowers are hermaphrodite, and pollination occurs by the help insects. The edible roots and shoots of Purple Salsify urged people to cultivate this plant worldwide (Davidson 1999). Cultivation started in Paris and Rome at the beginning of the 16th century. In Great Britain it was simply grown for its ornamental flower until its edible properties were discovered; nowadays it is a popular often grown vegetable. It is not known as a food item in the United States. Italians, French and Russians are known as frequent cultivators and consumers of *Tragopogon porrifolius* roots. People who tasted the plant claim that the root have an oyster-like taste, which explains the source of the plant's alternative name (Salsify, <http://www.innvista.com>). In all cases, it is recommended to eat the plant directly after it has been harvested because it will taste much sweeter than when it has been stored for a period of time. Preferably young roots are used in salads while older roots are better used cooked in soups or stews (Stephens 1994).

Latex-containing parts of the plant's roots can be used as a chewing gum. The flowering shoots, similar to asparagus, can be consumed



either raw or cooked, the flowers and the seeds can be used to flavor different kinds of salads, while the developed seeds can be used in sandwiches just like olives (Stephens 1994). Salsify is known to be rich in numerous valuable nutrients. Specifically it contains high concentrations of pyridoxine, magnesium, fibers, calcium, riboflavin, saponin, folate, inulin and most importantly phenols(Salsify, <http://www.innvista.com>).Inulin is a prebiotic, sugar able to enhance calcium and magnesium absorption, promote beneficial bacterial growth and favor weight loss (Niness 1999). Besides its affluent nutritional contents, many claimed that salsify contributed efficiently to herbal therapy since old times, due to its favorable effects on the liver, ulcer and gall bladder(Salsify, <http://www.innvista.com>). In addition to a potent role in resisting inflammation the stated plant proved to resist fatigue and tolerate anorexia in mice (ZhongguoZhong Yao Za Zhi1990).



Figure 1.1: Purple Salsify scientific classification

## Purple Salsify



### Scientific classification

Kingdom: Plantae  
Division: Magnoliophyta  
Class: Magnoliopsida  
Order: Asterales  
Family: Asteraceae  
Genus: *Tragopogon*  
Species: *Tragopogon porrifolius*

### Binomial name

*Tragopogon porrifolius*  
L.

<http://plants.usda.gov/java/profile?symbol=TRPO>

## 1.1. Gastric Ulcer

Peptic ulcer disease (PUD) is a multi-factorial disease that frequently targets the gastrointestinal (GI) tract causing lesions in the gut lining of the esophagus, stomach, or duodenum (Shokunbi et al. 2008). Peptic ulcer of the stomach, which is also termed gastric ulcer, causes gastritis which is known as the inflammatory response that causes open lesions of 3 mm or more in the stomach (Filaretova et al. 2007). Gastric ulcer develops when damaging aggressive factors defeat protective defending factors in our stomach (Bansal et al. 2009). Aggressive factors are numerous and comprise: infective agents such as bacteria and parasites; as well as irritation-producing substances such as non-steroidal anti-inflammatory drug NSAIDs, tobacco, alcohol, caffeine, acidic beverages and stomach acids (Bansal et al. 2009). The two leading causes of gastric ulcer are: *Helicobacter pylori* and NSAID (Bansal et al. 2009). NSAIDs lead to gastric ulcer by inhibiting the synthesis of prostaglandins, which are essential for preserving the integrity of gastric mucosa and triggering the secretion of mucous and bicarbonate (Chungag et al. 2009). Ethanol, which is often used to induce gastric ulcer, causes gastric mucosal injury by enhancing the production of reactive oxygen species and lipid peroxidation in the gastric mucosa (Shokunbi et al. 2008). Treating gastric ulcer is mainly dependent on its underlying cause. For example to treat *H. pylori* gastritis and ulcers, a "Triple therapy" procedure including two antibiotics and a proton pump inhibitor to decrease acid production is usually being followed (Bansal et al. 2009). Whereas to treat non-*H. pylori* gastritis various drugs are being used such as; Antacid to aid in the digestion process and relief heartburn, H<sub>2</sub> blockers and proton-pump inhibitors to reduce the production and secretion of gastric acid (Bansal et al. 2009). In addition to effective treatment, following an adequate diet free of disturbing acids and fats, rich in water, vitamins,

minerals, and anti-oxidants, will most definitely help in reducing pain and alleviating the symptoms of gastritis (Filaretova et al. 2007). Various pharmaceutical interventions proved to be highly efficient in the treatment of gastric ulcer however their positive therapeutic effects were undermined by their negative side effects. Based on this fact, light is being shed on herbal drugs nowadays as a source of cure for gastric ulcer, especially that herbal medicine is known for its potential curative capability in addition to its relatively limited damaging possibility with respect to synthetic medicine (Bansal et al. 2009). Several plants proved to be highly effective against gastric ulcer (Bansal et al., 2009), and act as an incentive for the future discovery of new herbal gastro-protective remedies. In the following study ethanol toxicity was used to assess the effect of the plant on gastric ulcer; however it should be noted that other assays such as acetaminophen and carbon-tetrachloride toxicity may be performed as further confirmation of the gastro-protective role of plants.

## **1.2. Inflammation**

Inflammation is a local protective reaction produced by living tissues in response to infection and injury (Chungag et al. 2009). This reaction can either be acute or chronic. While acute inflammation is characterized by a rapid onset during which vascular and exudative processes predominate; chronic inflammation is mainly characterized by the formation of new connective tissue in response to prolonged inflammatory reactions (Gupta et al. 2006). The inflammatory response is produced by the body as a defending mechanism that mainly serves to demolish or weaken both the injurious agent and the injured tissues (Paschapur et al. 2008). This defending mechanism takes place in various diseases such as arthritis, diabetes, cancer and atherosclerosis (Burk et al. 2010).



Inflammation predominantly involves a localized increase in the number of white blood cells in addition to the formation of oedema and granuloma (Paschapur et al. 2008). The inflammatory reaction is usually coupled with several undesired symptoms such as: pain, fever, swelling and loss of function which have urged specialist throughout the years to invest great efforts in the progress of anti-inflammatory drugs (Londonkar et al. 2010). These sign and symptoms are usually caused by potent inflammatory mediators such as prostaglandins and nitric oxide (Gupta et al. 2006). Mainly inflammation is treated using two types of drugs: steroidal and non-steroidal. Steroidal drugs are specific in their mode of action but exhibit several side-effect; non-steroidal drugs produce fewer side-effect but are less specific (Burk et al. 2010). Accordingly, continuous hard work is being employed in order to find an anti-inflammatory drug with adequate mode of action, however resulting in less adverse effects (Burk et al. 2010). In this study both acute and chronic inflammation were induced using carrageenan and formalin respectively, in order to study the anti-inflammatory potential of *Tragopogonporrifolius*.

### **1.3. Hepatotoxicity**

The liver is a highly important organ involved in numerous processes essential for life. The importance of the liver is predominantly attributed to its indispensable role in metabolism and detoxification (Li et al. 2010). Hepatic injury develops when the liver fails to protect the body against exogenous or endogenous damaging factors such as drugs, toxins, and alcohol (Setty et al. 2006). Liver damage is indicated by a significant elevation in the serum levels of several biochemical markers such as SGOT, SGPT, LDH, AST, triglycerides, cholesterol, and bilirubin; it can also be characterized by different factors like a decrease in tissue GSH levels, an increase in tissue lipid peroxidation or cellular necrosis (Li

et al. 2010). Sarcasically, the functional role of the liver is one of the major causes of hepatic disease which results from the death of hepatocytes due to extensive oxidative stress resulting from the detoxification of xenobiotics(Li et al. 2010). Although our cells contain various anti-oxidant agents, sometimes this endogenous mechanism fail to overpower severely imposed oxidative stress. In such cases, an efficient exogenous source of anti-oxidants is urgently needed to help the liver survive the resulting oxidative damage (Setty et al. 2006). Since long time ago plants are known to be rich in natural antioxidants such as phenols, saponins and flavanoids (Samudram et al. 2009). Accordingly, the hepatoprotective ability of *Tragopogonporrifolius* was tested by challenging the rats under study with a toxic dose of paracetamol.

#### **1.4. Lipid Profile**

Lipids are oily non-polar organic compounds that are soluble only in non-polar solvents. They are essential components of biological membranes, and important constituents of hormones, coenzymes, transporters and detergents (Vance and Vance 2004). They play a key role in numerous biological functions favoring cell growth and energy supply (Mehrota et al. 2009). A lipid profile measures total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides in order to assess the relative risk correlating it with certain diseases that show elevated blood lipid concentrations (Chahlia 2009).

Cholesterol is an amphipathic lipid present mainly in cell membranes and in outer layers of plasma lipoproteins (Mehrota et al. 2009). It plays an important role in cellular uptake and in preserving the integrity of cell membranes (Augusti et al. 2008). It exists in the body either as free cholesterol or as cholesteryl ester. Free cholesterol is transported through the blood bound to lipoproteins (LDL, HDL and VLDL) while

cholesteryl ester is found in biological membranes bound to long chains of fatty acids (Mehrota et al. 2009). LDL is known as the "bad cholesterol" because it transports cholesterol to the walls of blood vessels causing vascular damage (Oluwatosin et al. 2008). HDL Cholesterol is known as the "good cholesterol" because it transports cholesterol to the liver for harmless excretion (Giri et al. 2009). Regulation of the cholesterol in the blood is a complex process involving both endogenous and exogenous factors. Endogenously, cholesterol level is regulated by the liver and the lipoprotein receptors (Zulhairi et al. 2009). Exogenously, it is mainly regulated by diet and exercise (Oluwatosin et al. 2008). Enhanced activity of Lipoprotein receptors serve to reduce cholesterol levels in the blood by favoring its cellular uptake (Giri et al. 2009); accordingly great importance is attributed to LDL especially that cells sequester cholesterol predominantly from the mentioned lipoproteins (Oluwatosin et al. 2008). Hypercholesterolemia, which is a condition involving increased levels of cholesterol in the blood, is strongly correlated with an increased risk for cardiovascular diseases (CVD) such as atherosclerosis and myocardial infarction (Giri et al. 2009). Specifically, LDL cholesterol is highly correlated with CVD since it allows cholesterol to form atherosclerotic plaque on the walls of blood vessels causing the blockage of these vessels (Oluwatosin et al. 2008). Oppositely HDL cholesterol plays a protective role against atherosclerosis since it favors the transport of cholesterol from various body tissues to the liver for excretion, reducing the risks for CVD (Oluwatosin et al. 2008). Based on the high mortality and morbidity rates caused by CVD, extensive efforts have been put in order to find an adequate life-saving treatment (Chahlia 2009). However the various synthetic lipid lowering drugs discovered to date exhibit numerous undesired side-effect (Giri et al. 2009). Accordingly, specialists continuously seek potential lipid-lowering therapy conferring relatively less side-effect within the plant kingdom.



Triglycerides are lipids made up of one glycerol molecule attached to three fatty acids molecules. They represent the stored form of fat that constitutes the largest portion of the adipose tissue, VLDL and chylomicrons (Hayek et al. 1993). The long chains of high energy fatty acids present in triglyceride provide most of the energy required for normal cell function. A triglyceride level in the blood is affected by both external factors such as diet and internal factors such as the hepatic control over triglyceride synthesis. The excessive intake of alcohol and animal fats proved to cause a significant increase in the blood level of triglyceride (Goyal et al. 2003). Contraceptive pills and hormone-containing drugs might cause a moderate increase in triglyceride levels in some individuals. Very high levels are observed in metabolic syndrome which is considered as an important risk factor in CVD, diabetes and stroke (Goyal et al. 2003). Metabolic syndrome is the combination of high blood pressure, high blood sugar, low HDL and elevated amount of stored fat (Oluwatosin et al. 2008). Numerous studies were conducted in order to prove the efficacy of natural supplements in restoring normal blood lipid levels.

### **1.5. Glycemia**

Diabetes, which is a chronic metabolic disorder, is clinically indicated by hyperglycemia and hyperlipidemia (Chatterjee et al. 2009). Hyperglycemia, a condition characterized by elevated blood glucose level, result in serious complications like cancer, cardiovascular diseases and brain strokes (Liu et al. 2009). One of the main causes underlying the increased glucose level in diabetes is the decreased plasma insulin level (Chatterjee et al. 2009). In addition to controlling blood glucose levels, insulin also controls the activity of the enzymes regulating cholesterol production and lipolysis which explains the mutual existence of hyperglycemia and hyperlipidemia in diabetes



(Zhou et al. 2009). Various drugs are implicated in the treatment of diabetes such as: insulin, oligosaccharides, thiazalidinedimes, sulfonylureas and biguanides (Liu et al. 2009). Nevertheless, the undesired side effects of some of these drugs undermine their therapeutic potential on one hand, and empowered Herbal therapy on the other hand (Liu et al. 2009). Several plants proved to be effective against diabetes namely: *Tamarindusindica*, *Eugenia jambolana*, and *Cocciniaindica* (Chatterjee et al. 2009).

#### **1.6. Phenolic Content**

Phenolic compounds are secondary plant metabolites that proved to be effective antioxidative, anticarcinogenic, anti-hepatotoxic, anti-allergic, anti-ulcerative and anti-inflammatory agents (Alizadeh et al. 2009). Their abundant presence in the plants explained the increasing interest of specialists in screening the plant kingdom for effective natural remedies especially that natural supplements exhibit limited side effects when compared to synthetic drugs (Sharma et al. 2009). The anti-oxidant effect of phenolic compounds is mainly attributed to their free radical scavenging activities in addition to their ability to prevent the initiation and propagation of oxidative chain reactions (Hasan et al. 2009). Among the existing phenolic compounds flavonoids, flavonols and terpenoids were given extra importance due to their electron-rich content and their ability to donate these electrons to reactive oxygen species (ROS) in order to reduce the effect of these damaging chemical agents (Kruawan et al. 2006). In the performed study, the absorbance of the extract was compared to the absorbance of gallic acid standard solutions in order to assess the phenolic content of the plant.

## 1.7. Oxidation

Sarcastically, oxygen which is a fundamental requirement of survival can become under certain circumstances life-threatening (Hasan et al. 2009).

This occurs whenever oxygen forms ROS. ROS include both free and non-free radical species. Oxidative tissue damage is caused by a sequence of oxygen stealing chain reactions initiated by ROS (Zulkhairi et al. 2009). ROS are extremely damaging species that target biological membranes triggering lipid peroxidation which results in the loss of membrane structure and function (Sharma et al. 2009). These products often cause severe cytotoxic and genotoxic results (Kruawan et al. 2006). ROS are frequently representing the underlying cause of various health-threatening conditions such as cardiovascular diseases, cancer and neurodegenerative diseases (Alizadeh et al. 2010). Given the harmful effects of ROS nearly all organisms possess protective mechanisms against free radical attack; however these mechanisms are sometimes overpowered by excessive free radical production (Hasan et al. 2009). In such cases the body relies on exogenous sources of anti-oxidants in order to survive the imposed oxidative damage. The plant kingdom provides a rich source of potent anti-oxidants, with effective free radical scavenging activities, capable of preventing and treating free radical caused diseases (Luis et al. 2009). The anti-oxidative ability of plants is predominantly due to the rich presence of phenolic compounds that are able to neutralize free radicals, decompose peroxides and quench singlet and triplet oxygen (Sharma et al. 2009). There exists a directly proportional relationship between plant's phenolic content and its respective anti-oxidant effect (Luis et al. 2009). Accordingly plants represent a rich source of highly effective potential remedies against oxidative stress and its relevant damaging results. The ability of *Tragopogonporrifoliusto* scavenge reactive oxygen

species was demonstrated by performing a DPPH assay; in which a decrease in the absorbance denote the presence of an anti-oxidant in the plant's water extract. This observed decrease is due to the ability of one of the plant's supplement to act as an anti-oxidant, giving electrons to ROS; thus preventing them from causing oxidative damage.

Aim of the study:

Inspite of the presence of certain claims stating that *Tragopogonporrifoliuse* exhibit favorable effects on liver,ulcer and gall bladder (Salsify, <http://www.innvista.com>); there is no published scientific work dealing with the plant. In addition, in the South of Lebanon the stated plant is commonly consumed in salads and recommended as potential cure for cancer. This study serves to investigate the role of *Tragopogonporrifolius* in hyperlipidemia, hepatotoxicity, inflammation, oxidation, glycemia and gastric ulcer.



## Chapter 2

### Material and Methods

#### Extract Preparation

Shoots of *Tragopogonporrifolius* plants were dried in the shade and then cut into small pieces. To determine the plant water extract yield, 5 g of the dry plant shoot were soaked in 100 mL of pre-boiled water for 20 min. Subsequently, the solution was filtered using Whatman Nr.1 filter paper and the filtrate was first evaporated on a mild flame and then in an oven at 45°C overnight to ensure total water evaporation. Data indicated that every 1g of plant shoots yields 0.06g of dry water extract (6%).

#### Animal treatment

28 Male Sprague-Dawley rats weighing 250-280 g were used. Animals were obtained from the Lebanese American University stock. For a period of one month, rats were put on a diet consisting of standard rat chow in addition to 5 % coconut oil and were given the water extract of *Tragopogonporrifolius* in three different doses (50,100 and 250 mg/kg) via drinking water. They were randomly divided into four groups comprising 7 animals each. Group I represented the control group, Group II, III and IV represented the animals given the plant extract in 50, 100 and 250 mg/kg doses respectively. The purpose of adding 5% coconut oil was to obtain a high fat diet (Khouzami et al. 2009). After one month of extract intake, fasted (18h) animals were sacrificed after being anesthetized using diethyl ether. A midline abdominal incision was made allowing the withdrawal of 8 mL of blood from the inferior vena cava of each rat. The blood was then centrifuged (2000 g; 20 min; 4°C) in order to obtain the serum samples that were used for the analysis of serum lipid levels (TAG, total cholesterol, HDL-cholesterol

and LDL-cholesterol), glucose levels, and liver enzymes (SGOT, SGPT and LDH).

#### **Measurement of serum lipid, glucose, SGPT, LDH, SGOT.**

The serum concentration of total serum cholesterol, TAG, glucose, and liver enzymes SGOT SGPT and LDH were measured according to the SPINREACT kit protocol.

#### **Anti-Inflammatory effect**

Anti-inflammatory activity was assessed by carrageenan-induced acute and formalin-induced chronic paw edema in rats.

##### **(a) Carrageenan induced paw edema in rats:**

Animals were divided into five groups of six animals each. In all groups acute inflammation was produced by a single subplantar injection of 0.02 mL of freshly prepared 1% carrageenan in normal saline in the right hind paw of rats. Group 1 represented the positive control (no treatment), Groups II, III and IV represented the animals receiving the water extract of *T. porrifolius* intraperitoneally (*i.p*) at different concentrations of 50, 100 and 250 respectively 30 min prior to carrageenan injection, Group V received Diclofenac (10mg/Kg BW, *i.p*), as a standard reference drug, 30 min prior to carrageenan injection. The paw thickness was measured using vernier calipers before and 3 hours after carrageenan injection.

##### **(b) Formalin induced paw edema in rats:**

Animals are divided into five groups of six animals each. In all groups, chronic inflammation was produced by a single subplantar injection of 0.02 mL of 2% formalin in the right hind paw (Khouzami et al. 2009).

Thirty minutes prior to formalin injection, Groups I, II and III received the water extract of *Tragopogonporrifolius*(i.p.) at a concentration of 50, 100 and 250 mg/kg BW, Group IV received the standard reference drug Diclofenac (10 mg/ kg BW, ip), and Group V served as a positive control (no treatment). The administration of the extracts and Diclofenac was continued once daily for 6 consecutive days. The paw thickness was measured using vernier calipers before and 6 days after formalin injection (Khouzami et al. 2009).

The increase in paw thickness in both models was calculated using the formula:

$$Pt - P0$$

Where  $P_t$  is the thickness of paw at time  $t$  (that is, 3 h after carrageenan injection and 6 days after formalin injection) and  $P_0$  is the paw thickness at time 0. The percent inhibition was calculated using the formula:

$$(C - T)/C \times 100$$

Where  $C$  is the increase in paw thickness of the positive control and  $T$  is that of treatments.

### **Anti-Ulcerogenic Effect**

The anti-ulcerogenic effect of water extract of *Tragopogonporrifolius* shoots on ethanol induced gastric ulcer was determined in male rats.

Briefly, male rats of an average weight of 275g were randomly divided to 6 groups of 6 rats each. Group I represented the control, group II represented the reference group, groups III, IV and V represented the



groups receiving the three different doses of the water extract (50,100 and 250mg/kg BW)

and group VI represented the rats receiving the reference drug Cimetil (11.5mg/kg BW). Forty-eight hours before use, animals were starved to ensure an empty stomach. Furthermore, they were kept in cages with raised floors of wide wire mesh to prevent coprophagy. To prevent excessive dehydration during starvation, all groups were supplied with sucrose 8% (w/v) in NaCl 0.2% (w/v) which was removed 1 hour before experimentation (Alkofahi and Atta, 1999; Gharzouli, 1999). The Group I (reference group) received 2 mL of distilled water (10mL/kg) as the other groups but had no ulcer induction. Group II (control) received 3mL of distilled water (10 mL/kg). The treatment groups III, IV, V received 4 mL of *Tragopogonporrifolius* water extract in three different doses (50,100 and 250 mg/kg). Group VI received 4 mL of the reference drug Cimetil (Xu et al. 1998). Doses were administrated orally with water via a stainless steel intubation needle. Two doses were given on the first day at 9:00 h and 17:00 h; a third dose was given on the second day 1.5 h before induction of gastric ulceration. To induce gastric ulcer, the control (group II) as well as the treatment groups III, IV and V received by gastric gavage 10 mL/kg body weight ethanol 50% (v/v) in water. One hour after ethanol administration, all animals were sacrificed by an overdose of diethyl ether, stomachs rapidly removed, opened along their greater curvature and rinsed under running tap water. Long lesions were counted and measured along their greater length. Petechial lesions (very small lesions) were also counted and each five were considered as 1 mm of ulcer. The sum of total length of long ulcers and petechial lesions in each group of rats was divided by its number to calculate the ulcer index (mm).

The curative ratio was determined by the formula:



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

### **Anti-Hepatotoxic Effect**

Antihepatotoxic effect was assessed according to the method of Chattopadhyay followed by (Setty et al. 2007). Animals were divided into five groups of 6 animals each. Group 1 received saline 1 mL/kg for one week (control). Group II received saline 1 mL/kg for one week (positive control). The groups III, IV and V received *Tragopogonporrifolius* (50,100 and 250 mg / kg BW) respectively once a day for seven days. On the fifth day, after the administration of the respective treatments, all animals of groups II, III, IV and V received an overdose of paracetamol (2 g/kg) orally. On the seventh day, 2 h post-treatment, blood samples were collected for each rat for the estimation of liver enzymes (AST, ALP, ALT and LDH).

### **Anti-oxidant Effect**

#### **Free radical scavenging activity (DPPH assay):**

As followed by (Kruawan et al. 2006) the antioxidant activity of the water extract was tested using a stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described by Fukumoto and Mazza with some modifications. The extract was allowed to react with DPPH in order to evaluate the free radical scavenging activity.

The activity was monitored by a decrease in an absorbance at 520 nm. An aliquot of 22 µL of the extract or blank reagent (dimethyl sulfoxide, DMSO) or standard Trolox (0.04-1.28 mM in 80% methanol) was added to 200 µL of DPPH in 80% methanol (150 µM) in a 96-well flat-bottom microplate. After incubation at 37°C for 30 min, the absorbance of the solution was read in a microplate reader using a 520 nm filter.

The radical scavenging activity was calculated as a percentage of DPPH scavenging activity using the equation:

$$\% \text{scavenging activity} = 10 \times [1 - (AE/AD)],$$

where AE is the absorbance of the DPPH solution with an extract added, and AD is the absorbance of the DPPH solution with nothing added.

### **Determination of total phenolic compounds**

The total phenolic content of water extracts was determined according to the method described by Kruawan et al. (2006). Briefly, 10  $\mu\text{L}$  of each extract was transferred into a 96-well microplate containing 160  $\mu\text{L}$  of distilled water. After mixing the contents, 10  $\mu\text{L}$  of Folin-Ciocalteu reagent and 20  $\mu\text{L}$  of saturated sodium carbonate solution were added. The plate was mixed well and the absorbances of blue mixtures were recorded at 750 nm using a microplate reader after 30 min incubation. The readings of sample and reagent blanks were subtracted from the reading of reagent with extract. The total phenolic contents were calculated as a gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions (ranging from 25 to 800 mg/mL), and expressed as mg of gallic acid per gram of dry sample.

## Chapter 3

### Results

#### 3.1. Serum Lipid and Glycemic Profile

After one month of receiving the water extract of *Tragopogonporrifolius* (50, 100 and 250 mg/kg body weight) via drinking water, the animals exhibited significant dose-dependent decrease in the serum concentration of glucose, total cholesterol, HDL-cholesterol and triglyceride with respect to the control. Results are shown in Table 3.1.

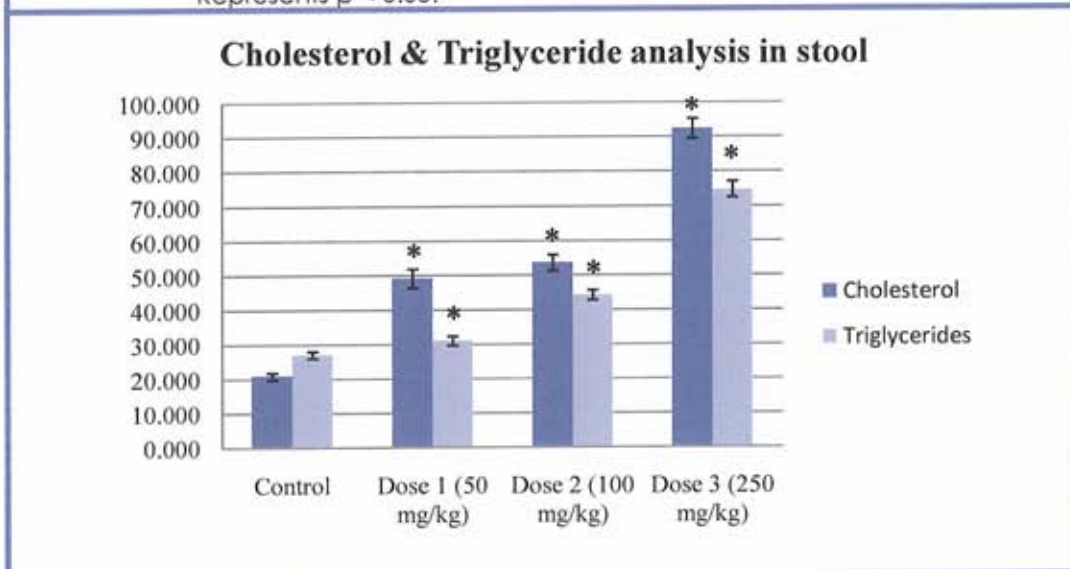
**Table 3.1** Serum concentration of total cholesterol, HDL cholesterol and TAG after 1 month of *Tragopogonporrifolius* water extract administration (50, 100 and 250 mg/kg body weight). Values are expressed as mean  $\pm$  SEM (n = 7)  
\* Represents p < 0.05.

Parameter in Serum	Control Group	Treatment Group		
		Dose 1 (50 mg/kg)	Dose 2 (100 mg/kg)	Dose 3 (250 mg/kg)
Total Cholesterol (mg/dl)	46.9 $\pm$ 1.6	36.2 $\pm$ 0.7*	31.3 $\pm$ 0.7*	23.1 $\pm$ 1.5*
Triglycerides (mg/dl)	109 $\pm$ 2.5	80.2 $\pm$ 2.9*	60.4 $\pm$ 1.3*	49.4 $\pm$ 1.2*
HDL Cholesterol (mg/dl)	39.4 $\pm$ 0.4	42.6 $\pm$ 0.4*	47.5 $\pm$ 0.5*	52.8 $\pm$ 0.6*
Glucose (mg/dl)	119.9 $\pm$ 0.7	111.1 $\pm$ 1.5*	101.4 $\pm$ 0.6*	90.5 $\pm$ 2.1*

### 3.2. Stool Analysis

The concentration of Cholesterol and Triglyceride clearly increased in the stools of animals representing experimental groups I, II and III with respect to the control group (Figure 3.3. Results are shown in Figure 3.1.

**Figure 3.1** Stool concentration of total cholesterol and TAG after one month of *Tragopogon porrifolius* water extract administration (50, 100 and 250 mg/kg body weight). Values are expressed as mean  $\pm$  SEM (n =7) \* Represents  $p < 0.05$ .





### 3.3. Liver Function Tests:

After one month of *Tragopogonporrifolius* water extract intake (50, 100 and 250mg/kg body weight) the serum levels of liver enzymes (ALT, ALP and LDH) were assessed in the experimental groups I, II, and III with respect to the control group. A substantial dose-dependent decrease was noticed in the experimental groups. Results are shown in Table 3.2.

**Table 3.2** Serum levels of hepatic enzymes after 1 month of *Tragopogonporrifolius* water extract administration (50, 100 and 250 mg/kg body weight). Values are expressed as mean  $\pm$  SEM (n = 7) \* Represents  $p < 0.05$ .

Parameter in Serum	Control Group	Treatment Group		
		Dose 1 (50 mg/kg)	Dose 2 (100 mg/kg)	Dose 3 (250 mg/kg)
AST (U/L)	27.6 $\pm$ 1.9	18.6 $\pm$ 0.8*	14.7 $\pm$ 0.6*	10.9 $\pm$ 0.4*
ALT (U/L)	30.6 $\pm$ 1.3	21.2 $\pm$ 0.9*	17.7 $\pm$ 0.3*	13.9 $\pm$ 0.7*
LDH (U/L)	28.3 $\pm$ 0.3	26.2 $\pm$ 0.4*	24.1 $\pm$ 0.4*	22 $\pm$ 0.3*

### 3.4. Anti-Ulcerogenic effect against ethanol induced gastric ulcer:

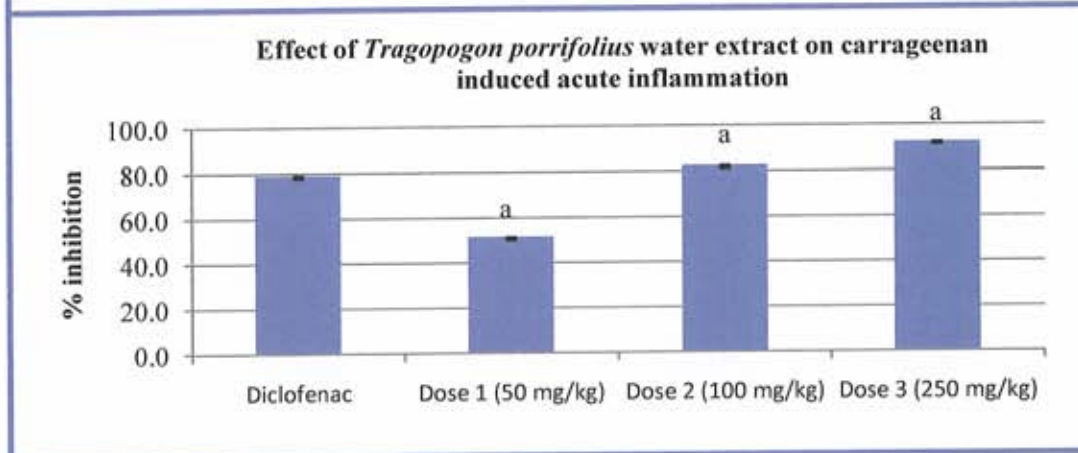
The water extract of *Tragopogonporrifolius* shoots proved to be extremely effective against ethanol induced gastric ulcer. The effectiveness of the extract was demonstrated by measuring long and short lesions in the glandular region of the stomach. The three experimental groups I, II and III revealed potent dose-dependent gastro-protective ability. Results are shown in Table 3.3.

Treatment Group	Ulcer Index (mm)	Curative Ratio (%)
Reference (Group I)	0	0
Control (Group II)	22.5	0
Extract (50 mg/kg) (Group III)	13	42.2*
Extract (100 mg/kg) (Group IV)	8	64.4*
Extract (250 mg/kg) (Group V)	5.3	76.3*
Cimetril (Group VI)	4.3	80.7

### 3.5 Anti-Inflammatory Effect:

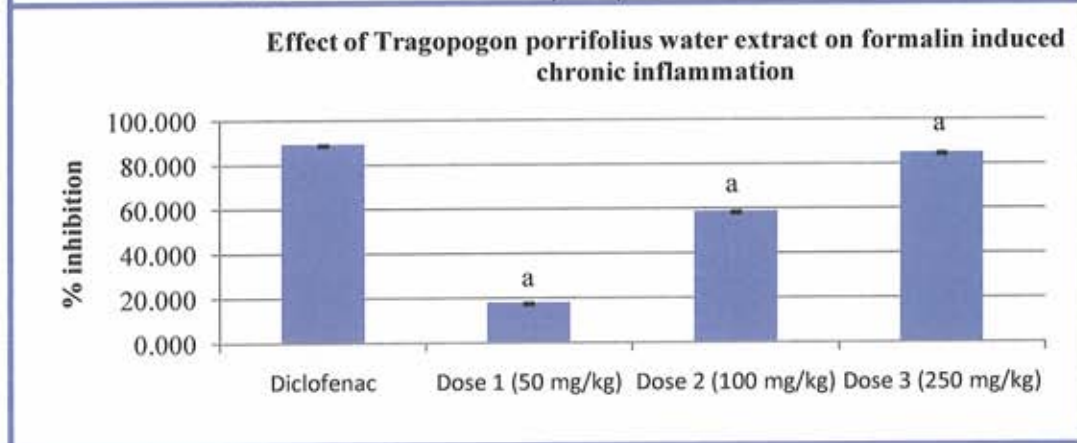
The water extract of *Tragopogonporrifolius* shoots confirmed a strong anti-inflammatory potential against both acute and chronic inflammation. The anti-inflammatory effect was significant in all experimental groups. The rats under study inhibited inflammation in a dose-dependent manner. Results are shown in Figures 3.2 and 3.3.

**Figure 3.2** Diclofenac, *Tragopogonporrifolius* extracts (50mg, 100mg, 250mg) and their percent inhibition on inflammation in carrageenan induced paw edema.  
<sup>a</sup>Significant difference ( $p < 0.05$ ) between extract and non treated control group.  
Values denote mean  $\pm$  SEM ( $n = 7$ ).





**Figure 3.3** Diclofenac, *Tragopogon porrifolius* extracts (50mg, 100mg, 250mg) and their percentage inhibitory rate on inflammation in formalin induced paw-edema.  
<sup>a</sup>Significant difference ( $p < 0.05$ ) between extract and non-treated control group.  
Values denote mean  $\pm$  SEM ( $n = 7$ ).



### 3.6 Anti-Hepatotoxic Effect

The administration of the water extract of *Tragopogonporrifolius* shoots to the experimental rats induced a noticeable dose-dependent decrease in the serum levels of the following liver enzymes: ALT, AST, ALP, and LDH. Therefore, it can be inferred that the aqueous extract of *Tragopogonporrifolius* is effective against paracetamol-induced hepatotoxicity. Results are shown in Table 3.4.

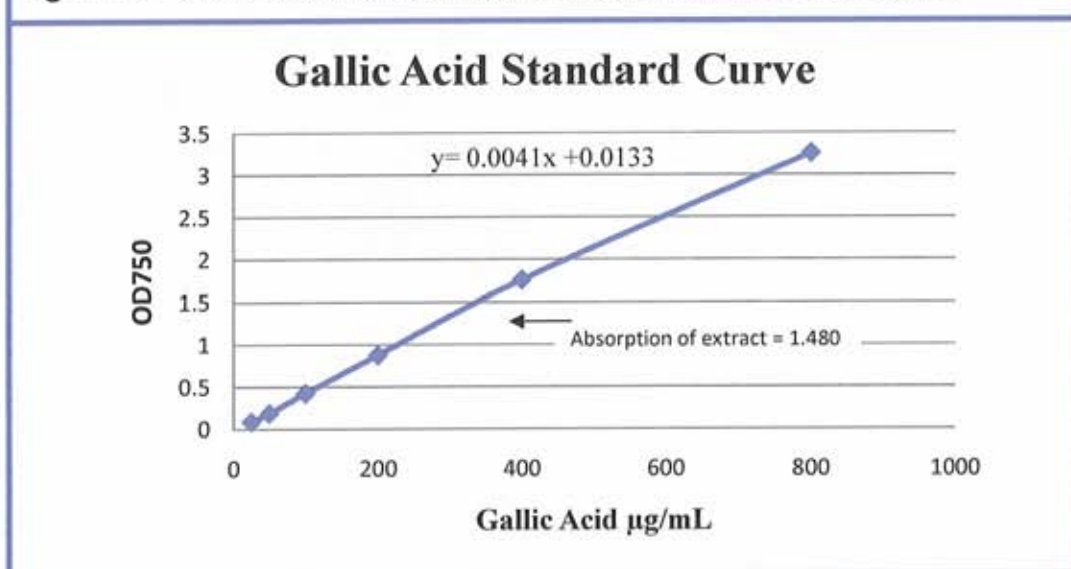
**Table 3.4** Effect of *Tragopogonporrifolius* water extract on paracetamol induced hepatotoxicity.  
Values are expressed as mean  $\pm$  SEM (n = 7)  
\* Represents  $p < 0.05$ .

Treatment	ALT U/L	AST U/L	ALP U/L	LDH U/L
Control	25.1 $\pm$ 1.8	31.8 $\pm$ 2.8	108.3 $\pm$ 3.5	136.2 $\pm$ 8.5
Paracetamol (2g/kg)	235.9 $\pm$ 5.1	221.3 $\pm$ 8.8	343.1 $\pm$ 10.7	398.9 $\pm$ 9.4
Dose 1 (50 mg/kg)	109.6 $\pm$ 2.8*	112.9 $\pm$ 4.2*	270 $\pm$ 6.4*	272.2 $\pm$ 8.6*
Dose 2 (100 mg/kg)	84.5 $\pm$ 2.6*	90.7 $\pm$ 1.8*	179.7 $\pm$ 11.2*	197.4 $\pm$ 2.9*
Dose 3 (250 mg/kg)	40.8 $\pm$ 2.1*	38.5 $\pm$ 2.3*	115.7 $\pm$ 4.5*	176.3 $\pm$ 8.9*

### 3.7. Total Phenolic Content and Anti-Oxidant Effect

With reference to the gallic acid standard curve (Figure 3.7), the total phenolic content of each gram of dry extract of *Tragopogonporrifolius* was approximately equal to 596mg of GAE/g. The water extract of *Tragopogonporrifolius* resulted in a relatively high DPPH scavenging activity (81.43 %) which related to a strong anti-oxidant ability. Results are shown in Figure 3.4 and Table 3.5.

**Figure 3.4** Gallic Acid Standard Curve ranging from 25 to 800 µg/mL.



**Table 3.5:** Total phenolic content and % scavenging effect

Extract	Total Phenolic content (mg of GAE/g of extract)	Antioxidant Activity DPPH (% scavenging effect)
<i>Tragopogonporrifolius</i>	596	81.43%

## Chapter 4

### Discussion and Conclusion

The world today is undergoing drastic evolution in all fields, especially medicine. Doctors, pharmacologists and scientific researchers contributed greatly to the marvelous progress of diagnosis, prognosis and treatment of different diseases and infections. However, the advantages of medicinal advancement are being constantly challenged by the disadvantages of their respective side effects; which revived great interest in herbal medicine and plant therapy. Accordingly numerous investigations and studies were conducted in search for natural remedies against various health conditions. Ethnopharmacologists considered the Mediterranean region as a powerful source of potential herbal remedies due to its affluent stock of medicinal plants (Saad et al. 2005). Commonly, the therapeutic potential of a plant is highly based on its bioactive ingredients especially aromatic compounds (Saad et. al 2005); hence it has been claimed that *Tragopogonporrifolius* which is rich in phenols, saponins and riboflavin in addition to several minerals and vitamins might exhibit a medicinal role (Salsify, <http://www.innvista.com>). This study served to investigate the therapeutic effect of *Tragopogonporrifolius* water extract in hyperlipidemia, glycemia, inflammation, hepatotoxicity, oxidation and gastric ulcer. Accordingly, twenty-eight rats were put on a diet for one month during which the water extract of *Tragopogonporrifolius* was administered to the rats via drinking water in three different doses (50, 100 and 250 mg/BW) along with normal rat chaw with the addition of 5% coconut oil. The coconut oil served to increase the concentration of lipids in the rats' blood in order to better assess the effect of the plant under study on hyperlipidemia.

At the end of the one month period, the results showed a potent decrease in the serum levels of CH, TAG, glucose, ALP, ALT and LDH in



addition to a significant increase in HDL-cholesterol. The decrease noticed in the levels of the liver enzymes serve as a proof of the absence of liver toxicity. No harmful adverse event was noticed in the rats during the period of experimental work inferring that *Tragopogonporrifolius* can be safely administered to rats even in high doses (250mg/kg BW).

The potent hypolipidemic and hypoglycemic role of *Tragopogonporrifolius* demonstrated in the experimental groups I, II and III in Table 3.1 after one month of *Tragopogonporrifolius* water extract administration is justified by the presence of inulin, saponins, vitamin B6 and riboflavins. Based on literature review the three previously stated herbal supplements play important roles in lowering blood lipids and glucose concentrations (Powers, 2003)(Warashina et al. 1991)(Niness, 1999).

Saponins are glycosides able to reduce the concentration of cholesterol in the blood either by slowing fat re-absorption through inhibiting pancreatic lipase, or by forming a complex with cholesterol in the intestinal tract preventing its re-absorption or by increasing the hepatic metabolism of cholesterol (Francis et al. 2002). Furthermore this herbal supplement is able to selectively reduce LDL blood levels by favoring its hepatic uptake through enhancing the activity of hepatic LDL receptors (Maurya et al. 2009). The exclusive LDL lowering ability of saponin explains the persistent high levels of HDL, despite the decrease in total cholesterol observed in the serum of the rats representing experimental groups I, II and III (Francis et al. 2002). The increase in the levels of HDL cholesterol conferred by the plant under study suggests a potent protective role against chronic heart diseases (Oluwatosin et al. 2008) since high HDL concentration is known to lower the risk for cardiovascular diseases through preventing the oxidation of LDL and inhibiting the formation of lipid hydroperoxides in addition to

decreasing the blood concentration of lipids by favoring the efflux of surplus cellular cholesterol (Oluwatosin et al. 2008).

In addition to their lipid lowering effect, saponins are also able to decrease glucose serum levels by restraining gastric emptying and preventing glucose transport from the stomach to the small intestine and restricting its passage through the intestinal brush border (Francis et al. 2002).

Riboflavin, being the major constituent of Flavin adenine dinucleotide (FAD) and Flavin mononucleotide (FMN), which are essential cofactors of various metabolic enzymes, exhibits an important lowering effect on the plasma concentrations of both glucose and lipids by breaking them down through activating specific glycolytic and lipolytic enzymes in order to generate the adequate amount of energy essential for the regular functions of the body (Powers 2003).

Inulin is known as a prebiotic fiber, predominantly found in plants, possessing a lipid-lowering ability implicated by absorbing excess amount of cholesterol from the blood (Niness, 1999). Inulin is considered as diabetic and diet-friendly because it provides the sweet taste and great energy of sugar without the increase in caloric and glucose intake. It is known to up-regulate the absorption of important minerals such as magnesium which is an important enzymatic cofactor, essential for the activity of vital metabolic processes such as glycolysis and lipolysis; thus justifying its glucose and lipids lowering ability (Niness, 1999).

Vitamin B6 (pyridoxine) is required for the metabolism of proteins, carbohydrates and lipids. Most importantly, this vitamin displays significant lipotropic activity that serves to decrease blood lipid levels and reduce the accumulation of hepatic lipid deposits (Francis et al. 2002).

The stool analysis performed after one month of *Tragopogonporrifolius* water extract administration revealed the plant's ability to induce both cholesterol and triglyceride fecal excretion in a dose-dependent manner. Probably these observed results are based on the plant's ability to increase the metabolic rate of lipids by the action of saponins, riboflavin and inulin, hence decreasing their concentration in the blood but increasing it in the stools.

It is well documented, that most of the therapeutic effects exhibited in plants can be attributed to their high phenolic content (Saad et al. 2005). After assessing the phenolic content of *Tragopogonporrifolius* (596mg/GAE) and testing its ability to scavenge DPPH (81.43%) it may be concluded that the high concentration of phenols strongly correlates with the effective DPPH scavenging ability (Alizadeh et al. 2009). The obtained data suggests that the water extract of *Tragopogonporrifolius* will succeed in limiting the damaging effect of oxidation through its phenolic compounds namely, saponins and riboflavin which will neutralize the free radicals of ROS (Kruawan et al. 2006).

Specifically, riboflavin is known to be the major constituent of the cofactors Flavin Mononucleotide (FMN) and Flavin Adenine Dinucleotide (FAD) which are required to activate a wide variety of oxidative enzymes like glutathione peroxidase that plays a key factor in combating free radicals in all body cells (Powers, 2003). The anti-oxidative potential of Flavins is achieved through their ability to accept a pair of hydrogen atoms, thus reducing the isoalloxazine ring present in FAD and FMN resulting in the production of FADH<sub>2</sub> and FMNH<sub>2</sub> (Powers, 2003). Whereas the anti-oxidative power of saponins is based on its ability to prevent the oxidative damage caused by free radicals by scavenging superoxides through producing hydroperoxide intermediates (Francis et al. 2002). Accordingly this plant can be



considered as a potent anti-oxidative natural remedy. This consideration is further stabilized by several published studies which revealed a tight correlation between the concentration of phenolic compounds present in plants in general and their anti-oxidative potential (Norhaiza et al. 2009).

*Tragopogonporrifolius* inhibited both carrageenan and formalin induced inflammation in rats in a dose-dependent manner. The highest dose of the water extract (250mg/kg) demonstrated a very high inhibitory percentage similar to that observed with Diclofenac, which is the common non-steroidal drug used to treat inflammation (Londonkar et al. 2010). The specific anti-inflammatory mode of action implicated by the plant's water extract is still unknown. However, logically it should be related either to inhibiting the release or altering the function of pro-inflammatory mediators such as histamine, serotonin and prostaglandins (Chungag et al. 2009). Furthermore, there exists a strong association between the anti-inflammatory ability and the phenolic content, especially flavonoid, saponins and polyphenols, of plants (Bhujbal et al. 2008). After thorough literature screening, it has been established that saponins exhibit a potent stimulatory effect on the specific immune response on one hand and a strong inhibitory effect on the non-specific immune system on the other hand (Francis et al. 2002). Inflammation is a vital non-specific immune response observed in various health conditions; based on that it is readily inhibited by saponins. The latter modifies the nuclear transcription factor- $\kappa$ B preventing it from promoting the transcription of certain pro-inflammatory genes (Francis et al. 2002). Nevertheless, further studies are needed in order to determine whether the anti-inflammatory mechanism of *Tragopogonporrifolius* depends on its phenolic content or its ability to inhibit pro-inflammatory agents.



The gastro-protective ability of *Tragopogonporrifolius* proved to be very effective against ethanol induced gastric ulcer in rats as shown in Table 3.3. This effectiveness was dose-dependent as the highest dose of the plant's aqueous extract demonstrated a curative ratio of 76% which is very close to the curative potential of Cimetril (80%) confirming the anti-ulcerogenic potential of *Tragopogonporrifolius*. The mechanism through which this plant protects the experimental rats against ethanol-induced gastric ulcer remains ambiguous. It may contain certain inhibitory constituents able of preventing gastric acid release (Adesanwo et al. 2009) or it may possess the ability to up-regulate the protective factors lining the stomach, such as mucous, thus enhancing their defensive capacity against ethanol (Shokunbiet al. 2008). In addition to treatment, usually doctors recommend all patients suffering from gastric ulcer to follow a diet rich in vitamins, fibers and minerals (Bansal et al. 2009). Since ancient times *Tragopogonporrifolius* is known for being loaded with magnesium, potassium, iron calcium, fibers, folate, vitamin B and riboflavin (Salsify, <http://www.innvista.com>). The fact that *Tragopogonporrifolius* is rich in riboflavin, which is known to enhance the production of mucous in the digestive tract, emphasizes that the gastro-protective effect conferred by the plant is based on its ability to promote gastric protection by up-regulating mucous formation in the stomach (Powers, 2003). The generous nutritional content of *Tragopogonporrifolius* might indeed justify the protective role played by the plant against ethanol induced gastric ulcer; yet the mechanism according to which these nutritional supplements work is still depending on future investigations to be accurately revealed.

The role of *Tragopogonporrifolius* in hepatotoxicity was established after challenging the rats under study with high doses of paracetamol. Overdose of paracetamol is widely known to induce liver damage by enhancing oxidative stress, elevating lipid peroxidation and diminishing hepatic GSH pools; hence extreme care must be taken while

consuming medications in general and paracetamol in particular (Akah and Ado 2010). The water extract of *Tragopogonporrifolius* conferred a substantial dose-dependent anti-hepatotoxic effect. This potent effect was indicated by the significant decrease in serum hepatic enzymes' levels (AST, ALT, APT and LDH) exhibited by the experimental groups I, II and III after administration of the plant's water extract. The hepato-protective mechanism demonstrated by the plant further support the strong association between hepato-toxicity and oxidation since the plant is rich in anti-oxidative agents such as saponins, riboflavins and polyphenols (Setty et al. 2006). Amongst the already stated anti-oxidants, it is speculated that riboflavin is the most effective anti-hepatotoxic agent. This speculation is based on the fact that riboflavin is required for the normal functioning of Glutathione reductase, which is responsible for the conversion of oxidized glutathione (GSSG) into reduced glutathione (GSH) (Powers, 2003). Consequently the newly formed GSH will replenish the previously depleted hepatic GSH pools due to paracetamol overdose.

The promising anti-hepatotoxic results attained in this study, should act as a great incentive for the conduction of future studies in order to certify the existing speculations and discover new potential anti-hepatotoxic agent amongst the herbal supplements of *Tragopogonporrifolius*.

In conclusion, *Tragopogonporrifolius* proved to be a potential remedy against hyperlipidemia, hepatotoxicity, inflammation and gastric ulcer. However, future investigations are needed to confirm the presence of beneficial herbal supplements and link each constituent to its respective therapeutic function accurately; revealing the specific mode of action followed by the plant in its ethno-pharmaceutical role.

## Chapter 5

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