Urtica dioica WATER EXTRACT AND ITS IMPACT ON GASTRIC ULCER AND COLONIC ULCER IN THE RAT MODEL

by

Dany Nachabe

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Thesis approval Form (Annex III)

Student Name: Dany Nachabe
I.D. #: 199936440

Thesis Title: *Urtica dioica* water extract and its impact on gastric ulcer and colonic ulcer in the rat model

Program: Molecular Biology

Division/Dept: Natural Sciences Division

School: School of Arts and Sciences

Approved by:

Thesis Advisor: Dr. Costantine Daher

Member: Dr. Georges Baroody

Member: Dr. Yollande Saab

Member: 

Date: June 22, 2006
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Dany Nachabe
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GLOSSARY

*ad lib*: ad libitum.

*ASA*: Aminosalicylic acid

*Cox*: Cyclooxygenase

*CT*: Chest - Thorax

*GI*: Gastrointestinal

*H. pylori*: Helicobacter pylori

*IBD*: Inflammatory bowel disease

*NF-kappa B*: Nuclear factor kappa B

*NSAID*: Non-steroidal anti-inflammatory drug

*SJS*: Stevens-Johnson Syndrome

*TEN*: Toxic epidermal necrolysis

*TLC*: Thin layer chromatography

*TNFα*: Tumor necrosis factor α

*U. dioica*: Urtica Dioica
Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction and aim of the project

Long before the discovery of new scientific medicine, therapeutic drugs and medical surgeries, the practice of folk medicine was common and has traditionally existed in every culture. Side effects or toxic reactions associated with scientific medicine in addition to its high cost have made people, over the last decade, to turn into plant extracts instead of, or in addition to, their doctors' prescription as they have shown to generate promising results for the cure of illness due to effective compounds they contain (Wolinsky, 1980). As there are various plant species (about 300,000 species of flowering plants) that are chemically distinct, about 10 percent of them have been carefully studied for their toxic and medicinal constituents (Duke, 1997). Some of the toxic plants have been known as actual poisons but if combined may be used for remedial purposes (Foster and Duke, 1990; Blumenthal, 1992). *Urtica dioica* (*U. dioica*), of the family of Urticaceae, also known as Stinging Nettle, is an annual plant (Akgül, 1993; Ziyyat et al., 1997). This herb grows in nitrogen-rich soils in deserted field and is a characteristic of the Mediterranean regional countries as well as Italy (Pignatti, 1982). Its leaves and seeds include minerals, amino acids and vitamins (Baytop, 1999; Wetherilt, 1989). *U. dioica* has been well known throughout history for its medicinal uses. Nevertheless, the Food and Drug Administration classifies nettle as a plant of vague safety (Fetrow and Avila, 2000). Several cultures have administered *U. dioica* internally and externally with its various parts for a diversity of reasons including its use as a general tonic (Brochers et al., 2000). Other studies of this plant described its anti-inflammatory, anti-rheumatic, and hypolipidemic effects (Obertreis et al., 1996; Riehemann et al., 1999; Daher, C. F. et al., 2006). Moreover, the water extract of the aerial parts has shown a diuretic and hypotensive effect provoked by an unknown component (Tahri et al.,
Cardiovascular effects have been also reported (Testai et al., 2002). The *U. dioica* effects are also evoked in the treatment of prostatic hyperplasia (Krzeski et al., 1993; Hiramo et al., 1994 and Lichius and Muth, 1997) and in the production of various pharmacological outcomes such as stimulation of lymphocyte proliferation in human (Wagner et al., 1989). Furthermore, nettle has been known in Oriental Morocco to have antidiabetic, hypotensive, and hypoglycemic effects (Ziyyat et al., 1997; Bnouham et al., 2002). Conversely, other studies stated no hypoglycemic action of this plant (Swanson-Flatt et al., 1989; Roman-Ramos et al., 1992). In his work, Lebedev mentioned that an aqueous combination of Mediterranean herbs including *U. dioica* prevents the harm of the liver tissue of the rat (Lebedev et al., 2001).

Till now, no reports have dealt with the effect of *U. dioica* water extracts upon the protection and treatment of gastric ulcer and/or colonic ulcer. Thus, the present study investigates the effect of water extracts of *U. dioica* in preventing and/or healing the mucosal lesions in the stomach and colon induced by indomethacin and iodoacetamide respectively.

1.2 Functional Anatomy of the Stomach

Located on the left side of the body, under the diaphragm, the stomach is an expanded section between the esophagus and the small intestine. It is a muscular sack like structure composed of a lumen surrounded by four principle layer of muscles: mucosa, submucosa, muscularis, and serosa (Junqueira et al., 1995). The stomach has four main regions: the cardia, fundus, body, and pylorus. The concave medial border of the stomach is called the lesser curvature, and the convex lateral border is called the greater curvature (Tortora et al., 2000). The parietal cells, when stimulated, secrete HCL at a concentration of roughly 160mM (equivalent to a pH of 0.8) (Yao et al., 2003). The epithelium of the stomach is intrinsically resistant to gastric acid and its damaging effects.
Nonetheless, excessive secretion is a problem leading to gastritis, gastric ulcer, and peptic acid disease (Samuelson et al., 2003).

Figure 1.1: Macroscopic Anatomy of the Stomach (Mintchev et al., 1993)
1.2.1 Gastric Ulcer

1.2.1.1 Background and Pathophysiology

Gastric ulcer is one of the most common diseases affecting the gastrointestinal (GI) tract. It causes inflammatory injuries in the gastric mucosa, with extension beyond the submucosa into the muscularis mucosa (Mintchev et al., 1993). A diagnosis can be difficult because it has a large spectrum of clinical presentations, ranging from asymptomatic to indistinguishable or unclear epigastric pain, nausea, iron deficiency anemia to acute life-threatening hemorrhage (Cho and Wang, 2002).

Normally, the stomach maintains equilibrium between the protective factors (blood flow, bicarbonate and mucus secretion) and aggressive factors (pepsin and acid secretion). Gastric ulcers build up when aggressive factors overcome the protective mechanism (Chan et al., 2005).

The three major etiological causes for gastric ulcer are mainly *Helicobacter pylori* infection, administration of nonsteroidal anti-inflammatory drugs (NSAIDs), and acid over secretion. At this time, 70% of all gastric ulcers occurring in the United States can be accredited to *H pylori* infection (Sonnenberg et al., 1997). Besides the increase in acid secretion, *H pylori* also predisposes patients to peptic ulcer disease by upsetting mucosal integrity. The bacterium spiral shape with flagella facilitates the penetration into the mucous and its attachment to the epithelial layer. Consequently, it liberates phospholipase and proteases, which lead to further mucosal damage (Graham et al., 2001).

1.2.1.2 Diagnosis

Currently, the most exact method to detect ulcers is videoendoscopy of the upper digestive tract. The physician uses a gastroscope, a thin tube containing a tiny camera, through the mouth and down to the stomach to observe the stomach lining (Alkofahi and Atta, 1999). The symptoms can be different and various from patient to patient (Zayachkivska et al., 2005). An ulcer can evolve without
causing pain, until extremely severe pain (Gharzouli et al., 1999). The risk of an ulcer is the complications it causes which could be life threatening as it leads to bleeding (hemorrhage), and perforation of the stomach wall (Lau et al., 2000). The diagnosis of gastric ulcer can be done based on a characteristic clinical history (Zayachkivska et al., 2005). Laboratory tests such as blood cell count and blood iron level can help detect anemia. Weight loss and anemia are signals for a possible ulcer in the stomach (Lau et al., 2000). Imaging studies, such as an upper GI radiography, has a high accuracy in diagnosing gastric ulcer (Gharzouli et al., 1999). Other tests such as H pylori testing could be beneficial. There is a strong relationship between peptic ulcer disease and H pylori infection. This bacterium can be diagnosed using various tests such as biopsy, blood culture, rapid urease test, stool antigen, etc... (Soll, 1996)

1.2.1.3 Treatment

The medical treatment of gastric ulcer is meant to restore the equilibrium between the aggressive acid secretion and the mucosal protective factors (Toma et al., 2005). In patients with H pylori infection, the most effective treatment is to exterminate the organism and to suppress acid secretion. In patients with bleeding ulcer, an intravenous proton pump inhibitor is started. This is followed by examination for acute anemia, thrombocytopenia, or coagulopathy, which needs to be supplemented with vitamin K (Suerbaum et al., 2002).

Treatment can be directed toward histamine release, such as H2 blockers, which selectively block the H2 receptors in the parietal cells. If this therapy is followed for a period of eight weeks, with a twice-daily dose of H2 blockers, healing rate will be higher than 70% (Steinbach et al., 1999).

Proton pump inhibitors are drugs that bind and irreversibly inhibit the H⁺/K⁺ ATPase pump, hence effectively inhibiting acid secretion. Omeprazole, lansoprazole, rabeprazole, and esomeprazole doses given once or twice-daily for four weeks heal 80-100% of gastric ulcers if H pylori infection is absent or has been eliminated. All proton pump inhibitors have almost equal efficacy (Vanderhoff and Tahboub, 2002). They should preferentially be taken on an
empty stomach to allow highest inhibition of the $\text{H}^+/\text{K}^+$ pumps. Studies have revealed that continuous intravenous infusion of omeprazole in patients with vigorous bleeding from gastric ulcer reduces the need for transfusion, mortality, and hospital stay (Lai et al., 2002).

Moreover, $\text{H} \text{ pylori}$ infection eradication using multiple regimens, including antibiotics and/or H2 blockers, has been evaluated and it was shown that the organism could be eradicated more than 90% of the time (Suerbaum et al., 2002).

Another therapy using mucosal protectors can also be effective, such as Bismuth and sucralfate, in healing gastric ulcers; however, they are not as efficient as H2 blockers. Patients taking NSAIDs should stop them if possible, and if it is not, omeprazole should be given concurrently at a dose of 40 mg (Lassen et al., 2000).

Endoscopic evaluation of the bleeding ulcer can reduce the duration of the hospital stay by identifying the patients at low risk for rebleeding. It decreases also the likelihood of repeated bleeding and reduces the need for surgery. Ulcers with stigmata (overlying blood clot, visible vessels) require an endotherapy, whereas ulcers with a clean base crater do not need to be treated endoscopically (Lassen et al., 2000).

Most ulcers can be managed effectively using medicinal drugs or with medical treatment, however, surgery still has an important role in hemorrhage that can not be controlled with medical management alone. Additional indications for surgery are ulcer perforation, giant gastric ulcer, and gastric outlet obstruction. This therapy is based on acid reduction with vagotomy. If vagotomy is not necessary, selective or truncal vagotomy is done routinely (Lau and Chung, 2000).

### 1.3 Functional Anatomy of the Colon

The colon, or large intestine, is part of the digestive tract, which is a series of organs from the mouth to the anus. The colon is approximately 5 feet long. It joins to the small bowel, which is known as the small intestine. The major
functions of the colon are water and salt absorption from partially digested food that enters through the small bowel and then sends waste out of the body via the anus (Stein and Hanauer, 1999; Leducce, 2002). After absorption, remains the feces which pass from the colon to the rectum and out through the anus. The colon consists of several segments including the cecum (just after the small bowel), the ascending colon, transverse colon, descending colon, sigmoid colon (S-shaped), and the rectum (store of feces until defecation).

![Anatomic Structure of the Colon](image)

**Figure 1.2: Anatomic Structure of the Colon (Marc, 2002)**

The colon is created during the first three months of embryonic development. As the bowel extends, part of it passes into the umbilical cord, which joins the fetus and the mother. The colon and the small bowel are held in position by a tissue called the mesentery. The descending colon and ascending colon are fixed in
place in the abdominal cavity. The transverse colon, cecum and sigmoid colon are suspended from the back of the abdominal wall by the mesentery (Beart et al., 2005).

1.3.1 **Colonic Ulcer**

1.3.1.1 **Background and Pathophysiology**

Colonic ulcer is a disease that causes sores and inflammation in the lining of the rectum and colon. Inflammation kills the cells that usually line the colon, forming ulcers, which then bleed and form pus (Meyers, 1999; Bernstein, 2000). It also induces frequent emptying of the colon, causing diarrhea (Bernstein, 2000). If the inflammation takes place in the rectum and lower part of the colon it is called ulcerative proctitis. If the left side of the colon is affected it is known as distal or limited colitis. If the entire colon is concerned with the inflammation it is called pancolitis (Lee et al., 2005). The general name for diseases that cause inflammation in colon and small intestine is known as inflammatory bowel disease (IBD). Diagnosis could be hard because its symptoms are similar to other intestinal disorders and to another type of IBD known as Crohn's disease (Sachar, 1995). Crohn's disease is however different because it leads to deeper inflammation within the intestinal wall and can occur in other parts of the digestive tract such as the mouth, esophagus, stomach, and small intestine (Truelove and Jewell, 1974). Colonic ulcer can occur in people of any age, but it starts usually between the ages of 15 and 30, and affects men and women equally (Jarnerot et al., 1985). The most common symptoms of colonic ulcer are bloody diarrhea and abdominal pain. Patients may also experience anemia, weight loss, fatigue, rectal bleeding, loss of appetite, skin lesions, loss of body fluids and nutrients, joint pain, and growth failure especially in children. Colonic ulcer can also cause problems such as inflammation of the eye, liver disease, arthritis, and osteoporosis (McIntyre et al., 1986). Yet it is not clear why these problems take place outside the colon. Scientists assume that the inflammation
triggered by the immune system might have led to such complications (Jamerot et al., 1985).

Patients with colonic ulcer have a defective immune system. Scientists wonder if this abnormality is a result or a cause of the disease. Colonic ulcer is not caused by sensitivity to certain foods or emotional distress, but these factors can trigger symptoms in some people (Lee et al., 2005).

![Colonoscopy diagram](image)

Figure 1.3: Colonic ulcer as visualized with a colonoscope (Al Ataie et al., 2005)

1.3.1.2 Diagnosis

Several tests are performed to diagnose colonic ulcer. A medical history and a physical examination are usually the first step (Robert et al., 1990). One of the tests is a blood test that may be done to check whether the patient is anemic, which could indicate bleeding in the rectum or colon, or to check the white blood cell count, which, if elevated, is a sign of inflammation somewhere in the body (Askling et al., 2001). White blood cells can also be revealed by a stool sample, presence here indicates colonic ulcer or inflammatory disease. Moreover, the stool sample allows the detection of bleeding or infection in the rectum or colon caused by bacteria, parasites, or a virus (Froehlich et al., 1999). A colonoscopy
is the most precise method for making a diagnosis of colonic ulcer. This method is performed by insertion of an endoscope, which is a long flexible lighted tube joined to a TV monitor, into the anus to see the inside of the rectum and colon (See Figure 1.3). Using a colonoscope, inflammation can be detected, as well as a bleeding, or ulcers on the colon wall (Arner, 1971). During the therapy, the physician may do a biopsy (take a sample of tissue from the lining of the colon) to view it under the microscope. Sometimes X-rays like barium enema or CT scans are performed to diagnose colonic ulcer and its complications (Froehlich et al., 1999)

1.3.1.3 Treatment

Treatment of colonic ulcer differs from person to person because it depends on the severity of the disease. Either a drug therapy is followed and is enough to cure the patient, or else a surgery would be a feasible solution. The purpose of drug therapy is to stimulate and maintain remission, and to recover the quality of life for people with colonic ulcer (Sandborn and Targan, 2002).

To help control inflammation, aminosalicylates drugs that contain 5-aminosalicylic acid (5-ASA) should be taken. A combination of 5-ASA and sulfapyridine is the sulfasalazine. The anti-inflammatory 5-ASA is carried by the sulfapyridine component in the intestine. This latter may lead to side effects such as vomiting, diarrhea, nausea, heartburn, and headache. People who can not take sulfasalazine may use other 5-ASA agents such as balsalazide, olsalazine, and mesalamine, which have a different carrier. Depending on the location of inflammation, 5-ASAs can be given in a suppository, or orally. The majority of people with mild or moderate colonic ulcer are treated with this group of drugs first (Jess et al., 2006). Other drugs that can also help decrease inflammation are corticosteroids, such as methylprednisone, prednisone, and hydrocortisone. Patients who do not respond to 5-ASA drugs or who have moderate to severe colonic ulcer may use these drugs. Corticosteroids, which are also known as steroids, can be given intravenously, in a suppository, or orally, depending on the
location of the inflammation. Side effects that might be caused by these drugs are acne, weight gain, hypertension, facial hair, mood wings, diabetes, bone mass loss, and an increased risk of infection. Although they are considered very effective, they are not recommended for long-term use (Sands, 2000).

Inflammation reduction could be also successful using immunomodulators such as 6-mercaptopurine and azathioprine which act by affecting the immune system. People who do not respond to 5-ASA or corticosteroids use these drugs. Immunomodulators are given orally. They act slowly; they may take up to six months to be fully successful. Patients using these drugs are examined for complications involving hepatitis, pancreatitis, an increased risk of infection, and a white blood cell count reduction (Rioux et al., 2000).

Some patients have remissions (period when the symptoms go away) that remain for months or even years, however, symptoms eventually return in most people. Around 25 to 40 percent of colonic ulcer patients must eventually undergo a proctocolectomy (colon removal) because of severe bleeding, massive illness, rupture of the colon, or risk of cancer. Physicians also recommend colon removal if the patients are suffering from the side effects of the drugs administered during treatment period (Kornbluth, 2001).

1.4 Indomethacin

Indomethacin is a non-steroidal drug with anti-inflammatory property, used to decrease fever, stiffness, pain, and swelling. Its mode of action is to inhibit the production of prostaglandins, which are molecules known to cause these symptoms (Hart et al., 1963).

1.4.1 Clinical Pharmacology

Indomethacin is an indole derivative and a component of the aryalkanoic acid class of non-steroidal anti-inflammatory drugs (NSAIDs).
Indomethacin can not be considered a simple analgesic and must not be given in conditions other than those recommended under indications. Clinical indications for indomethacin include rheumatoid arthritis, arthritic gout, osteoarthritis, pseudogout, dysmenorrhea (menstrual cramp), pericarditis, bursitis, tendinitis, nephrogenic diabetes insipidus, and fever and pain associated with malignant diseases (bone metastasis, tumors) (Hart et al., 1963).

The use of indomethacin in conjunction with other salicylates such as aspirin and/or other NSAIDs is not recommended. Studies have shown that this combination does not produce any higher therapeutic effect than the use of indomethacin alone. Moreover, the frequency of gastrointestinal side effects was considerably elevated with combined therapy (Ferreira et al., 1971).

Indomethacin is contraindicated in patients with asthma, or allergic-type reactions after being given aspirin or other NSAIDs. Suppositories indomethacin should not be given to patients with a history of rectal bleeding. Indomethacin, just like other NSAIDs, can cause serious gastrointestinal problems including bleeding, inflammation, and ulceration, perforation of the esophagus, stomach, small or large intestine, which can be fatal (Phelan et al., 2003).
In addition to the risk of ulceration and perforation, indomethacin can lead to the onset of hypertension which may contribute to the elevated incidence of cardiovascular events. It also should be given with caution in patients with fluid retention or heart failure, as well as in patients with known cardiovascular disease because they have shown an elevated risk of serious myocardial infarction and stroke which can be fatal (Scherzer et al., 1992).

Indomethacin, like other NSAIDs, can lead to serious skin adverse events such as Stevens-Johnson Syndrome (SJS), exfoliative dermatitis, and toxic epidermal necrolysis (TEN), which can be fatal. Administration of NSAIDs has also resulted in the onset of renal injury such as renal papillary necrosis after a long-term treatment. Treatment should be initiated with caution in patients with significant dehydration. They should be rehydrated before any therapy start with indomethacin (Phelan et al., 2003).

1.4.2 Mechanism of Action

Indomethacin can non-selectively inhibit the cyclooxygenase (COX) 1 and 2 which are enzymes involved in prostaglandin synthesis from arachidonic acid (Chandrasekharan et al., 2002). NSAIDs are the most widely prescribed drugs on the market and are effective for decreasing pain and inflammation. Originally, their mechanism of action was discovered to be the inhibition of cyclooxygenase.

A second form of cyclooxygenase has been discovered, the gene cloned, and inhibitors synthesized (Silva et al., 2003). Prostaglandins derived from the action of COX-1 are considered to be the constitutive prostaglandins present in all tissues. They are important for platelet aggregation, renal blood flow in the impaired kidney, and cytoprotection in the stomach (Portanova et al., 1996). Prostaglandins derived from COX-2 are inducible and upregulated in areas of inflammation. They do not exist in the basal state. The genetics of COX-1 and COX-2 shows that although they share 60% structural homology, they constitute two completely different systems with different functions in the human body (Raz et al., 1988). Inhibition of COX-1 is not necessary to achieve the anti-
inflammatory and analgesic effects of NSAIDs, but doing so significantly contributes to the risk of GI ulceration, bleeding, inhibition of renal blood flow, and inhibition of platelet aggregation (Reingold et al., 1981). Inhibition of COX-2 with new selective agents yields equal anti-inflammatory and analgesic effects without the above-mentioned side effects (Vane, 1971). Prostaglandins have several effects, some of which cause pain, inflammation, and fever. They also lead to uterine contractions in pregnant women. Indomethacin is able to postpone or delay labor through the decrease of uterine contractions by inhibition of prostaglandin synthesis in the uterus and probably via calcium channel blockade. In addition to its mode of action, indomethacin also inhibits the motility of polymorphonuclear leucocytes like colchicine, and uncouples oxidative phosphorylation in mitochondria like salicylates (Phelan et al., 2003). Indomethacin can cross the placenta easily, and can decrease fetal urine production to treat polyhydramnios, by decreasing renal blood flow and increasing renal vascular resistance (Scherzer et al., 1992).

1.4.3 Animal Toxicity and Human Overdose

Indomethacin has an elevated acute toxicity for both animals (12 mg/kg in rats and 50 mg/kg in mice) and humans. There are no exact human data, but some fatal human cases, especially in children and adolescents, have been observed. Human overdose leads to dizziness, drowsiness, severe headache, paraesthesia, mental confusion, nausea, and vomiting. It might also cause severe gastrointestinal bleeding. In children, cases of cardiac arrest and cerebral edema have been seen (Lum et al., 1997).

1.5 Iodoacetamide
Little is known about iodoacetamide; it is made up of yellow-brown crystals. Iodoacetamide is highly toxic and may act as a human carcinogen. This chemical has shown to induce tumors in laboratory animals when applied on the skin. It may also cause reproductive damage. Concerning its stability, iodoacetamide showed to be stable but light sensitive. It is not compatible with strong bases, strong oxidizing agents, reducing agents, and acids (Tremaine, 2000).

1.6 *Urtica dioica*

1.6.1 Plant taxonomy

Family: Urticaceae  
Genus: *Urtica*  
Species: *dioica*

*Urtica dioica* is also called: Nettle, stinging nettle, big string nettle, common nettle, isirgan, gerais, ortiga, kazink, nabat al nar, kerreiss, grande ortie, ortie, and urtiga.

Leaves and roots are the valuable parts of this herb (Thornhill and Kelly, 2000).
*U. dioica* is considered as a highly nutritious food that can be easily digested. It is rich in vitamins A and C and in minerals such as iron (Allardice, 1993; Bown, 1995). In brief, nettle is a very important addition to the diet (Phillips and Foy, 1990).

![Urtica dioica](image)

*Figure 1.6: Urtica dioica* (Thornhill & Kelly, 2000)

### 1.6.2 Nurturing and Cultivation

*U. dioica* grows best in a soil rich in nitrogen and phosphates. A deep rich soil is preferable if good quality fiber is needed (Grieve, 1984). It rises up to 7 feet from
a creeping rootstock. Nettle stem is square in shape, leaves are sharply toothed and opposite, and both are enveloped with stinging hairs. Flowers are green in color and are found in inflorescences in the leaf axils, with female and male flowers on separate plants. Flowers develop mostly during the period between June and September (Chevallier, 1996). A various number of insect species feed on this herb (Carter, 1982). *U. dioica* was shown to improve the strength of soft fruits growing nearby hence it was found to be a good companion plant to grow (Hatfield, 1997).

1.6.3 *U. dioica* constituents

Plant chemicals include alkaloids, acetylcholine, agglutinins, butyric acid, carbonic acid, caffeic acids, coumaric acid, chlorophyll, formic acid, histamine, linolenic acid, linoleic acid, palmitic acid, serotonin, and xanthophylls (Wagner et al., 1989).

*U. dioica* grows in tropical and temperate wasteland areas around the world (Grieve, 1984; Lust, 1983). In Lebanon, it is an annual plant that rises 0.5 to 1 meter high and makes white to yellowish flowers and piercing or pointed leaves (Fetrow and Avila, 2000). Nettle or stinging nettle is well known for giving a savage throb whenever the skin gets into contact with the hairs and bristles of the leaves and stems (Wagner et al., 1989). This throb or sting leads to irritation of the skin (Lust 1983; Randall et al., 2000). The skin can be treated by thorough drying or by heat (Huxley, 1992). Nettle usually produces either female or male flowers, which define its species name "dioica" meaning "two houses" (Facciola, 1990).

1.6.4 Medicinal Effects

*U. dioica* was screened for its ability to possess some medicinal effects. An aqueous extract from the leaves has traditionally been used as a blood purifier and cleansing tonic and hence the herb is frequently used in the treatment of high fever, anemia, and arthritis (Chevallier, 1996). Another study revealed that
this plant is antiasthmatic, diuretic, antidandruff, hypoglycemic, haemostatic, and a stimulating tonic (Laurent, 1981; Grieve, 1984; and Bown, 1995). *U. dioica* infusion is highly important in stopping internal bleeding (Grieve, 1984). It has also been shown to treat hemorrhoids, excessive menstruation, skin complaints, and rheumatism. As for external use, nettle has been reported to treat arthritic pain, skin complaints, gout, neuralgia, sciatica, and problems of the hair (Bown, 1995).

Treatment of rheumatoid arthritis can be applied by rubbing fresh leaves of *U. dioica* onto the skin. This practice leads to severe irritation of the skin as it is throbbed or stung by the nettles. The mode of action is summarized as follows: On one hand, more blood is brought to the area which helps remove toxins causing rheumatism, thus it acts as a counter-irritant, and on the other hand, formic acid produced by the nettle is believed to have a positive effect upon rheumatic joints (Moerman, 1998).

1.6.4.1 Hypoglycemic Effect

Nettle was shown to enhance the insulin concentration of blood sera in normal and streptozotocin-induced diabetic rats that were injected intraperitoneally with the nettle water extract. The aqueous extract of nettle leaves could increase the secretion function of Islets of Langerhans as observed by perifusion experiment. The effect revealed to be concentration dependent. That is, the administration of half concentrated extract causes the insulin level to decrease to its half value (Farzami et al., 2003). The effect of *U. dioica* aqueous extract on alloxan-induced diabetic rats revealed that it may act on glucose homeostasis via the pancreas. It was indicated that the presence of insulin is needed for the hypoglycemic activity of the nettle (Bnouham et al., 2003).

1.6.4.2 Microbiological Activity

Another study revealed that water extract of *U. dioica* was tested against Gram-positive and Gram-negative bacteria and have been shown to possess potent
antimicrobial activity when compared with standard and strong antimicrobial compounds such as amoxicillin-clavulanic acid (augmentin), miconazole nitrate, netilmicin, and ofloxacin (Gülçin et al., 2004). Eventhough, Sokmen et al. (1999) have reported that seeds of *U. dioica* do not have antibacterial effects on some members of *Enterobacteriaceae*, the addition of different parts of this plant has revealed a significant effect on the *Enterobacteriaceae* count (Aksu and Kaya, 2004).

### 1.6.4.3 Anti-aggregant Characteristic

Tannins and flavonoids have been reported to be present in *U. dioica* (Bellakhdar et al., 1991; Bruneton, 1993 and Hhamouchi, 1999). Flavonoids are almost ever-present in plants and are identified as the pigments responsible for the leaves color. Hence, the anti-aggregant characteristic of the plant can be attributed to these chemical components. Consequently, several studies have reported that flavonoids significantly reduce platelet aggregation, adhesion, and secretion (Middleton et al., 2000).

### 1.6.4.4 Hypotensive Property

Possible vasorelaxant effect was also investigated by many researchers and the results showed that *U. dioica* can produce hypotensive responses mediated by the discharge of endothelial nitric oxide and potassium channels opening, and through a negative inotropic action (Testai et al., 2002). An increase of the concentration of KCl (40–60 mM), i.e. by the enhancement of the levels of membrane depolarization, was found to reduce the vasorelaxing action of *U. dioica*. These findings suggested the probable binding of hyperpolarization factors to potassium channels opening (Magnon et al., 1998). Hypotensive action of aqueous extract of nettle reveals a direct consequence on the cardiovascular system. Furthermore, natriuretic and diuretic effects were also studied, proposing an action on the renal function (Tahri et al., 2000).
1.6.4.5 Anti-inflammatory Effect

The immune system is a highly complex, specialized group of cells, whose integrated role is to clear infection from the body. Cells of the immune system may interact in a cell-cell manner and may also respond to intercellular messages including cytokines, hormones, and eicosanoids (local hormones that have a role in inflammation, blood pressure, and fever) elaborated by different cells. Cells that will differentiate into many types of more specialized cells that circulate all over the immune system are generated in the bone marrow. This soft nutrient-rich tissue is located in the center shafts of certain flat, long bones of the body, including the bones of the pelvis. The immune system can be adjusted by diet, environmental pollutants, pharmacologic agents, and naturally occurring chemicals, such as flavonoids and vitamins. Flavonoids have an influence on the function of T cells, B cells, basophils, macrophages, neutrophils, mast cells, eosinophils, and platelets (Middleton et al., 2000). An inflammatory process necessitates that local endothelial cells become stimulated and express adhesion molecules on their surface; these interact with associated molecules on the surface of activated leukocytes, which then attach tightly to the endothelium and transmigrate into the site of inflammation (Aplin et al., 1998). An acute inflammation may be due to chemical substances, physical damage, microorganisms or other agents. The inflammatory response involves changes in blood flow, enhanced permeability of blood vessels and migration of cells from the blood into the tissues. In the early stages, fibrin, edema fluid, and neutrophil polymorphs gather in the extracellular spaces of the harmed tissue (Cope, 2002). The major phenolic ingredients in U.dioica extracts are caffeic and malic acid, which restrain eicosanoid formation but, compared with U.dioica extract, are rather ineffective in avoiding cytokine production in peripheral blood cells (Obertreis et al., 1996). Gene expression of cytokines is controlled by an essential transcription factor known as NF-kappaB (NF-kB). It also controls chemokines, growth factors, and cell adhesion molecules as well as some acute phase proteins (Barnes and Karin, 1997). The inhibition of NF-kB may be
attributed to an antioxidant activity of *U.dioica* extract (Schulze-Osthoff et al., 1995).

The inhibitory effect on NF-κB activation may as a result grant simple means to
discover the active antirheumatic compound in nettle extracts (Schulze-Osthoff et
al., 1995; Riehemann et al., 1999).

### 1.7 Aim Of The Project

The aim of the present work is to investigate the chronic effect of water extracts
of *U.dioica* upon healing of induced ulcerative gastritis and colitis in the rat
model. Parameters were assessed after administration of the plant extract firstly
prior to ulcer induction, to study the presence of any possible preventive effect,
and secondly after ulcer induction to evaluate for its effectiveness as a treatment
in both stomach and colon.
Chapter 2

MATERIALS AND METHODS

2.1 Animal Treatment

Male Sprague-Dawley (Rattus norvegicus) rats weighing 200-250g (Lebanese American University stock) are used in all experimental procedures. Animals were maintained and experimental protocols compiled with the Guide for the Care and Use of Laboratory Animals (National Research Council of the United States 1985). All animals were sacrificed using diethyl ether, at the end of the procedures described, without recovery from anaesthesia.

2.2 Collection of the Plant

The aerial parts of Urtica dioica (U. dioica) were collected, dried and powdered. Afterwards, the dry weight of water extract per grams dry leaves was determined.

2.3 Calculation of dry weight of Urtica dioica water extract

Procedure

To calculate the dry weight of the water extract obtained from decoction as described in section 2.2, 1g of plant material were added to 100ml pre boiled distilled water and simmered for 20 min, after which they were filtered and collected in an empty beaker (weight of empty beaker was recorded DW). Water was totally evaporated at 65°C, and the weight of the beaker with the dried filtrates was recorded (BE).

Calculation

\[ \text{Dry weight of water extract of } Urtica dioica (g) = \text{BE} - \text{DW} \]

It was found that the dry weight of water extract of U. dioica dried leaves was 17.3%.
Three different doses of extract, 50mg, 100mg, and 250mg/kg body weight, were prepared by simmering for 20 min 2.9g, 5.8g, and 14.5g respectively of dried leaves in 1L of preboiled hot water with occasional stirring. After decantation, the filtrates were given to rats in the different groups.

2.4 Group Allocation

The present study aims at assessing the role of U.dioica water extract intake upon protection from and treatment of stomach and colonic ulcers. To study the protective role, water extract of U.dioica was given prior to stomach and colon ulcer induction. However, in the treatment of gastric and colonic ulcer, the extract was given after ulcer induction. In order, to determine the ideal dose along with the best administration technique of the extract, three doses of the extract were used which were administered to the animals through either an intraperitoneal injection or via drinking water. Animals of all studies were maintained at an ambient temperature of 20 - 22°C, under 12 hours photoperiod, and fed a normal rat chow diet.

2.4.1 Water Extract Via Drinking Water

Animals, in this study, were subdivided into two sets of 3 groups each containing 7 rats. All animals received U.dioica extract via drinking water ad lib. The first group was given a dose of 50mg/Kg body weight, while the second and third group received 100mg and 250mg/Kg body weight doses respectively. All doses were based on the fact that a rat consumes 10 ml of drinking water per 100 g body weight per day (Waynfforth and Flecknell, 1992).
Study Design

Group Allocation - Gastric Ulcer

Prevention Experiment (6 days)  Treatment Experiment (repeated twice: 24 hrs & 48 hrs)

Oral Administration of water extract of Utiça dioica  IP Administration of water extract of Utiça dioica  Oral Administration of water extract of Utiça dioica  IP Administration of water extract of Utiça dioica

Total: 35 rats  Total: 28 rats  Total: 35 rats  Total: 35 rats

Negative Control (7 rats)  Negative Control (7 rats)  Negative Control (7 rats)  Negative Control (7 rats)
Positive Control (Cimetidine) (7 rats)  Group I: 50mg/kg (7 rats)  Positive Control (Omeprazole) (7 rats)  Group I: 50mg/kg (7 rats)
Group II: 50mg/kg (7 rats)  Group II: 100mg/kg (7 rats)  Group II: 100mg/kg (7 rats)  Group II: 100mg/kg (7 rats)
Group III: 250mg/kg (7 rats)  Group III: 250mg/kg (7 rats)  Group III: 250mg/kg (7 rats)  Group III: 250mg/kg (7 rats)

Figure 2.1: Experimental protocol and group allocation for gastric ulcer study

Group Allocation - Colonic Ulcer

Prevention Experiment (6 days)  Treatment Experiment (15 days)

Oral Administration of water extract of Utiça dioica  IP Administration of water extract of Utiça dioica

Total: 28 rats  Total: 28 rats  Total: 21 rats

Negative Control (7 rats)  Negative Control (7 rats)  Negative Control (7 rats)
Group I: 50mg/kg (7 rats)  Group I: 50mg/kg (7 rats)  Oral Group: 250mg/kg (7 rats)
Group II: 100mg/kg (7 rats)  Group II: 100mg/kg (7 rats)  IP Group: 250mg/kg (7 rats)
Group III: 250mg/kg (7 rats)  Group III: 250mg/kg (7 rats)

Figure 2.2: Experimental protocol and group allocation for colonic ulcer study
2.4.2 **Negative Control Group**

All studies conducted without exception included a negative control group containing 7 rats. Animals of this group were treated similar to other groups but received regular drinking water instead of the extract and 0.9% NaCl instead of the drug used to induce ulcer.

2.4.3 **Positive Control Group**

All studies conducted on gastric ulcer included a positive control group containing 7 rats with the exception of the intraperitoneal pre-treatment method. In the oral pre-treatment study, 11.5 mg/kg Cimetidine (commercially known as Tagamet) (XU 1998) was used as a reference drug. Doses were administered orally via a stainless steal intubation needle. Two doses were given per day with a 12 h interval. In the treatment experimental study, animals of the positive control group received omeprazole at a dose of 15 mg/kg (Gastrimut) (LABORATORIOS NORMON, S.A., Nieremberg, SPAIN) as a medication for gastric ulcer treatment (Poulsen et al., 1999). Omeprazole was dissolved in 0.25% methylcellulose and 0.9% saline. Animals received the drug intraperitoneally through two daily injections of 3mg doses each every 12 hours (Michael et al., 2001). No medication or a positive control group for the animals undergoing colonic ulcer was included in the study. The reason for that is that the treatment is usually a combination of drugs like prednisolone, azathioprine, and pentoxyfilline, and needs a period of about 10 to 12 months to give good results (Kasahara et al., 2002). In some cases, patients need a set of 4 regulating intestine prescriptions in order to be cured, and this might not be enough to eliminate colonic damage, therefore patient undergoes a surgery for ulcer removal (Fan et al., 2005).
2.5 Ulcer induction

2.5.1 Procedure for Gastric Ulcer induction

Rats were fasted for 18 - 24 hours before experimentation. Chemicals used for gastric ulcer induction are Indomethacin, minimum 99% TLC (SIGMA Chemicals, St. Louis, USA) and Tris (ACROS ORGANICS, New Jersey, USA). Gastric damage was induced by intragastric installation of indomethacin (10 mg/kg) dissolved in Tris buffer (50mM). After 12 hours, animals were sacrificed and their stomachs removed and opened by an incision along the greater curvature. The lumens of the stomachs were washed under running tap water. Using an illuminated stereomicroscope (10 x), long lesions were counted and measured along their greater length. Petechial lesions (very small lesions) were also counted and each five petechial lesions were considered equivalent to 1mm of ulcer. In order to calculate the ulcer index (cm) in each group, the sum of the total length of long ulcers and petechial lesions in each group of rats was divided by the number of rats in the group (Souza et al., 2004). The curative ratio was determined by the formula:

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

Figure 2.3: Intragastric installation of indomethacin to induce gastric ulcer
2.5.2 Procedure for Colonic Ulcer induction

Male rats were fasted for 18 - 24 hours prior to ulcer induction. Ulcerative colitis was induced by intrarectal administration of 0.2 ml of 3% iodoacetamide (SIGMA Chemical, St. Louis, Mo, U.S.A) with 1% methylcellulose (SIGMA Chemical, St. Louis, Mo, U.S.A). Animals were sacrificed 12 hours later and their colons were removed and opened (Levine et al., 2002). An incision was done exactly at the borders of the ulcerative bloody area, isolating it, in order for this surface area to be drawn on a blank white paper, and then photocopied with a resolution of 200%. The hemorrhagic surface areas were thus collected in terms of cut papers, and weighed.

2.6 Administration of the water extract of U.dioica

2.6.1 Prior to ulcer induction

In order to test the effectiveness of the water extract of Urtica dioica in preventing or reducing the occurrence of either gastric or colonic ulcer, the extract was given to five groups of 7 animals each for a period of 6 days prior to ulcer induction. Administration of the extract was done through drinking in a first trial, and by intraperitoneal injections in a second trial.

2.6.1.1 Rats that underwent Gastric Ulcer and Colonic Ulcer induction

2.6.1.1.1 Oral Administration

Procedure

Different concentrations were prepared for the different groups of animals as mentioned in section 2.3. Animals of the different groups received the appropriate doses (50, 100 and 250 mg/kg body weight) of Urtica dioica water extract via drinking water for a period of 6 days, until 24 hours before ulcer induction.
2.6.1.1.2 Intraperitoneal Administration

Procedure
In order to prepare the concentrated solution or the major solution from which the more dilute solution were obtained, 27g of dried plant leaves were simmered in pre-boiled 300ml of 0.9% NaCl for 20 min. After filtration and collection of the filtrate, a further purification was done by suction filtration and the final sterile solution was obtained through syringe filtration. A volume of 4 ml of the final solution injected intraperitoneally to rats weighing 250 g on average, is equivalent to 250 mg/kg body weight. Thus, according to their body weight animals received the appropriate injectable volumes. Using sterile 0.9% NaCl solution, dilution of the concentrated solution was performed to obtain the solutions that can be used as the 100 and 50 mg injectable doses. Thus, animals of the different groups received the set doses via intraperitoneal injections once daily for 6 days, until 24 hours before ulcer induction.

2.6.2 Post ulcer induction treatment
In order to test for its effect on the healing of the mucosal lesions in the stomach and in the colon after ulcer induction, Urtica dioica water extract was administered to rats either orally or intraperitoneally 12 hours post ulcer induction.

2.6.2.1 Gastric Ulcer induced in rats

2.6.2.1.1 Oral Administration
In this experiment, 35 rats were randomly allocated into 5 groups of 7 rats each. 12 hours after ulcer induction, when ulcer is at its maximum, animals started receiving Urtica dioica water extract through drinking water (50, 100 and 250 mg/kg body weight). After 24 h of the treatment (or 36 h post ulcer induction)
animals were sacrificed, and the ulcer prognosis was determined. In a second experiment, similar groups of rats were allocated, but in this experiment the plant extract was administered after 12 h and 36 h of ulcer induction. Animals were sacrificed 24 hours after the last treatment dose. Normally, gastric ulcer starts to heal automatically 48 hours after ulcer onset, therefore extending further the treatment with the water extract is not of importance (Poulsen et al., 1999).

2.6.2.1.2 Intraperitoneal Administration
The same protocol was followed as in section 2.6.1.1.2 to prepare the solutions to be injected intraperitoneally. The experimental protocol was similar to that described in section 2.6.2.1.1 with the exception that the animals received the different doses of the extract through an intraperitoneal injection rather than through drinking water.

2.6.2.2 Colonic Ulcer induced in rats
Colonic ulcer treatment is much more complicated and requires an unlimited period of time to be effective. In the present study, the effect of _U. dioica_ water extract, as a treatment remedy, was assessed after 15 days of ulcer induction and treatment. A dose of 250mg of _U. dioica_ water extract per kg body weight was selected since in the prevention experiment data showed that this dose exhibited maximum antiulcer activity in the colon. Animals were divided into 3 groups of 7 rats each. One group served as a control (no treatment), and the two remaining groups received the plant extract as a treatment through drinking water or via a single daily intraperitoneal injection for 15 days. Treatment was started 12 hours after ulcer induction, and animals were sacrificed 24 hours after the last daily dose received.
2.7 Ulcer Index Calculation

In gastric ulcer studies, the sum of the total length of long ulcers and petechial lesions in each group was divided by the number of rats in order to calculate the ulcer index (cm).

In colonic ulcer studies, the sum of the weight of papers representing the ulcerative areas in each group was divided by the number of rats in order to calculate the ulcer index (g).

2.8 Data handling and statistical methods

Values of the different tested parameters within each group are presented as mean ± SEM. Comparison between each two groups was made by independent t-test. A p value of less than 0.05 (p<0.05) was considered significant.
Chapter 3

RESULTS

3.1 Effects of Water Extract of *U. dioica* on preventing the occurrence of Gastric Ulcer in Rats

Animals were maintained for a period of 6 days on water extract of *U. dioica* and had a regular diet. At the end of the treatment, all rats were fasted for 24 hours, and gastric ulcer was induced. Hemorrhagic or ulcerative lesions were counted and the ulcer index calculated in the control and in the treated groups.

3.1.1 Oral Administration of Water Extract of *U. dioica*

Table 3.1 summarizes the ulcer index obtained in the stomach of control and experimental groups. Data have shown that all groups showed a significant decrease (P<0.05) in the ulcer index with respect to the negative control group.

3.1.2 Intraperitoneal Administration of Water Extract of *U. dioica*

Determination of the ulcer index in the intraperitoneally treated animals showed also a significant effect (P<0.05) in all groups with respect to the negative control group. Table 3.1 indicates that Group III showed that the (250mg/kg body weight) dose had the most significant effect when given intraperitoneally, in contrast to what was observed with oral treatment, where the lowest dose showed the most significant change.
<table>
<thead>
<tr>
<th></th>
<th>Negative Control (No treatment)</th>
<th>Positive Control (Cimetidine)</th>
<th>Group I 50 mg/kg</th>
<th>Group II 100 mg/kg</th>
<th>Group III 250 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Administration of <strong>U. dioica</strong></td>
<td>7.47 ± 0.22</td>
<td>4.78&lt;sup&gt;a&lt;/sup&gt; ± 0.25</td>
<td>2.63&lt;sup&gt;b&lt;/sup&gt; ± 0.20</td>
<td>3.19&lt;sup&gt;c&lt;/sup&gt; ± 0.25</td>
<td>5.23&lt;sup&gt;a&lt;/sup&gt; ± 0.30</td>
</tr>
<tr>
<td>Curative Ratio (with respect to Negative Control)</td>
<td>-</td>
<td>36 %</td>
<td>64 %</td>
<td>57 %</td>
<td>30 %</td>
</tr>
<tr>
<td>Intraperitoneal Administration of <strong>U. dioica</strong></td>
<td>5.03 ± 0.39</td>
<td>-</td>
<td>2.41&lt;sup&gt;b&lt;/sup&gt; ± 0.56</td>
<td>1.27&lt;sup&gt;d&lt;/sup&gt; ± 0.23</td>
<td>1.14&lt;sup&gt;d&lt;/sup&gt; ± 0.11</td>
</tr>
<tr>
<td>Curative Ratio (with respect to Negative Control)</td>
<td>-</td>
<td>-</td>
<td>52 %</td>
<td>74 %</td>
<td>79 %</td>
</tr>
</tbody>
</table>

**Table 3.1**: Effect of water extract of *U. dioica* given orally or intraperitoneally in preventing gastric ulcer induced by indomethacin.

Values denote mean of ulcer index (cm) ± SEM (n=7)

<sup>a</sup>: Significant difference (p<0.05) with respect to Negative control, Group I, and Group II.

<sup>b</sup>: Significant difference (p<0.05) with respect to Negative control, Positive control, Group II, and Group III.

<sup>c</sup>: Significant difference (p<0.05) with respect to Negative control, Positive control, Group I, and Group III.

<sup>d</sup>: Significant difference (p<0.05) with respect to Negative control, and Group I.
Figure 3.1: Ulcer shown in removed stomachs of orally pre-treated groups and negative control group

3.2 Effects of Water Extract of *U. dioica* on preventing the occurrence of Colonic Ulcer in Rats

Animals underwent the same conditions as the oral groups, but at the 6\textsuperscript{th} day, colonic ulcer was triggered by intrarectal administration of lodoacetamide. 24 hours later, animals were sacrificed and the ulcer index calculated.

3.2.1 Oral Administration of Water Extract of *U. dioica*

Determination of ulcer index in the pretreated groups did not reveal any significant changes with respect to control (Table 3.2).
3.2.2 Intraperitoneal Administration of Water Extract of *U. dioica*

In comparison with values of the control group, the mean ulcer index of Group I and Group II have slightly decreased, however, no significant changes have been observed. A significant (P<0.05) decrease was only reached between the high dose group (250mg/kg body weight) and both the control and the remaining groups (Table 3.2).

<table>
<thead>
<tr>
<th>Preventive Treatment for Colonic Ulcer</th>
<th>Negative Control (No treatment)</th>
<th>Positive Control</th>
<th>Group I 50 mg/kg</th>
<th>Group II 100 mg/kg</th>
<th>Group III 250 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Administration of <em>U. dioica</em></td>
<td>0.08 ± 0.004</td>
<td>-</td>
<td>0.09 ± 0.006</td>
<td>0.10 ± 0.006</td>
<td>0.08 ± 0.005</td>
</tr>
<tr>
<td>Curative Ratio (with respect to Negative Control)</td>
<td>-</td>
<td>-</td>
<td>-13 %</td>
<td>-25 %</td>
<td>0 %</td>
</tr>
<tr>
<td>Intraperitoneal Administration of <em>U. dioica</em></td>
<td>0.08 ± 0.01</td>
<td>-</td>
<td>0.09 ± 0.008</td>
<td>0.05 *</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Curative Ratio (with respect to Negative Control)</td>
<td>-</td>
<td>-</td>
<td>-13 %</td>
<td>38 %</td>
<td>-13 %</td>
</tr>
</tbody>
</table>

Table 3.2: Effect of water extract of *U. dioica* given orally or intraperitoneally in preventing colonic ulcer induced by iodoacetamide.

Values denote mean of ulcer index (g) ± SEM (n=7)

\*\*\* Significant difference (p<0.05) with respect to Negative control, Group I, and Group II.
Figure 3.2: Ulcer shown in removed colons of orally pre-treated groups and negative control group.

3.3 Effects of Water Extract of *U. dioica* in the Treatment of Gastric Ulcer in Rats

The effect of *U. dioica* water extract was evaluated for gastric ulcer treatment in two sets of 5 groups of animals each. Evaluation was performed after 24 hours for the first set and 48 hours for the second set post-treatment.

3.3.1 Treatment for 24 hours

Treatment was started 12 hours after ulcer induction using indomethacin. At this stage gastric damage is at maximum level. 24 hours later, animals were sacrificed, stomachs removed and ulcer indexes were calculated.

3.3.1.1 Oral Administration of Water Extract of *U. dioica*

A significant decrease in the ulcer index was detected in animals of the group III (250mg/kg body weight) with respect to the negative control group. However, a
significant increase in ulcer index was observed in animals of group I (50mg/kg body weight) when compared to animals of the positive control group (animals treated with Omeprazole as an anti-ulcerative drug). Animals of group II (100 mg/kg body weight) and positive control group had similar ulcer index. Results are shown in table 3.3.

3.3.1.1 Intraperitoneal Administration of Water Extract of *U.dioica*

Determination of ulcer index in animals of the groups III revealed a significant decrease in ulcer index in comparison with the negative control group. However, the ulcer index in group I, II, and the negative control group were similar but significantly higher with respect to group III and the positive control group (Table 3.3).

3.3.2 Treatment for 48 hours

After 2 days of treatment with *U.dioica* water extract via drinking water or intraperitoneal injections, the ulcer index in the different groups was determined.

3.3.2.1 Oral Administration of Water Extract of *U.dioica*

Data in Table 3.4 reveals a decrease in the ulcer index for group III attaining significance with respect to group I, II, and negative control group. Animals of the group I, II, and negative control group had similar ulcer index.

3.3.2.2 Intraperitoneal Administration of Water Extract of *U.dioica*

Data in Table 3.4 shows that there are no significant changes between group I, group II, group III, and negative control. However, only positive control group showed a smaller ulcer index with respect to group I, and negative control group.
<table>
<thead>
<tr>
<th></th>
<th>Negative Control (No treatment)</th>
<th>Positive Control (Omeprazole)</th>
<th>Group I 50 mg/kg</th>
<th>Group II 100 mg/kg</th>
<th>Group III 250 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral Administration of U. dioica</strong></td>
<td>3.21 ± 0.38</td>
<td>2.32&lt;sup&gt;a&lt;/sup&gt; ± 0.46</td>
<td>3.70&lt;sup&gt;b&lt;/sup&gt; ± 0.34</td>
<td>2.76&lt;sup&gt;c&lt;/sup&gt; ± 0.34</td>
<td>1.90&lt;sup&gt;d&lt;/sup&gt; ± 0.25</td>
</tr>
<tr>
<td><strong>Curative Ratio (with respect to Negative Control)</strong></td>
<td>-</td>
<td>28 %</td>
<td>-2 %</td>
<td>14 %</td>
<td>41 %</td>
</tr>
<tr>
<td><strong>Intraperitoneal Administration of U. dioica</strong></td>
<td>3.34 ± 0.21</td>
<td>1.82&lt;sup&gt;d&lt;/sup&gt; ± 0.36</td>
<td>3.43&lt;sup&gt;e&lt;/sup&gt; ± 0.22</td>
<td>2.76&lt;sup&gt;f&lt;/sup&gt; ± 0.33</td>
<td>2.06&lt;sup&gt;g&lt;/sup&gt; ± 0.29</td>
</tr>
<tr>
<td><strong>Curative Ratio (with respect to Negative Control)</strong></td>
<td>-</td>
<td>46 %</td>
<td>-3 %</td>
<td>18 %</td>
<td>38 %</td>
</tr>
</tbody>
</table>

Table 3.3: Effect of *U. dioica* water extract as a treatment given orally or intraperitoneally after 24 hrs of gastric ulcer induction using indomethacin. Values denote mean of ulcer index (cm) ± SEM (n=7)

<sup>a</sup>: Significant difference (p<0.05) with respect to Negative control and Group I.
<sup>b</sup>: Significant difference (p<0.05) with respect to Positive control, Group II, and Group III.
<sup>c</sup>: Significant difference (p<0.05) with respect to Group I, and Group III.
<sup>d</sup>: Significant difference (p<0.05) with respect to Negative control, Group I, and Group II.
<sup>e</sup>: Significant difference (p<0.05) with respect to Positive control, and Group III.
<sup>f</sup>: Significant difference (p<0.05) with respect to Positive control.
<sup>g</sup>: Significant difference (p<0.05) with respect to Negative control, and Group I.
<table>
<thead>
<tr>
<th>Treatment of Gastric Ulcer for 48 hrs</th>
<th>Negative Control (No treatment)</th>
<th>Positive Control (Omeprazole)</th>
<th>Group I 50 mg/kg</th>
<th>Group II 100 mg/kg</th>
<th>Group III 250 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Administration of <em>U. dioica</em></td>
<td>0.62 ± 0.12</td>
<td>0.29&lt;sup&gt;a&lt;/sup&gt; ± 0.08</td>
<td>0.67&lt;sup&gt;b&lt;/sup&gt; ± 0.13</td>
<td>0.48&lt;sup&gt;b&lt;/sup&gt; ± 0.07</td>
<td>0.25&lt;sup&gt;a&lt;/sup&gt; ± 0.06</td>
</tr>
<tr>
<td>Curative Ratio (with respect to Negative Control)</td>
<td>-</td>
<td>54 %</td>
<td>-8 %</td>
<td>23 %</td>
<td>60 %</td>
</tr>
<tr>
<td>Intraperitoneal Administration of <em>U. dioica</em></td>
<td>0.48 ± 0.13</td>
<td>0.22&lt;sup&gt;c&lt;/sup&gt; ± 0.11</td>
<td>0.50&lt;sup&gt;d&lt;/sup&gt; ± 0.15</td>
<td>0.39 ± 0.07</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>Curative Ratio (with respect to Negative Control)</td>
<td>-</td>
<td>54 %</td>
<td>-4 %</td>
<td>19 %</td>
<td>30 %</td>
</tr>
</tbody>
</table>

Table 3.4: Effect of *U. dioica* water extract as a treatment given orally or intraperitoneally after 48 hrs of gastric ulcer induction using indomethacin.

Values denote mean of ulcer index (cm) ± SEM (n=7)

<sup>a</sup>b: Significant difference (p<0.05) with respect to Negative control, Group I, and Group II.

<sup>b</sup>c: Significant difference (p<0.05) with respect to Positive control, and Group III.

<sup>c</sup>d: Significant difference (p<0.05) with respect to Negative control, and Group I.

<sup>d</sup>e: Significant difference (p<0.05) with respect to Positive control.
3.4 Effects of Water Extract of *U. dioica* in the Treatment of Colonic Ulcer in Rats

Data presented in Table 3.5 revealed that neither oral nor intraperitoneal treatment with *Urtica dioica* water extract have any significant effect upon colonic ulcer treatment.

<table>
<thead>
<tr>
<th>Treatment of Colonic Ulcer for 15 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control (No treatment)</td>
</tr>
<tr>
<td>Oral Administration of water extract of <em>U. dioica</em></td>
</tr>
<tr>
<td>Intraperitoneal Administration of water extract of <em>U. dioica</em></td>
</tr>
<tr>
<td>0.092 ± 0.004</td>
</tr>
<tr>
<td>0.082 ± 0.004</td>
</tr>
<tr>
<td>0.091 ± 0.006</td>
</tr>
</tbody>
</table>

**Table 3.5**: Effect of *U. dioica* water extract as a treatment given orally or intraperitoneally after 15 days of colonic ulcer induction using iodoacetamide. Values denote mean of ulcer index (g) ± SEM (n=7)
DISCUSSION

The present study investigates the medicinal effects of water extracts of *U. dioica* upon indomethacin-induced gastric ulcer as well as on 2-cidoacetamide-induced colonic ulcer. The role of the plant extract was assessed for its role in prevention and treatment of both types of ulcers. In all studies, extract was administered via two ways, drinking water or intraperitoneal injections. In order to better evaluate the effect of the water extracts of *U. dioica* on ulcerative lesions in stomach and colon, three different doses were assigned (50, 100, and 250 mg/kg body weight). The rat model was chosen in order to avoid any possible toxic effect on human since no human studies have been reported, and because of the tissue damage that need to be induced in different studies. Also, human studies are conflicted by differences such as diet, smoking, physical activity, and other confounding factors.

*U. dioica* or nettle has been extensively studied pharmacologically. It has been shown to exhibit anti-hypertensive (Garnier et al., 1961), antirheumatic, anti-inflammatory (Obertreis et al., 1996; Riehemann et al., 1999), cardiovascular (Testai et al., 2002), natriuretic activities (Tahri et al., 2000), and stimulation of proliferation of human lymphocytes (Wagner et al., 1989). The effects of *U. dioica* are also suggested in the therapy of the prostatic hyperplasia (Krezeski et al., 1993; Hiramo et al., 1994; Lichius and Muth, 1997), and lately Gülçin et al. (2004) reported that the plant showed protection against ethanol-induced gastric ulcer and analgesic effect on acetic acid-induced stretching. In the present study, gastric ulcer was differently induced and the role in prevention and treatment of ulcer was investigated.
Pre-treatment over a 6 day period via oral administration of water extract of *U. dioica* showed significant reduction in gastric lesions produced by indomethacin. However, there was an inverse relationship between the extract dose and the protection against gastric ulcer. Results revealed that the protection against ulcer was 64% (dose of 50 mg/kg body weight), 57% (dose of 100 mg/kg) and 30% (dose of 250 mg/kg) for the extract of *U. dioica* used while the group that received cimetidine, as a reference drug, produced 36% protection. The 50 mg/kg body weight dose was about two times more effective in protection against gastric ulcer with respect to the reference drug. Since the lowest dose used exhibited the highest protection, further studies using smaller doses of the extract may be needed to locate the optimum dose. On the other hand, pre-treatment over a 6 day period via intraperitoneal administration of the aqueous extract of *U. dioica* resulted in a dose-dependent protection against gastric ulcer. Unlike the data observed in the oral pre-treatment study, the extract showed a direct relationship between the dose administered and the protection against gastric damage. Also, the intraperitoneal pre-treatment appeared to be more effective than the oral pre-treatment. Results revealed that the protection against gastric ulcer was 52% (dose of 50 mg/kg), 74% (dose of 100 mg/kg) and 79% (dose of 250 mg/kg) for the extract groups. The discrepancy observed between the intraperitoneal and oral pre-treatment methods may be due to a possible irritation of the mucosal lining of the stomach after continuous exposure of the mucosa to high doses of the extract. Alkofahi et al. (1999) tested the ability of many plants to prevent induced gastric damage and pointed out that all of the effective plant extracts contain tannins and/or flavonoids to which the anti-ulcerogenic activity could be attributed. Khennouf et al., (2003) showed that this inhibition might be related to the inhibition of the acid secretion before gastric ulcer induction. This property was also proved by other authors whose findings suggested that tannins extracted from *S. cumini* exhibited gastroprotective and anti-ulcerogenic effects (Ramirez 2003).

Furthermore, Tannins are used in medicine primarily because of their astringent properties (Dar et al., 1976; Ikram, 1977; Mammela et al., 2000), which are due
to the fact that they react with the proteins of the layers of tissue with which they come into contact. Tannins are known to "tan" the outermost layer of the mucosa and to render it less permeable and more resistant to chemical and mechanical injury or irritation (Borrelli and Izzo, 2000). In correlation with another study that tested the gastroprotective effect of other species of plants (Gharzouli et al., 1999; Khennouf et al., 1999), it could be inferred that protection of the gastric mucosa against hemorrhagic lesions produced by indomethacin was partly due to the presence of tannic acid in these species. Although tannins seem to be one of the important factors that help in gastric protection via tanning the stomach lining, the present study reveals that other mechanisms are important as well since the intraperitoneal pre-treatment was more effective than oral pre-treatment.

In the treatment experiment of gastric ulcer that was conducted for a period of 1 and 2 days, results showed that the groups receiving the dose of 250 mg/kg body weight of aqueous extract of *U. dioica* orally as well as intraperitoneally had the highest protection against gastric damage. In the oral treatment period for 1 day, the group that received the highest dose (250 mg/kg body weight) produced a significant curing effect of 41% (P<0.01 with respect to negative control) compared with the group that received Omeprazole as a reference drug and which showed 28% protection (P<0.05). Extract doses smaller than 250 mg/kg body weight were ineffective. Similar results were observed with the intraperitoneal treatment period for 1 day where significance was only reached in the group that received the highest dose (250 mg/kg body weight) and showed a curative effect of 38%. However, Omeprazole appeared to be more effective when given intraperitoneally and showed a 46% curative effect.

After two days of ulcer induction, all ulcers in control and experimental groups showed substantial healing where ulcer index regressed from about 3.2 cm to about 0.6 cm. However, the experimental groups receiving the highest dose of extract showed an ulcer index of 0.25 and 0.33 cm for the oral and intraperitoneal
treatment methods respectively. These values were comparable with Omeprazole, which showed an ulcer index of about 0.25 cm on the average. These results support the consensus that induced gastric ulcers in rats heal within a period of 48 hours. On the other hand, the extract appeared to have a promising effect on healing gastric ulcers when given orally or intraperitoneally. However, one should keep in mind that doses smaller than 250 mg/kg body weight are not effective in this respect.

Gastric ulcer induced by ethanol has been widely used for the experimental evaluation of anti-ulcerogenic activity. Ethanol, similarly to indomethacin, causes damage to gastric mucosa, disturbances in gastric secretion, permeability alteration, free-radical production and gastric mucus depletion (Salim, 1990). There are several important targets that should be dealt with during treatment of gastric ulcer. One of them is the reduction of aggressive acid in the stomach lumen which irritates the preformed lesion. This also must be supported with mucosal protection as suggested by Toma et al. (2005). Khennouf et al., (2003) have previously reported that the plant extract shows protection against gastric ulcer via inhibition of gastric acid secretion. In addition, U.dioica is known to contain tannins (Bellakhdar et al., 1991) which offers a mucosal protection since it "tans" the outermost layer of the mucosa and makes it less permeable and more resistant to chemical and mechanical injury or irritation (Borrelli and Izzo, 2000). Therefore, the extract meets the basic requirement of treatment of gastric ulcer; inhibition of gastric acid secretion and mucosal protection.

Gastric ulcer is also characterized by bleeding lesions. Therefore, one of the important steps in the treatment is to stop bleeding which may lead to anemia. Previous studies revealed that U.dioica is an important haemostatic agent since it stops internal bleeding (Grieve, 1984). Therefore, this characteristic further supports the fact that the plant is important in gastric ulcer treatment.

Treatment of gastric ulcer should also target the histamine release by blocking the H2 receptors in the parietal cells. Histamine is known to be one of the primary causes of inflammation. Therefore, reduction of inflammation is one of the key factors in the treatment of gastric ulcer (Steinbach et al., 1999). This is true for
U.dioica water extract which has been shown to have a potent anti-inflammatory action (Obertreis et al., 1996; Riehemann et al., 1999). A recent study by Erdelyi et al. (2005) demonstrated that tannins have an anti-inflammatory activity manifested by the inhibition of transcription of nuclear factor kappa B. The nuclear factor kappa B is known to induce the transcriptional up-regulation of various inflammatory responses such as interleukins and tumor necrosis factor alpha (TNFα) (Choi et al. 2003). In addition, the mechanism underlying the anti-inflammatory effect of tannins include the scavenging of radicals (antioxidant effect) and inhibition of expression of the mediators of the inflammatory response including cytokines, inducible nitric oxide synthase and cyclooxygenase-2 (Erdelyi et al., 2005). Moreover, it is generally known that flavonoids such as kaempferol and quercetin present in U.dioica are able to inhibit gastric injury through their anti-oxidant activity (Shirwaikar et al., 2003; Ligumsky et al., 1995). Therefore, it is likely that the anti-oxidant property of U.dioica could be also linked to its healing effect.

In this study, intrarectal administration of iodoacetamide induced colitis in all rats. Rectal bleeding and diarrhea were observed during the experiment, which confirm the statement of Lee (2005). In the oral pre-treatment study using the water extract of U.dioica for 6 days, results were found to be similar in the different groups. However, during the intraperitoneal pre-treatment study, it was shown that iodoacetamide induced colitis has significantly decreased in the group having the highest dose of the extract (250 mg/kg body weight) and was 44% below control. Flavonoids such as kaempferol and quercetin present in U.dioica can act as anti-oxidant agents (Shirwaikar et al., 2003; Ligumsky et al., 1995). These compounds can be behind the partial prevention of colonic ulcer induction since Seidner (2005) reported that antioxidants play a protective role against colonic inflammation and preserve mucosal integrity. However, Seidner (2005) added that a nutritional supplement including fibers and fish oil may ease ulcerative colitis. This is in contradiction with the present study since the oral pre-treatment, which dictates the presence of water-soluble fibers in the colon, was
ineffective with respect to the intraperitoneal pre-treatment where fiber do not reach the colon. Another study by Tasman (2006) contradicted that of Seidner (2005) concerning the appropriate nutritional diet for patients with colitis and supported the present study since it recommended that during acute colitis, a diet low in fat and fibers should be followed. Further experiments are needed in order to confirm whether fibers are beneficial for patients with colitis or not and possibly one should discriminate between different types of fibers that may have variable effects on ulcerative colitis.

Based on the data observed in the pre-treatment study of ulcerative colitis using the aqueous extract of *U. dioica*, only the highest dose of extract (250 mg/kg body weight) was chosen for the treatment of ulcerative colitis experiment. This study covered both the oral and the intraperitoneal treatment of colonic ulcer and came out with similar results among the different groups after 15 days of treatment. These findings confirm the consensus that ulcerative colitis needs a long-term therapy in order to be cured and in some cases surgery or proctoectomy might be necessary (Kasahara et al., 2002).

Briefly, the crude aqueous extract of *Urtica dioica* displayed various important and promising biological activities in the rat model. Although the different activities observed may be the result of action of different active ingredients, however the present study constitutes the backbone of future studies where further purification and assessment of the activity of each component may be done taking into consideration the preliminary results obtained in the present investigation. The extract showed potent protection against indomethacin-induced gastric ulcer. This protection was more effective with the intraperitoneal method of pre-treatment, although the oral pre-treatment method was also effective. However, in treatment of gastric ulcer only the 250 mg/kg body weight dose was effective regardless of the method of treatment used. Substances responsible for the anti-inflammatory activities such as tannins appeared to have
an anti-ulcerogenic effect. In addition, antioxidants present in the extract were shown to be protective against both gastric and colonic ulcers. An overall conclusion can be drawn here, that water extracts of *U. dioica* can be used as an inexpensive remedy for the prevention and treatment of gastric ulcer. Finally, these findings remain to be backed up with further work especially on the part concerning ulcerative colitis treatment.
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