GASTROPROTECTIVE ACTIVITY OF HYDROALCOHOLIC EXTRACT OF THE AERIAL PARTS OF WATERCRESS (Nasturtium officinale) ON ASPIRIN-INDUCED ULCER IN MALE WISTAR RATS (*Rattus norvegicus*)

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ABSTRACT

Several medicinal plants are used in folk medicine to treat gastrointestinal disorders. In this study, the hydroalcoholic extract of the aerial parts of Nasturtium officinale (HAENO) was used to investigate its gastroprotective effect in Aspirin-induced ulcer in male Wistar rats (Rattus norvegicus) which were randomly divided into 5 groups. The rats were orally pre-treated with water (ulcer control group), Omeprazole® 20mg/kg (reference group), 100,200 and 300 mg/kg of HAENO (experimental groups) before oral administration of Aspirin® 30mg/kg to generate gastric mucosal injury. The rats were sacrificed to determine presence of ulcer in their stomachs. The ulcer control group exhibited severe mucosal injury, the reference group exhibited severe bleeding but no visible ulcer formed, whereas groups pre-treated with HAENO exhibited protection in the rats' gastric mucosa. These results suggest that HAENO promotes ulcer protection as ascertained grossly by significant reduction of ulcer area.

Key words: *watercress, gastroprotective, gastric ulcer, hydroalcoholic, wistar, aspirin-induced ulcer*

INTRODUCTION

The stomach plays a pivotal role in the chemical breakdown of food and it also produces a thick mucus that keeps acids from damaging the stomach lining (Zatorski, 2017). If not enough mucus is produced or if too much acid is produced, open sores on the stomach walls develop which may heal on its own over time, but deeper and larger erosions termed as an ulcer can cause serious harm.

Most common causes of peptic ulcer disease are bacterial infection caused by Helicobacter pylori and long-term use of non-steroidal anti-inflammatory drugs (NSAIDs). Alcohol abuse, smoking, stress, eating spicy foods and excessive consumption of coffee can worsen these ulcerations and may make the healing process of already existing ulcerations slower. The main symptom of a stomach ulcer is an upper abdominal pain, which can be a dull, sharp, or a burning sensation (Marks, 2017). According to the latest World Health Organization (WHO) data published last May 2014, Peptic Ulcer Disease Deaths in the Philippines reached 6,234 or 1.20% of total deaths. The age adjusted Death Rate is 10.98 per 100,000 of population with the Philippines have been ranked as no. 21 in the world. As to the growing population of the Philippines, these figures are expected to rise even more.

There is a variety of approaches to treat peptic ulcer disease but some of the available commercial ulcer medications were found to have side effects. Long-term use of proton pump inhibitors may increase the risk of hip, wrist and spine fracture and the use of antacids can result in constipation and diarrhea (Mayo Clinic, 2018). It is therefore important to investigate potential gastroprotective agents against possible ulcer development. Since organic plants are widely used in the traditional systems of medicine, it might provide an efficient ulcer management.

Watercress (*Nasturtium officinale*) which belongs to the family Brassicaceae can be found in shallow waters and small streams. It is known for its folkloric use as an aid in digestion and can improve appetite. To the researchers' knowledge, there are still no study proving the effect of watercress on the stomach, therefore the present study is an attempt to assess the efficacy of the hydroalcoholic extract of the aerial parts of this plant for its gastroprotective activity on the Aspirin-induced ulcer in male albino rats of Wistar strain (*Rattus norvegicus*).

The Centers for Disease Control and Prevention (CDC) categorizes watercress as a "powerhouse" vegetable in a nutritional profiling done last 2014. Powerhouse fruits and vegetables pack a lot of key nutrients into each calorie and are linked with a reduced risk of chronic diseases. Of 47 foods studied, 41 satisfied the powerhouse criterion and watercress tops the list.

Research Questions

Generally, this study aimed to determine the gastroprotective activity of the hydroalcoholic extract of the aerial parts of watercress (*N. officinale*) on the Aspirin-induced ulcer in male Wistar rats (*R. norvegicus*).

Specifically, it aimed to answer the following questions:

1. What is the gastroprotective activity exhibited by the different treatments against the Aspirin-induced ulcer in male Wistar rats (*R. norvegicus*)?
2. Is there a significant difference between the gastroprotective activity of the positive control Omeprazole® to the experimental watercress (*N. officinale*) hydroalcoholic extract against the Aspirin-induced ulcer in male Wistar rats (*R. norvegicus*)?
3. Is there a significant difference in the gastroprotective activity of the different doses of the experimental control watercress (*N. officinale*) hydroalcoholic extract against the Aspirin-induced ulcer in Wistar rats (*R. norvegicus*)?

Hypothesis

1. There is no significant difference between the gastroprotective activity of the positive control Omeprazole® to the experimental watercress (*N. officinale*) hydroalcoholic extract against the Aspirin-induced ulcer in male Wistar rats (*R. norvegicus*).
2. There is no significant difference in the gastroprotective activity of the different doses of the experimental control watercress (*N. officinale*) hydroalcoholic extract against the Aspirin-induced ulcer in Wistar rats (*R. norvegicus*).

Significance of the Study

This research study will be beneficial to the community by increasing the awareness of the public to an alternative and natural remedy for peptic ulcer disease. Also, this study will provide immediate medication to those people in remote areas where medical services are hard to avail since they can grow their own watercress plant. This research aims to safeguard good health against peptic ulcer disease which concerns the Filipino people. This endeavor could become a useful tool in the medical field for further understanding of the therapeutic effect of this plant. The researchers will also benefit from this by gaining learning experiences throughout the whole research process.

Literature Review

Ethnobotany of Watercress (*Nasturtium officinale*)

The leaves and aerial stems of watercress are rich sources of proteins, dietary fibers, vitamins, minerals, other essential nutrients, and phytochemicals, and inflammation.

Watercress is generally used in traditional medicine as a treatment of diabetes, bronchitis, abdominal pain, asthma, chest pain, skin afflictions, facial scars, iron deficiency. It is also used to purify the blood, to stop hemorrhages and to eliminate excess bile. It is found to be good for the liver, gallbladder, kidneys, and lungs (Pandey, Bhatt, & Debbarma, 2018).The study of Meriem, Soumia, and Fairouz (2017) affirms that the high concentrations of *N. officinale* extract can exert an intersecting in vitro antioxidant potential and it may be attributed to its components' effectiveness as scavengers of free radicals which is helpful in dealing with inflammations. Watercress is rich in many vitamins such as Vitamin C (62 mg / 100 g servings), Vitamin A (420 µg / 100 g servings, Vitamin E (1.46 mg / 100 g servings), Vitamin K (250 µg / 100 g servings), gluconasturtine, folate acid, iodine, iron, protein, and calcium. Watercress contains flavonoid compounds (quercetin and kaempferol) which can act as antioxidants (Haro et al., 2018).

Traditional and Alternative Medicine Act of 1997

Using herbal medicine was a practice a long time ago. The community utilizes their available resources in order to prevent and manage their diseases such as infections. Herbal medications are not only considered for their effective and cost-effective way of preventing or managing infections but also, due to their constant availability in the community.

Republic Act 8423 (RA 8423) also known as the Traditional and Alternative Medicine Act of 1997, focuses on developing different traditional health-related management in the country. Drugs for prevention, cure, lessening signs and symptoms, diagnosis and maintaining a healthy lifestyle with lower price are needed to be explored and developed. The alternative medications undergo methods of proper compounding.

This law encourages the indigenous people to share their traditional medicines and for people to study more about the safety and effectiveness of these alternative medicines. The healthcare professionals should become aware of these alternative medications and promote to their patients. By this, our countrymen would encounter more alternative medicines coming from that cost much lesser than the existing drugs. The cheaper the medicines get, the more patients will comply with medication.

Pathophysiology of Peptic Ulcer Disease

Under normal conditions, a physiologic balance exists between the protective factors — mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis, normal tissue microcirculation, nitric oxide and the aggressive factors — gastric acid, abnormal motility, pepsin, bile salts, oxidative stress, use of alcohol and nonsteroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (Helicobacter pylori and others). When the balance between these factors is disrupted, it can lead to the development of ulcerations (Silva & de Sousa, 2011). If left untreated, PUD may lead to penetration, which occurs when the ulcer goes through the wall of the digestive tract and into another organ, such as the pancreas; perforation, which occurs when the ulcer creates a hole in the wall of the digestive tract; obstruction (blockage) in the digestive tract, which is due to swelling of inflamed tissues or stomach cancer (Marcin, 2017).

Omeprazole®

This medication is a proton pump inhibitor that is used to treat gastroesophageal reflux disease (GERD), heartburn, possible injury of the esophagus, conditions in which the stomach produces too much acid, ulcers and to treat and prevent the return of ulcers caused by a certain type of bacteria (Helicobacter pylori). Omeprazole® may cause side effects such as constipation,

gas, nausea, vomiting, and headache. It is not an immediate relief of heartburn symptoms. It may take 1 to 4 days for you to feel the full benefit of the medication. In some cases, long-term use of Omeprazole® may develop weakening of the stomach lining (MedlinePlus, 2107). This antisecretory drug is known for its ability to protect gastric mucosa against the indomethacin-induced gastric ulcer (Morjan, Al Laham, & Atieh, 2013).

Aspirin®

Aspirin® is a non-steroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic activity. It is a nonselective inhibitor of the two cyclooxygenase isoforms ─ COX-1 and COX-2. Cyclooxygenase enzyme is responsible for catalyzing the rate-limiting step in prostaglandin synthesis via the arachidonic acid pathway. Inhibition of the action of cyclooxygenase results in suppression of a number of prostaglandin-related protective functions. Furthermore, prostaglandins play a role in maintaining adequate blood flow in mucosal microcirculation (Zatorski, 2017). Aspirin directly and irreversibly inhibits the activity of cyclooxygenase enzymes which is its distinguishing characteristics from other NSAID drugs.

Research Paradigm

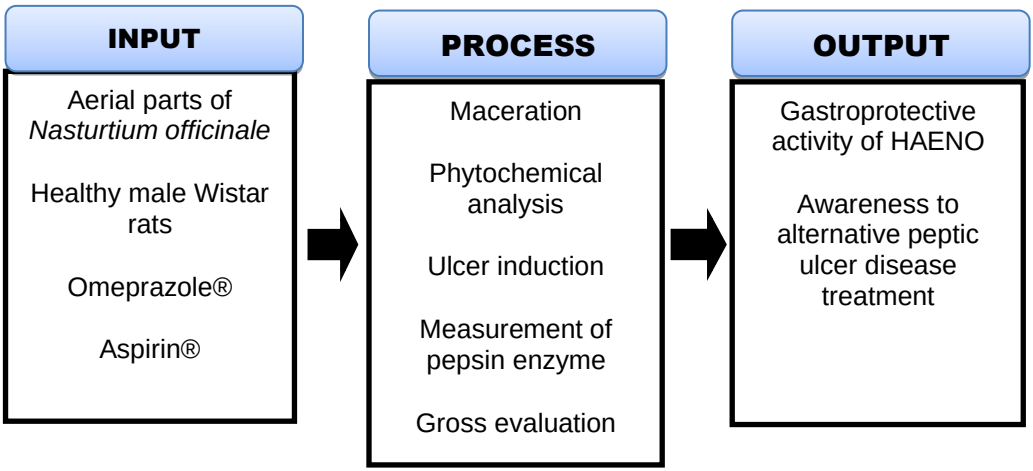

Figure 1. *Research Paradigm*

The paradigm of this study is presented using the IPO model ─ Input, process, and output. The inputs reflect the resources that the researches will use which include the plant N. officinale, the reference drug Omeprazole® and the NSAID drug Aspirin® that will be used to induce ulcer in the experimental rats. The process includes; obtaining the hydroalcoholic extract of N. officinale, quantitation of pepsin enzyme activity and gross evaluation of the excised stomach.

METHODS

Research Design

Experimental method was used in this research study. The diagram below presents the entire process that the researchers conducted.

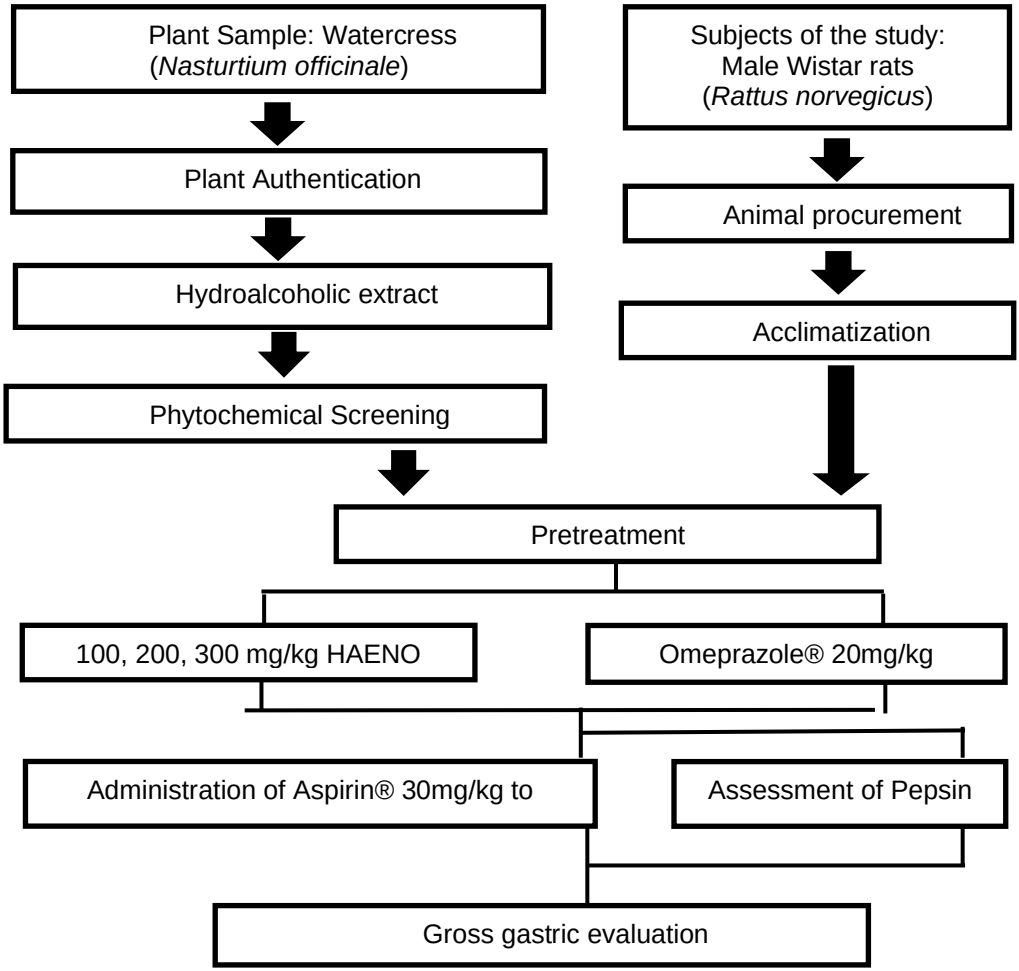

Figure 2. *Methodological Flow*

Subjects of the Study

Healthy male albino rats of Wistar strain were used in this study because according to the 2013 Philippine Health Statistics, from ages 25-34 years, gastric peptic ulcer occurs most commonly in male than in the female. For feeding, conventional laboratory diet (GMP1) was used with an unlimited supply of drinking water. The rats were separated randomly per cage, 3 rats each group.

Data Gathering Procedure

1. Collection of Plant Material: Watercress plant (*Nasturtium officinale*)

The plant material was collected by the researchers in Tabuk City, Cagayan where Watercress plants are usually grown. After collection, the aerial parts of Watercress plants were washed with water to remove foreign matter and were allowed to shade dry until a crisp texture is obtained. The botanical verification and the authentication of the plant was done at the Bureau of Plant Industry located at Carig Sur, Tuguegarao City, Cagayan.

2. Extraction of Plant Material

Extraction of plant material was done according to the method used by Sadeghi, Mostafazadeh, Naderian, Barmak, Talebianpoor and Mehraban (2013).The shade-dried aerial parts of Watercress plants were powdered using an electrical blender. 300 g of the powdered plant were extracted with a 1000ml mixture of EtOH-H_2O (7:3) for 48 hr. It was filtered using Whatman no.1 filter paper and the organic solvent was completely evaporated by putting the filtrate in a water bath at 65ºC.

3. Phytochemical Screening

A preliminary phytochemical screening was performed with the hydroalcoholic extract of *N. officinale* (HAENO) for the detection of various phytochemicals and it was conducted by the Department of Science and Technology.

4. Gastroprotective studies

The procedure was conducted according to the method utilized by Khazaei & Salehi (2006) with some modifications.

4.1. Ulcer induction by Aspirin

Food was withdrawn for 24 hours and water one hour before the pre-treatment. Three treatment groups received different doses of the plant extract orally via stainless steel intubation needle. Two doses were given at 08:00am and 04:00pm

and a third dose was given on the second day 1.5 hours before the induction of gastric ulceration with Aspirin® 30mg/kg.

- Group I: Ulcer control group received water
- Group II: Positive control pre-treated with Omeprazole® 20mg/kg
- Group III: Pre-treated with HAENO (100mg/kg)
- Group IV: Pre-treated with HAENO (200mg/kg)
- Group V: Pre-treated with HAENO (300mg/kg)

The following day, the rats were sacrificed (only one rat per group, chosen randomly) and dissected. The rats' stomachs were excised and opened along the greater curvature for gross evaluation.

4.2. Measurement of pepsin activity

Blood samples were collected from the animal subjects after the pre-treatment and after the ulcer induction by Aspirin®. Quantitation of pepsin enzyme activity was performed using a spectrophotometer. Results of the drug control and the treatment control was compared to the normal control to check any deviation.

4.3. Gross gastric evaluation

The rats' stomachs were washed in a running tap water to remove any waste and gastric juice to evaluate the ulcer areas in the gastric wall.

Disposal of the Experimental Animals

The Philippine Institute of Traditional and Alternative Health Care (PITAHC) have their own policy on how to dispose experimental animals. The researchers entrusted the experimental animals under their care after conducting the experiment.

Ethical Considerations

The researchers requested the authentication of the plant material and animal subjects that were utilized in the study. Authentication of the animal model was done in the United Doctors Animal Clinic located in Santiago City and authentication of the plant model was done in Bureau of Plant Industry located in Carig Sur, Tuguegarao City, Cagayan.

The researchers requested permission at Philippine Institute of Traditional and Alternative Health Care regarding the housing of the animal models, in which proper cages and animal disposal were taken in consideration to avoid contamination and spread of disease.

The researchers asked permission from the University for the conduction of this study and to request permit for the utilization of laboratory equipment and the medical technology laboratory.

And lastly, the researchers asked permission from a registered Veterinarian in the extraction of blood samples and dissection of the animal models taking in consideration the laws that are being implemented in proper utilization of laboratory rat models.

RESULTS

Table 1. *Phytochemical Screening of Hydroalcoholic Extract of Watercress*

CONSTITUENT	RESULT
Flavonoid	(+)
Tannins	(+)
Saponin	(-)

Phytochemical screening of the hydroalcoholic extract of Watercress (N. officinale) is positive for the presence of Flavonoids and Tannins.

Table 2. *Test of Significant Difference in the Gastroprotective Activity between Hydroalcoholic Extract of Watercress and Omeprazole*

Treatment	t-value	p-value	Decision
Watercress 100 mg/kg	-1.000	.423	Accept Ho
Watercress 200mg/kg	-17.321	.003	Reject Ho
Watercress 300mg/kg	-35.000	.001	Reject Ho

The table 2 above shows the paired result of the positive control Omeprazole that has a dosage of 20 mg with experimental control Watercress (N. officinale) which has doses of 100 mg/kg, 200 mg/kg and 300 mg/kg. The calculated results are 0.423, 0.003 and 0.001 respectively. This means that there is a significant difference between the Omeprazole and the Watercress. With a 300 mg/kg of experimental extract Watercress, it yields more potency in treating an aspirin-induced ulcer than that of the 20 mg of positive control Omeprazole.

Table 3. *Test of Significant Difference in the Gastroprotective Activity between the different concentrations of the Hydroalcoholic Extract of Watercress*

Treatment	t-value	p-value	Decision
Watercress 100 mg/kg – 200mg/kg	-10.583	.009	Reject Ho

Watercress 100 mg/kg – 300mg/kg	-34.000	.001	Reject Ho
Watercress 200 mg/kg - 300mg/kg	-3.464	.074	Accept Ho

DISCUSSION

Various factors are playing a pivotal role in pathogenesis of peptic ulcer disease. Imbalance between protective factors ─ mucus secretion, bicarbonate production, gastroprotective prostaglandin synthesis, normal tissue microcirculation, nitric oxide and the aggressive factors ─ gastric acid, abnormal motility, pepsin, bile salts, oxidative stress, use of alcohol and nonsteroidal anti-inflammatory drugs (NSAID), as well as infection with microorganisms (Helicobacter pylori and others) cause damage to the gastric mucosa.

Aspirin® caused severe damage to the gastric mucosa as seen in Figure 3a (ulcer control group). Rats pretreated with Omeprazole® (Figure 3b) has no visible ulcer formation instead, it exhibited severe hemorrhage. Rats pretreated with 100mg/kg and 200mg/kg HAENO (Figure 3c and 3d respectively) showed no visible ulcer formation, instead flattening of the gastric mucosa can be seen. Lastly, rat pretreated with 300mg/kg HAENO exhibited moderate injuries to the gastric mucosa.Aspirin-induced ulcer is due to its ability to block the action of cyclooxygenases. Inhibition of cyclooxygenases will result in the suppression of prostaglandin-related protective functions. For instance, prostaglandins play an important role in maintaining adequate blood flow in mucosal microcirculation, it increase the mucus production and also modulates gastric acid secretion (Zatorski, 2017). Omeprazole®, a proton pump inhibitor, offered a fairly protected gastric mucosa and has been widely used to treat disorders of gastric secretion.

In an attempt to correlate the effects of the extract observed in this study with the chemical compounds present in *N. officinale*, the researchers carried out a literature review and found that flavonoids and tannins have been extensively confirmed to have an anti-ulcerogenic efficacy (Borelli et al., 2000). Tannins have an astringent action that can help in precipitating micro proteins on the ulcer site, thereby forming an impenetrable layer over the lining that protects the underlying mucosa from toxins and other irritants (Berenguer et al., 2005). While flavonoids have the ability to reduce free radical formation and scavenge free radicals owing to its anti-oxidant property (Pietta, 2000).

CONCLUSION

In conclusion, an aspirin-induced ulcer in Male Wistar rats treated with the hydroalcoholic extract of the aerial parts of the watercress (*N. officinale*) and the

positive control Omeprazole® has no signs of abnormality. Using a spectrophotometer and gross examination, it shows that the 300 mg/kg of Watercress (*N. officinale*) yields more potency among the three different concentration of the HEANO. Thus, the ulcer formation in the gastric mucosa lining has been lessen better with the 300 mg/kg Watercress than that of the 20 mg Omeprazole®. This plant could significantly protect the gastric mucosa against aspirin-induced ulcer in Male Wistar rats. This study proves that the Watercress plant *(N. officinale*) has a gastroprotective effect which is related to the preservation of the gastric mucosa secretions.

RECOMMENDATIONS

Based on the findings of the researchers' on the Gastroprotective Activity of Hydroalcoholic Extract of the Aerial parts of Watercress (*N. officinale*) on the Aspirin-induced Ulcer in Male Wistar Rats (*R. norvegicus*), here are the recommendations for the enhancement of this study.

- Perform further studies in order to elucidate fully the biochemical constituents of the plant material;
- Perform histopathological examination on the excised stomach;
- Perform additional studies to determine other uses of Watercress

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