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# PHYTOCHEMICAL SCREENING AND IN VIVO ANTI-ULCER ACTIVITY OF METHONOLIC EXTRACT OF CRESSA CRETICA (LINN)

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#### **ABSTRACT**

An ulcer is defined as "disruption of the mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation". Ulcers may range in size from several millimeters to several centimeters starting from small, painful sores in the mouth to bedsores and serious lesions of the stomach or intestine. Different types of ulcers are seen such as, Peptic ulcer, Duodenal Ulcer, Gastric ulcer, Esophageal ulcer, Bleeding ulcer, Refractory ulcer, Stress ulcer. Most of patients with peptic ulcers present with abdominal discomfort, pain, or nausea. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases.

Preclinical experiments are done to screen the anti ulcer activity in herbal drugs using various animal models. *Cressa cretica* (Linn) belonging to family Convolvulaceae, commonly known as Rudravanti is a erect, small, dwarf shrub, usually grows in sandy or muddy saline habitats. *Cressa cretica* is a plant that is referred to by the name that reflects the features of *Sanjeevani*. So this plant is commonly known as Sanjeevani in Sanskrit as it prolongs the life and prevents the onset of old age. It used as antibilious, antitubercular and expectorant. anthelmintic, stomachic, tonic and aphrodisiac purposes, enriches the blood and is useful in constipation, leprosy, asthma and urinary discharges, in the treatment of diabetes.

**KEYWORDS:** Peptic ulcer, Herbal medicine, *Cressa cretica*, Pylorus ligation.

# INTRODUCTION

A peptic ulcer is erosion in the lining of the stomach or duodenum. The word "peptic" refers to pepsin, a stomach enzyme that breaks down proteins and it is also known as "ulcus pepticum". [1] Most ulcers occur in the first layer of the inner lining. Peptic ulcer results from lesions in the gastric or duodenal mucosa caused or exacerbated by gastric acid and pepsin, or as the result of a confrontation between acid aggression and mucosal defence. The lining of the duodenum is at continuous risk of erosion by the acidic juices produced by the stomach walls. The lower part of the esophagus is at risk only if and when the reflux of the acidic juices from the stomach takes place. Peptic Ulcers arise in the jejunum, only when there is a massive secretion of the gastric juices. Some of the main causes of the occurrence of peptic ulcers mostly could be by the consumption of a lot of alcohol, or by excessive coffee drinking which induces high caffeine intake, or even by regular ingestion of aspirin. [2,3] Other irritants could also be bile and bacteria among others. They are also caused by an increase in acid secretion and a reduction in mucus production. For some people, peptic ulcers occur due to genetics, as they are pre-disposed to developing these ulcers hereditarily. If the occurrences are frequent in the family's medical history, it is important to take preventive measures. Psychological stress also plays a major part in aggravating an existing ulcer, thus making it even worse. Even smoking worsens ulcers, as the nicotine in tobacco increases the amount and concentration of acids in the stomach and thus intensifying the existing ulcer or it could also lead to the creation of more ulcers. Smoking may also slow down the treatment and healing process of ulcers. Both men and women are equally prone to the incidence of gastric ulcers, but when it comes to duodenal ulcers, more men are likely to suffer from them than women.[3]

Plant based drugs have been in use against various diseases since time immemorial. The primitive man used herbs as therapeutic agents and medicament, which they were able to procure easily. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. <sup>[4]</sup> Although herbal medicine has existed since the dawn of time, our knowledge of how plants actually affect human physiology remains largely unexplored. *C. cretica* L., belonging to the family Convolvulaceae, is a perennial plant with a lifecycle that continues in the summer period when the salt marsh area drains. It is reported to be antibilious, antitubercular, and expectorant. The plant is used as anthelmintic, stomachic, tonic and aphrodisiac purposes, enriches the blood, and is useful in constipation, leprosy, asthma, and

urinary discharges. The plant is traditionally used in Bahrain as expectorant and antibilious agent. Dry leaves of *C. cretica* crushed with sugar are used as emetic.<sup>[5]</sup> In the present study methanol is used for the extraction process which helps to yield photochemical present in the plant.

# MATERIALS AND METHODS

# **Collection and Authentication of Plant Material**

Plants were collected from the fields of Kakumanu Village, Guntur Disrtict, Andhra Pradesh, India. The plant was authentificated as The plants were authentified by Head of the Botany department Professor G.Pulla Rao Pragathi Jr college Sattenapalli by observing the morphological and microscopic characters the plant was identified as *Cressa cretica*. It was shade dried in room temperature and then made into coarse powder. The powdered plant material was extracted by methanol by using soxhlet extraction apparatus and the solvent is completely removed under reduced pressure by rotavapour apparatus. Methanolic extract of *Cressa cretica* plant extract was used in the entire study.

# **Preliminary Phytochemical Analysis**

The methonolic extract of *Cressa cretica* was subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents, it revealed that the presence of Alkaloids, Glycosides, Carbohydrates, Tannins, Proteins, Amino acids.<sup>[5]</sup>

#### **Animals**

Healthy Male Albino wistar rats weighing between 150-200 gm were used for the study. A Total of 24 animals was maintained in ployacrylic cages with standard lab conditions (temperature 25±2°C and relative humidity under a 12 hr light/dark cycle) and fed with standard pellet diet and water *ad libitum*. The rats were acclimatized to laboratory conditions a weak before the work.

# In vivo Study

The Albino Wistar rats weighing about 150-200 gms were divided into six groups consisting of five each. All the four groups were followed the pylorus ligation method for induction of ulcers. Group- I was given 0.9%(w/v) saline and served as normal control and group-II was served as standard control which administered with Cimetidine (10mg/kg I.P) remaing III and IV groups treated with Alcoholic extract 200mg and 400mg/k.g b.w *p.o*)respectively. V and VI groups treated with Polyherbal extract 200mg and 400mg/k.g b.w *p.o*)respectively.

The animals were fasted for 48 hrs before the operative procedure was started. Anaesthetize the fasted rat with anesthetic ether. Secure the rat on the operating table. Give an incision of 1cm long in the abdomen just below the sternum. Expose the stomach and pass a thread around the pyloric sphincter and apply knot tightly. While putting the knot care should be taken so that from blood vessel is tied along the knot. Close the abdomen wall by putting the sutures. Clean the skin from any blood spots and bleeding. Keep the rat in a separate cage and allow it to recover. [6,7]

After 15 min perform pylorus ligation was done. The test drug is administered either orally and the animals are placed in plastic cylinders. About 17 – 19 hrs after pyloric ligation, the animals are sacrificed and the stomach was dissected out. The contents of the stomach are drained into a graduated centrifuge tube and their acidity is determined by titrating it with 0.1N NaOH. The stomach is opened along its greater curvature.<sup>[8]</sup>

# **Acute toxicity**

At a dose of 1.6 g/Kg the extract of the *Cressa cretica* did not exhibit any toxic effects as shown by workers. Dose ranging from 200 - 400mg/Kg of the extract of the drugs was used for studies. Hence based on all those studies the dose ranging from 10mg/Kg upto 400 mg/Kg was selected for this study.<sup>[5]</sup>

# **Ulcer Index**

The pylorus is ligated and the abdominal wall is closed by sutures. 3 hours after pyloric ligation, the animals were sacrificed. The abdomen was opened and a ligature was placed around the esophagus. The stomach was removed and fixed on a cork plate and the number of and severity of ulcers was registered with a stereo-microscope. [9] The percentage of ulcer inhibition was determined as follows:

 $Ulcer\ Index\ = \frac{Arithmetic\ mean\ of\ intensity\ in\ a\ group\ x\ number\ of\ ulcer\ positive\ animals}{Total\ number\ of\ animals}$ 

# **Total acidity and Free Acidity**

A known amount of gastric residue was titrated with 0.1 N sodium hydroxide to a pH of 3.5. If pH meter is not available, add two drops of Topfer's reagent which changes to a salmon colour when all the free hydrochloric acid is neutralized. The total acidity however was determined by titration using phenolphthalein as indicator and titrated with 0.1 NaOH from a burette, mixing was done after each addition until the last trace of red colour disappeared and

was replaced by a canary yellow colour. The numbers of milliliters of NaOH used was read from the burette. This represents the amount of free hydrochloric acid. [10,11]

# pН

The abdomen was opened and a ligature was placed around the esophagus. A small nick along the greater curvature adjacent to the pyloric ligation was done. The stomach was removed and the contents were drained into a graduated centrifuge tube. The tubes were centrifuged at 3000 rpm for 10 minutes and the centrifuged samples were decanted and analyzed for pH.<sup>[12]</sup>

#### Gastric volume

The abdomen was opened and a ligature was placed around the esophagus. The stomach was removed and the contents were drained into graduated centrifuge tube through a small nick along the greater curvature adjacent to pyloric ligation and the volume of the juice was measured.<sup>[13,14]</sup>

# **RESULTS**

Table 1: Results of Alcoholic extract of Poly Herbal extract in curing Peptic ulcer.

| S.NO | PARAMETERS               | CONTROL    | STANDARD     | TEST 1       | TEST 2       |
|------|--------------------------|------------|--------------|--------------|--------------|
| 1    | Ulcer index              | 12.66±0.75 | 3.83±0.75**  | 4.5±0.54**   | 3.66±0.51**  |
| 2    | Total acidity (mEq/l)    | 67.8±2.63  | 30.3±4.14**  | 30.08±4.51** | 30.8±3.60**  |
| 3    | Free acidity (mEq/l)     | 44.0±3.22  | 19.66±2.06** | 20.0±1.67**  | 19.83±1.47** |
| 4    | pН                       | 1.61±0.17  | 4.13±0.25**  | 3.95±0.18**  | 4.05±0.25**  |
| 5    | Gastric volume (ml/100g) | 3.78±0.21  | 2.0±0.52**   | 1.83±0.64**  | 1.88±0.70**  |

Control: Saline solution (0.9% w/v), standard: Cemetidine (10mg/kg), Test 1: 200mg/kg plant extract and Test 2: 400mg/kg. plant extract p value is 0.01 \*\* - significant 0.05 \*\*\* - highly significant.

Table 2: Results of Aqueous extract of Poly Herbal Extract In curing Peptic ulcer.

| S.NO | PARAMETERS               | CONTROL     | STANDARD    | TEST 1      | TEST 2      |
|------|--------------------------|-------------|-------------|-------------|-------------|
| 1    | Ulcer index              | 25.67±0.84  | 7.24±3.38** | 21.06±0.61  | 10.10±0.01* |
| 2    | Total acidity (mEq/l)    | 114.24±0.22 | 34.65±0.13  | 100.18±0.34 | 30.8±3.60** |
| 3    | Free acidity (mEq/l)     | 90.17±5.28  | 20.24±6.78  | 84.61±0.31  | 36.71±0.11  |
| 4    | pН                       | 1.58±0.15   | 4.90±3.12   | 3.01±1.32   | 4.7±0.31    |
| 5    | Gastric volume (ml/100g) | 3.28±0.10   | 2.27±0.20   | 2.10±0.27   | 2.85±0.52   |

Control: Saline solution (0.9% w/v), standard: Cemetidine (10mg/kg), Test 1: 200mg/kg plant extract and Test 2: 400mg/kg. plant extract p value is 0.01 \*\* - significant 0.05 \*\*\* - highly significant.

# **DISCUSSION**

# **Estimation of pH of Gastric Contents**

In control animals, without any drug the mean was 1.61 and 1.58. Alcoholic extract showed significant rise in pH 3.95 to 4.05 as compared to control. The rise in pH shown by the aqueous extract was 3.01. Cimetidine, a standard drug raised the pH to 4.13. This was more potent than the extracts used. The results are shown in table no. 1 & 2.

# Estimation of Free Acidity of Gastric Contents In Terms of Ml Of 0.1N Hcl/ 100ml Of Gastric Contents

Gastric free acidity was increased to 44.0 & 90.17mEq/litre in control animals. Alcoholic Extract 20.0 mEq/litre showed significant decrease in free acidity as compared to control. The decrease in free acidity by aqueous extract was 84.61 mEq/litre respectively. When compared with Cimetidine, a known anti-ulcer drug, Alcoholic extract showed good results 19.66 mEq / litre, where as other extracts were less potent in decreasing gastric acidity. The results are tabulated in table no.1 & 2.

# Estimation of Total Acidity of Gastric Contents In Terms Of Ml Of 0.1N Hcl / 100ml Of Gastric Contents

Gastric total acidity was increased to 67.8 &114.24 mEq/litre in control animals. Alcoholic extract 30.08 mEq/litre showed significant decrease in total acidity as compared to control. The decrease in total acidity by aqueous extract was 100.04 mEq/litre respectively. The results are tabulated in table no.1&2.

# **Determination of Ulcer Index**

The ulcer index in control animals was 12.66 & 25.6. Alcoholic extract 4.5 significantly reduced the ulcer index as compared to control respectively Cimetidine, a standard anti-ulcer drug showed ulcer index 3.83 & 7.24 respectively. The results were tabulated in table no.1&2.

# **Determination of Gastric Volume**

The gastric volume has been decreased 3.78 in alcoholic extract to 2.10. The results are more comparable with the standard drug Cimetidine which results in gastric volume of 2.27 in alcoholic extract.

# **CONCLUSION**

On the basis of aforesaid studies, it was concluded that Alcoholic extract of *Cressa cretica* provides significant anti-ulcer activity and possess various bioactive compounds. Further studies will be needed for the structural elucidation of extracted bioactive compound.

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