Gastro-Protective Potential of *Trichosanthes Dioica* in Experimental Animals

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ABSTRACT

Ulcer is tissue erosion, either on skin or inside the body, for e.g. lining of gastrointestinal tract. The diverse causes for ulcer development range from, Genetic factors to Physiological/Environmental factors. Helicobacter pylori recently found to be responsible for majority of peptic ulcer cases. Peptic ulcer diseases are extremely common in developing and developed countries with annual incidence of 1 to 3 per 1000\(^{th}\) of the population. PUD is an important cause of morbidity and healthcare costs; estimates of expenditures related to work loss, hospitalization, and outpatient care are $5.65 billion per year in US. It affects 1 in 10 men & 1 in 15 women in Europe. Due to side effects from conventional drug therapy and non-specificity of therapeutic choices, there is inclination towards *Traditional medicines* with low cost and low adverse effect incidences. Many photochemical substances have been found to be effective against pathogenic factors responsible for ulcer formation and its severity. Alkaloids, Flavonoids, Terpenoids, & Tannins have been researched to possess potent Gastroprotective activities. Glycosidal components have been found effective against *Helicobacter pylori* gram negative strains. *Trichosanthes dioica* (Roxb.) found in flora and fauna of Uttar Pradesh have high concentrations of terpenoids, flavonoids, cucurbitacins, which have been researched for having anti-inflammatory and wound healing activities. The present study clearly shows the effectiveness of *Trichosanthes dioica* (Roxb.) in having Gastroprotective activity. This gives clear indication that *Trichosanthes dioica* shall be researched upon to bring effective drug in market against ulcer diseases.

**Keywords:** Trichosanthes dioica, Peptic Ulcer Diseases (PUD), Gastroprotective potential, Anti-ulcer drugs, Trichosanthes species, Methonolic extract of Trichosanthes dioica (MeTD)

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INTRODUCTION

Ulcer is defined as deep lesions in the specified area or in complete lining and spreading across the thickness of the specific area. The prevalence and spread of ulcers especially Peptic ulcer (ulcer of stomach lining or gastro-intestinal tract), varies wide across the Geographical dimensions across the world. Peptic ulcer or Peptic ulcer diseases (PUD), are caused by various factors responsible for it, mainly due to imbalance between the aggressive factors (Helicobacter pylori, NSAID’s, Gastric Acid) and protective factors (Mucin, Bicarbonate, and Prostaglandins), leading to disruption of overall environment of the gastrointestinal tract or stomach. The types of Ulcers are Peptic Ulcer which is broad term for defining the ulcers of digestive tract like stomach and the duodenum, Aphthous ulcer (oral ulcers), esophageal ulcers. Etiology of ulcers, are H.Pylori (Helicobacter pylori) which is considered as the main cause of Peptic ulcer; found by the two Australian scientists in 1982. (Figure 1) Gastric-acid secretion, almost 50 % of gastric acid patients are pepsin and acid hypersecretors. NSAID’s (Non-steroidal anti-inflammatory drugs), widely used therapeutics in medical conditions, which range from Anti-inflammatory to Analgesics & Antipyretic conditions. Nearly 25 % of chronic users of these drugs develop gastric ulcer disease. Reserpine, is one of the drugs which is derived from the roots of Rawolfia serpentine reported to have pivotal role in progression of ulcer. Ethanol, has been determined to cause severe damage to the gastrointestinal tract, which starts with microvascular injury resulting in increase vascular permeability, edema formation, and epithelial lifting. Moreover, by decreasing the secretion of bicarbonate ($\text{HCO}_3^-$) and mucus production, ethanol produces the necrotic lesions in gastric mucosa. Cytokines, Imbalance in VEGF functioning, Vascular endothelial growth factor, has pivotal role in gastric ulcer healing, when this mechanism gets disturbed, it results into ulcers. Decrease in NO (Nitric oxide) NO is barrier to ulcer causation, Leukotrienes & Imbalance in apoptosis i.e. Imbalance in normal cell growth and apoptosis results in increasing the severity of ulcers in the body.¹ There is evidence that Peptic ulcers occur at higher rates in people having blood group ‘O’ compared to other blood groups like A and B.² (Figure 2)
The worldwide role of plants in the cure of diseases is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. For an instance, Western medicines with origins in Mesopotamia and Egypt; the Unani (Islamic) and Ayurvedic (Hindu) systems are centered in western Asia and the Indian subcontinent source is Material Medica. A complete understanding of medicinal plants involves a number of disciplines including commerce, botany, horticulture, chemistry enzymology, genetics, quality control and pharmacology. The relatively lower incidence of adverse reactions of plant preparations compared
to the modern conventional pharmaceuticals, coupled with reduced cost, is encouraging to consider plants as alternative to synthetic drugs. India has a rich flora of Herbal medicines that have been the basis of treatment and cure for various diseases and physiological conditions, practiced widely in Ayurveda, Unani and Siddha systems. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications. The Indian subcontinent represents one of the richest diverse genetic resources. Of the estimated 250,000 species of flowering plants at global level, about 3000 are regarded as food source; out of which only 200 species have been domesticated. Global diversity in vegetable crops is estimated to be about 400 species of which about 80 species of major and minor vegetables are reported to have originated in India. Plants have been very beneficial in terms of medicinal properties they possess and variety of treatments options they give us to evaluate and use in medical practices. Some ulcer healing plants studied are, Pandya et al., 2011 studied antiulcer potential of Oxystelma esculentum against aspirin and ethanol induced ulcer models. It found compound classes like cardenolides, flavonoids, phenolics, sterols and triterpenoids depicting antiulcer activities in the rats induced ulcer models; Taha et al., 2012 evaluation gastroprotective activity in Turnera diffusa Wild and found pre-treatment of arbutin to have significant antiulcerogenic activity comparable to omeprazole in dose dependent manner thus arbutin has significant reduction in ulcer area and mucosal content followed by reduction in edema and inflammation in respective animal models; Yang et al., 2012 studied the gastroprotective activities of Pleurotus ostreatus.

**TRICHOSANTHES DIOICA**

Pointed gourd, *T. dioica* (Roxb.) is one of the most nutritive cucurbit vegetables, and it holds a coveted position in the Indian market. It is a perennial crop, highly accepted due to its availability for eight months in a year (February–September). Being very rich in protein and vitamin A, it has certain medicinal properties, and many reports are available regarding its role in lowering of blood sugar and serum triglycerides. The fruits are easily digestible and diuretic in nature. They are also known to have anti-ulcerous effects. It is prescribed to improve appetite and digestion. The decoction of TD is useful as a valuable alternative tonic, and as a febrifuge, which is given for boils and other skin diseases. The juice of the leaf is applied as patches for alopecia areata. The root are used as hydragogue cathartic tonic and as febrifuge. The fruits are used as a remedy for spermatorrhoea, and the juice of unripe fruits and also tender shoots, are used for cooling and as a laxative. The fruits and seeds have some prospects in the control of some cancer-like conditions and haem-agglutinating activities. It has been shown to be effective in Amlapitta.
Trichosanthes dioica, has been found to possess numerous pharmacological activity or actions, mainly are, it has Ameliorative effects, Anti-diabetic effects, Anti-oxidant, Anti-inflammatory, Anti-pyretic effects, Anti diarrheal effects, Anti-microbial activity, Cholesterol lowering activity, Hypoglycemic activity, Anti-nociceptive activity, Wound healing activity, Anti-worm activity & laxative action.

MATERIALS AND METHOD

Collection and Preparation of Plant:
The leaves of Trichosanthes dioica (Roxb.) were collected from farms of Hastinapur district & arrounding locations. Around 2 Kg of leaves were collected & kept in shade (Shade drying). The significance of shade drying is to preserve the vital constituents of the leaves as drying in sun leads to degradation of these constituents.

Preparation of Extract:
The Fresh dried leaves were subject to grinding with the help of Philips mixer with Blade no. S - 7/09, Model No. HL-1606; manufactured under the certificate no. HP/14/001/0008 dated 28/04/2004, leading to fine coarse powder leaves. The leaves were then put through Methanolic solvent extraction using Soxhlet apparatus.

Choice of Solvent:
Successful determination of biologically active compounds in the plant material is largely dependent on the type of solvent. Properties of a good solvent includes low toxicity, ease of evaporation at low heat, promotion of rapid physiological absorption of the extract, preservative action etc. Methanol having alcoholic property would dissolve organic chemical constituents. Polyphenols, Flavones, Tannins, Terpenoids, Glycosides & Saponins have antidiarrhoeal activity. The phytoconstituents having anti-diarrhoeal activity would also show possible anti-ulcer activity.

Defating:
Defatting of the plant material is important to remove excess of lipid present in the raw plant material. This would help in separating out phospholipids, which would make the solvent difficult to extract the main constituents. Petroleum ether was used for defatting leaves extract. Defatting is done in three successive stages of changing the solvent with consecutive maceration for 48 hrs each. Filter the drug in the first stage, and then re-macerate the drug with fresh petroleum ether. Repeat it for the third consecutive stage.

Soxhlet Apparatus Extraction:
apparatus was invented by Franz von Soxhlet. Soxhlet extraction is only required where the desired compound has a limited solubility in the solvent, and the impurity is insoluble in that solvent. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds. After extraction the solvent is removed, typically by means of a rotary evaporator. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded. (Figure 3)

![Soxhlet Apparatus Diagram](image)

**Figure 3: Soxhlet Apparatus ; A- Water outlet, B- Condenser, C-Thimble, D-Siphone tube,\nE-Round bottom flask, F-Heating mental, G- Water inlet.**

**Drying of Extract:**
The liquid extract of plant were collected in petri-dish for drying them using hot water bath maintaining temperature to avoid degradation of the extract.

**PHYTO-CHEMICAL SCREENING:**
Extracts obtained after drying were subjected to phyto-chemical screening using standard procedures.

**Detection of Alkaloids:**
Extracts were dissolved in HCL and then filtered. Mayers test: Formation of yellow coloured precipitate indicates the presence of alkaloids. Dragendorff’s reagent test were carried out. Formation of red precipitate confirms the presence of alkaloids in the given extract sample.

**Detection of Carbohydrates:**
Molisch’s test Formation of the violet ring at the junction indicates the presence of carbohydrates, Fehlings tests were carried out. Appearance of a brownish red precipitate indicates the presence of reducing sugars in the sample.

**Detection of Flavonoids:**
Alkaline reagent tests Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids. Lead acitrate tests were carried out Formation of yellow colour precipitate indicates the presence of flavonoids.

**Detection of Glycosoids:**
Modified Borntrager tests Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides, Legals test were carried out Formation of pink to blood red colour indicates the presence of cardiac glycosides.

**Detection of Phytosterols/triterpenoids:**
Salkowski tests Appearance of golden yellow colour indicates the presence of triterpenes, Libermann Burchards tests Formation of brown ring at the junction indicates presence of phytosterols, triterpenoids tests Formation of pink colour indicates the presence of triterpenoids., were carried out.

**Detection of tannins:**
Gelatin tests Formation of white precipitate indicates the presence of tannins were carried out.

**ANTI-ULCER MODELS USED IN PRESENT STUDY**

**Pylorus Ligation Method:**
The model was published by *(Shay et al. 1945)* as a reliable and simple method for production of gastric ulcer based on ligation of pylorus. The procedure involves accumulation of gastric juice in the stomach. Which involves starving for 48 hrs. with access to drinking water. They are kept in single housed cages with raised bottoms of wide wire mesh in order to avoid *cannibalism* and *coprophagy*. The pylorus is ligated by having midline incision on the abdominal under ether anesthesia. The pylorus ligation is made keeping in care neither blood vessels are damaged nor traction of pylorus occurs. Another precaution is to avoid grasping the stomach with any instrument else it will induce ulcer. The abdominal walls are closed by sutures, followed by oral gavage of test compounds or via injection. The animals are placed for 19 hours in plastic cylinders with inner diameter of 45 mm being closed on both ends by wire mesh. Later, the animals are sacrificed in CO₂ anesthesia. The abdomen is opened and a ligature is placed around the esophagus close to the diaphragm. The stomach is removed, and the contents are drained in a centrifuge tube. Along the greater curvature the stomach is opened and pinned on a cork plate. The mucosa is
examined with a stereomicroscope. In the rat, the upper two fifths of the stomach form the rumen with squamous epithelium and possess little protective mechanisms against corrosive action of gastric juice. Below a limiting ridge, in the glandular portion of the stomach, the protective mechanisms are better in the mucosa of the medium two fifths of the stomach than in the lowest part, forming the antrum. Therefore, the lesions occur mainly in the rumen and in the antrum. The number of ulcers is noted and severity recorded with the following scores,

0 = No Ulcer; 1 = Superficial Ulcer; 2 = Deep Ulcer; 3 = Perforation

The volume of gastric content is measured. After the centrifugation, acidity is determined by titration with 0.1 N NaOH.

**Evaluation of Ulcer**

The Ulcer index is calculated:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

$U_N$ = Average number of ulcer per animals; $U_S$ = Average of severity score; $U_P$ = Percentage of animals with ulcers. Ulcer index and acidity of the gastric content of treated animals are compared with controls. Using various doses, dose-response curves can be established for ulcer formation and gastric acid secretion.

**Percentage protection index:**

$$\frac{C - T}{C} \times 100$$

Where

$C$ – Ulcer Index of Control group,

$T$- Ulcer index of Test group.

**Indomethacin induced ulcers in rats**

Non-steroidal anti-inflammatory agents, like Indomethacin and acetyl-acetic acid, induce gastric lesions in man and in experimental animals by inhibition of gastric cyclo-oxygenase resulting in less formation of prostacyclin, the predominant prostanoid produced in the gastric mucosa. Procedure includes taking group of 8 to 10 groups of weight ranging from 150 – 200 g. The test drugs are administered orally in 0.1 % tween 80 solution 10 min. prior to oral Indomethacin in a dose of 20 mg/kg (4mg/ml dissolved in 0.1 % tween 80 solution). Six hours later, the rats are sacrificed in CO$_2$ anesthesia and their stomachs removed. Formal saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs are opened.
along the greater curvature, then washed in warm water, and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions are measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated.

**Ethanol Induced mucosal damage in rats**

Intra-gastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals *Robert et al. 1979* and *Szabo et al. 1981*. These lesions can be at least partially inhibited by various drugs, such as some prostaglandins. The protective effect against various irritants has been called cytoprotective activity. The procedure includes, selecting male wistar rats weighing 250-300 g deprived of food 18 h prior to the experiment but are allowed free access to water. During this time they are kept in restraining cages to prevent coprophagy. The rats are administered either the appropriate vehicle or the cytoprotective drug, e.g. a prostanoid, intragastrically 30 min prior to administration of 1 ml of absolute ethanol. Untreated animals are included as control. One hour after administration of ethanol, the animals are euthanized with CO₂ the stomachs are excised, cut along the greater curvature, and gently rinsed under tap water. The stomachs are stretched on a piece of foam core mat, mucosal site up. The subjective scores of the treated tissues are recored, the graded response is reflecting the least -0, to most damage-3. A circular full thickness area, about 13 mm diameter, is cut with a cork borer from each lobe of the fundus just below the ridge dividing the glandular from the non-glandular portion of the stomach. A plexiglass template (19*14*0.3 cm), burnished on one side with emery cloth, and with four rows with six holes 13 mm in diameter is placed on a sheet of clear glass, burnished side up, and bound to the glass with photographic tape along the periphery. The excised pairs of tissue from each stomach are placed into the holes of the template. Pairs of tissue from each stomach are examined to minimize sampling errors. The template is positioned on a rectangular central open area of an Aristo model T-16 cold cathode transilluminator (38*38 cm) containing a W-45 blue-white lamp. A camera is mounted on a copy stand directly above the template. Photographs are taken, the film processed in a standard manner and a contact sheet is made from negatives.

**EXPERIMENTAL STUDIES**

The animals were procured from premises of animal department and were divided into following groups

**Control group/ Group I:**

The animals taken in this group were given simple distilled water for five days as a study protocol for Gastro-protective activity. The aim being to get ideal figures for comparing the variation due to ulcerogents and antiulcerogen activity. Therefore, neither ulcer induction is done in rodents of this
group nor ulcer protective agents are given. The readings would give normal behavior of gastric content as well as of stomach lining.

**Negative control/ Group II:**

The animals taken in this group were given ulcerogent i.e. agent which induces ulcer. In the present case, ulcer induction was carried out by Ethanol, Indomethacin and pylorus ligation given at the end of five day study protocol. Thus on the fifth day of simple oral administration of distilled water ulcer induction was done. Ulcer protective agent was not given to get the readings of stomach and gastric content in the absence of ulcer protective agent. This would thus help in figuring out the degree of protective activity possessed by the experimental drug in the present study.

**Test I/ Group III:**

The animals taken in this group were subject to both ulcerogent and anti-ulcerogent. The difference is the dose of antiulcerant was taken as 100 mg/ml. In this group the animals were given experimental drug for five days before the administration of ulcer inducing agent on the last day of study protocol. The results were computed for dose of 100mg/ kg body weight of experimental drug i.e. MeTD. The parameters selected for study protocol were then carried out.

**Test II/ Group IV:**

The animals taken in this group were subject to higher dose of experimental drug i.e. MeTD, which was 200 mg/kg Body weight. The parameter for gastroprotective activities were recorded on the fifth day of the study protocol.

**Standard Control/ Group V:**

The animals taken in this group were given standard drug which was Omeprazole in the present study at the dose of 20mg/kg body weight. The rationale behind having standard control, is to find out degree of ulcer protective action compared to antiulcer drug already marketed and available for the disease symptom. (Table 1)

<table>
<thead>
<tr>
<th>Table 1: Experimental Study Design</th>
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<tbody>
<tr>
<td><strong>Experimental Protocol Animal Groups / Ulcer Models</strong></td>
</tr>
<tr>
<td>Group 1—Control</td>
</tr>
<tr>
<td>Group 2—(-ve) Control</td>
</tr>
<tr>
<td>Group 3— Test 1 (100mg/kg)</td>
</tr>
<tr>
<td>Group 4—Test 2(200 mg/kg)</td>
</tr>
<tr>
<td>Group 5—Standard Control (Omeprazole)-- 20 mg/kg</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Percentage yield/ Percentage Recovery:
The leaves of experimental plant *Trichosanthes dioica* (Roxb.) were coarsed into powdered form followed by extraction via soxhlet apparatus. The extract was dried by collecting the resultant mixture in petri dish using heating mental to the extent viscous fluid is formed. The resultant material is known as recovered extract of the plant *Trichosanthe dioica* (Roxb.). Percentage yield / Percentage Recovery was calculated using following formulae:

\[ P/B = R/100 \]

\[ R = 100 \times P / B \]

Here, Base value i.e. original weight of *Trichosanthes dioica* coarse powdered drug had been weighed around 260 grams (*used for extraction process*). Therefore, B = 260 grams which is base value. Recovered weight or processed weight/ yield was recorded 40.732 grams. There, P = 40.732 grams which processed weight. Thus, Recovered value = P/B, i.e. 40.732/ 260 = 0.1566615. Hence % Recovery = 0.1566615 \* 100 = 15.66615 %.

*Percentage recovery or percent yield on successive Soxhlet extraction is 15.66615 %.*

Phytochemical screening:
The crude extracts were subject to different tests for phytochemical presence/absence following standard testing procedures. Upon various tests and screening of extracts of *Trichosanthes dioica*, it was found that, Alkaloids were present in intermediate levels in the extract, Carbohydrates were present in Intermediate levels in the extract, Flavonoids were present in abundant levels in the extract, Glycolides were present in intermediate levels, Terpenoids were present in abundance level, Tannins. Fats or Fixed oils and Saponins are present in minor quantities or at very minimal level.

Toxicological Studies:
The Methanolic extract of *Trichosanthes dioica* (Roxb.) were subjected to toxicological studies at the dose of 1000mg/2000mg administered to mice in divided groups as per doses (n=6). No significant changes in the anatomy and physiological conditions of mice were noticed post five day treatment with the respective doses.
**Table 2: Phytochemical Screening of Trichosanthes dioica (Roxb.)**

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Tests</th>
<th>Procedure</th>
<th>Color</th>
<th>MeTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Alkaloids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>2ml ext. + Dragendorff’s Reagent</td>
<td>Dark reddish brown precipitate</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Mayer’s test</td>
<td>2ml ext. + Mayer’s Reagent</td>
<td>Off white precipitate precipitate</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td><strong>Carbohydrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molish’s Test</td>
<td>Small amount of ext. + Molish’s reagent + conc. Sulphuric acid added from sides</td>
<td>Dark purple ring appears</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Fehling test</td>
<td>Ext. + Fehling’s sol. A and B, then heated</td>
<td>Light Brick red precipitate</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td><strong>Flavonoids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alkaline reagent test</td>
<td>Few drops of ext. + NaOH solution</td>
<td>Yellow precipitate</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Lead acetate test</td>
<td>Few drops of lead acetate + drops of lead acetate</td>
<td>Yellow precipitate</td>
<td>++++</td>
</tr>
<tr>
<td>4.</td>
<td><strong>Glycosides</strong></td>
<td></td>
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<tr>
<td></td>
<td>Modified boormtragers test</td>
<td>Few ml of ext.+ Ferric chloride+ ammonia to benzene separated part</td>
<td>Slight brownish to light pinkish precipitate</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Legals test</td>
<td>Ext. + sodium nitroprusside in pyridine and NaOH</td>
<td>Brick red precipitate</td>
<td>++</td>
</tr>
<tr>
<td>5.</td>
<td><strong>Terpenoids</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Salkowski test</td>
<td>Ext.+ chloroform followed by chloroform filtrate+ conc. Sulphuric acid</td>
<td>Dark yellow precipitate</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Libermann Buchard’s test</td>
<td>Chloroform filtrate of ext.+ acetic anhydride</td>
<td>Brown ring followed by darkish brown precipitate</td>
<td>++++</td>
</tr>
<tr>
<td>6.</td>
<td><strong>Tannins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gelatin test</td>
<td>Ext.+1% gelatin solution having NaCl solution</td>
<td>Creamy white precipitate</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td><strong>Saponins</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Froth test</td>
<td>Ext. + Distilled water = Shaking well</td>
<td>Froth formation</td>
<td>_</td>
</tr>
<tr>
<td>8.</td>
<td><strong>Fats and Fixed oils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stain test</td>
<td>Ext.+ Pressed in between filter papers</td>
<td>Staining of filter paper</td>
<td>+</td>
</tr>
</tbody>
</table>

**Test Results**

**Ulcer Index and Percentage protection**

Ulcer Index and Percentage protection (Table 3: Ulcer Index and Percentage inhibition within groups and between ulcer models) Values are expressed as mean + S.E.M. of six rats in each treatment group where, *P< 0.5, **P< 0.01, ***P< 0.001 vs. control (Figure 4)
Figure 4: Ulcer Index in Pylorus Induced, Indomethacin Induced, and Ethanol Induced Ulcer Models.

Table 3: Ulcer Index and % Protection within and between the Groups; Values are expressed as mean ± S.E.M. of six rats in each treatment group where, *$P<0.5$, **$P<0.01$, ***$P<0.001$ vs. control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pylorus Ligation Induced Model</th>
<th>Indomethacin Induced Model</th>
<th>Ethanol Induced Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ulcer Index %age Protection</td>
<td>Ulcer Index %age Protection</td>
<td>Ulcer Index %age Protection</td>
</tr>
<tr>
<td>Group I – Control</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Group II – Neg. Control</td>
<td>33.70 ± 0.12**</td>
<td>33.59 ± 0.12**</td>
<td>21.47 ± 0.11*</td>
</tr>
<tr>
<td>Group III- Std. Control</td>
<td>2.13 ± 0.14**</td>
<td>3.11 ± 0.17**</td>
<td>2.09 ± 0.13*</td>
</tr>
<tr>
<td>Control/ Omp. 20mg/kg/bw</td>
<td>1.14 ± 0.16**</td>
<td>2.7 ± 0.21**</td>
<td>0.13 ± 0.09**</td>
</tr>
<tr>
<td>Group IV- MeTD 100mg/kg/bw</td>
<td>15.62 ± 1.37*</td>
<td>19.92 ± 0.17***</td>
<td>15.08 ± 0.10***</td>
</tr>
<tr>
<td>Group V – MeTD 200 mg/kg/bw</td>
<td>5.68 ± 0.25*</td>
<td>9.33 ± 0.09**</td>
<td>6.51 ± 0.21*</td>
</tr>
</tbody>
</table>

Gastric Juice studies:

Values are expressed as mean ± S.E.M. of 6 rats in each treatment group where, $^aP<0.05$, $^bP<0.01$, $^cP<0.001$ vs. control. (Table 4)
Table 4: Gastric pH & Acid volume within and between the groups; Values are expressed as mean ± S.E.M. of 6 rats in each treatment group where, \(^aP<0.05\), \(^bP<0.01\), \(^cP<0.001\) vs. control.

<table>
<thead>
<tr>
<th>Groups</th>
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<th>Indomethacin Induced Model</th>
<th>Ethanol Induced Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric pH (ml/100 g)</td>
<td>Gastric pH (ml/100 g)</td>
<td>Gastric pH (ml/100 g)</td>
</tr>
<tr>
<td>Group I – Control</td>
<td>2.29±0.25</td>
<td>3.18±0.49</td>
<td>2.91±0.04</td>
</tr>
<tr>
<td>Group II – Neg. Control</td>
<td>1.02±0.16</td>
<td>0.98±0.12</td>
<td>1.03±0.19</td>
</tr>
<tr>
<td>Group III- Std. Control/Omp. 20mg/kg/bw</td>
<td>5.86±0.13</td>
<td>5.02±0.28</td>
<td>5.47±0.10</td>
</tr>
<tr>
<td>Group IV- MeTD/100mg/kg/bw</td>
<td>1.93±0.37</td>
<td>2.02±0.88</td>
<td>2.81±0.23</td>
</tr>
<tr>
<td>Group V – MeTD/200 mg/kg/bw</td>
<td>3.80±0.11</td>
<td>3.47±0.17</td>
<td>3.60±0.08</td>
</tr>
</tbody>
</table>

**Acidity Studies:**

Acidity studies were carried out. The results are given in Table 5. (Table 5) Values are mean ± SEM for 6 rats where, \(^xP<0.001\) compared to respective control group, \(^aP<0.05\), compared between groups, \(^bP<0.01\) compared between groups, \(^cP<0.001\) compared to between groups.

**Ethanol Induced Ulcer Model Result:** Refer to Figure 5

![Figure 5: Ethanol Induced Ulcer Rat Stomach Study.](http://www.ajptr.com)
Indomethacin Induced Ulcer Model Result: Refer to Figure 6.

Figure 6: Indomethacin Induced Rat Stomach Study.

Pylorus-igation induced Ulcer Model Result: Refer to Figure 7.

Figure 7: Pylorus ligation induced Ulcer Results
Peptic ulcer disease represents a worldwide health problem because of its high morbidity, mortality and economic loss. In the United States, approximately, 5 million adult life suffer annually from peptic ulcer disease and 500,000 new cases with 4 million recurrences are reported each year, (Sonnenberg., 1995) in India around 1.47 million lives are lost per year due to peptic ulcer diseases (PUD"). In *Helicobacter pylori* (H.P) infected subjects; the lifetime prevalence appears to be 10% to 20%. (Soll., 1998) Variation in the prevalence of peptic ulcer occurs between geographic regions. The annual prevalence of active gastric and duodenal ulcers in the United States in males and females is reported 1.8 %. In a large Japanese survey of male office workers above the age of 40, duodenal ulcer prevalence was reported 4.3 % at endoscopic surveys. In the mid-1950s, the male: female ratio for deaths due to duodenum ulcers (DU) was about 5:1, but during the past decade this ratio was decreased to about 1.3:1. (Isenberg et al., 1991) The prevalence of peptic ulcer has shifted from being a disease predominant in males to one with a nearly comparable prevalence in both sexes.

Factors which play a role in the pathogenesis of peptic ulcer are endogen (acid and pepsin hypersecretion, reduce in mucosal resistance etc.), exogen (HP, smoking, NSAID etc.), emotional stress and genetic predisposition. (Leoci et al., 1995) Most studies reveal a strong positive association between cigarette smoking and ulcer incidence, mortality, complications, recurrences, and delayed healing rates. The quantity of smoking is also important. In fact, large retrospective studies reported that cigarette smokers were about two fold more likely to have peptic ulcer than nonsmokers with a dose-response phenomenon.

The use of NSAID is an important risk factor for elderly people especially. It is estimated that approximately 10 % patients receiving NSAID daily have active gastric ulcer. NSAID represent one of the major causes of life threatening complications such as upper gastrointestinal hemorrhage and perforation. In one study, the relative risk ratios for GU and DU in chronic NSAID users were about 45 and 8 fold greater, respectively. (Sonnenberg., 1995)

Alcohol, as a noxious agent causes gastric mucosal damage, stimulates acid secretion and increases serum gastrin levels. *H pylori* are one of the most common pathogens worldwide. Approximately sixty per cent of the world’s population gets infected by the bacteria causing gastritis and peptic ulcer. It is also strongly associated with gastric adeno-carcinoma and mucosa associated lymphoid tissue lymphoma. Possible routes of infection include either oral-oral or fecal-oral, iatrogenic spread with inadvertent use of unsterile pH probes and endoscopes and vectorial spreads by flies. The majority of individuals acquire HP early in life. An inverse relationship between socioeconomic status and prevalence of HP has been observed in most studies. (David., 1997)
The lower incidence of adverse reactions of plant parts used for medicinal purposes and high degree of suitability and effectiveness among patients and people, the natural remedies have an edge over the conventional medicines available alongwith advantage of being economical to reach masses. (Nair et al., 2005)\textsuperscript{35}. Soxhlet extraction under methanolic solvent, yielded 40.73 grams form 260 grams crude drug taken initially accounting to yield percentage of 15.66 %. The subsequent recovered extract was subjected to phytochemical screening for the detection of various chemical components. The respective chemical tests revealed presence of Alkaloids, Carbohydrates, Flavonoids, Glycosides, terpenoids and tannins. The parameters chosen for gastroprotective analysis were Ulcer index, percentage inhibition or protection, gastric pH, gastric volume, free acidity, total acidity, catalase, lipid peroxidation and histopathology of rat stomach section from the respective groups among three different models under study namely Pylorus ligation, Indomethacin and Ethanol induced ulcer models.

Ulcer Index shows dose dependent effectiveness of Methanolic extract of leaves of \textit{Trichosanthes dioica} at 200mg/kg B.wt. comparable to negative control. Dose of MeTD (Methanolic extract of \textit{Trichosanthes dioica}) at 200mg/kg B.wt showed 83.14 % inhibition or protection in Pylorus ligated model, 72.22 % in Indomethacin induced model and 69.67 % in Ethanol induced model compared to standard Omeprazoles similar from Pylorus ligated, Indomethacin Induced and Ethanol Induced i.e. 93.67 %, 90.74 % and 90.26 %. Also MeTD at dose of 200mg.kg B.wt showed significant increase in gastric pH mainly 3.80 ± 0.11, 3.47 ± 0.17 and 3.60 ± 0.08 for pylorus ligation, Indomethacin induced and Ethanol induced Ulcer models. The higher dose of Methanolic extract showed increase in free acidity and total acidity figures showing increased suppression of ulcer forming factors. The oxidative factors mainly catalase was shown to dose dependently increase for test 2 i.e. at dose of 200 mg/kg B.wt compared to test 1 i.e. 100 mg/kg B.wt., and lipid peroxidation levels showed dose dependent reduction in their subsequent levels. The histopathological report clearly marks the ulcer protective effects of MeTD at dose of 200mg/kg B.wt. where it showed near to normal photomicrographic characteristics of the stomach cross-section. Thus, clearly \textit{Trichosanthes dioica} Methanolic extract has significant GASTROPROTECTIVE ACTIVITY. Therefore, it should be thoroughly researched to launch a better ulcer healing prospect for the ulcer patients.

CONCLUSION

Gastric Ulcer is a serious gastro-intestinal disorder that requires a well targeted therapeutic strategy
as many factors have been associated with the etiology of this disease. As number of drugs like H₂ – receptor blocker and proton-pump inhibitor are available commercially for the treatment and healing of gastric ulcer but incidence of relapses, low degree of specificity, side effects and drug interactions have been major drawback and hindrance towards effective treatment goal. To overcome this menace, increased interest in the traditional sources and alternative therapies to seek ultimate targeted therapy for disease etiologies is gaining importance from all walks of medicinal research and professionals. Methanolic extract of (Trichosanthes dioica) showed dose dependently boost to the path of selecting traditional route for better cure. The tests and parameters to check effective gastroprotective effect had been found significant and effective results at MeTD 200 mg/kg B.wt. thus highly recommend the plant, to carry out research for developing commercially available targeted medicinal leads for better prospect in Ulcer treatment.

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