AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Anti-ulcer activity in *Trichosanthes Dioica* (Roxb.): A Histopathology Report Analysis

Devansh Mehta[1*], Anuj Kumar Sharma[2]
1: Kharvel Subharti College of Pharmacy, Subharti University, Meerut, U.P., India. Pin code: 250110.
2: Translam Institute of Pharmaceutical Education and Research, Meerut, UP. Pincode: 250001

ABSTRACT

Ulcer is lesion on skin or internal linings of organs or Gastro-intestinal tract, which leads to degradation of cells in the area of occurrence which may be followed by bleeding or cancer at later stages. Peptic Ulcer Disease known as *Ulcus pepticum* have mucosal erosions equal to or greater than 0.5 cm of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. The present study was to evaluate Anti-ulcer activity in *Trichosanthes dioica* (Roxb.) via experimental ulcer models in animals through Histopathology study of Rat's stomach. The leaves of *Trichosanthes dioica* (Roxb.) were collected from farms in and around Hastinapur district. Around two kilograms of the leaves of plant shrub were collected and were kept in shade for the purpose of drying the leaves completely. Sprague-Dawley rats (150 – 200 g) were procured from the animal house of Subharti University (*n=90*). The three animal models were used for the present study, mainly, Pylorus ligation, Ethanol Induced and Indomethacin (NSAID's) ulcer models. Five groups of Animals were made, with n=6 for each group. The histopathology study of rat's stomach was carried out for each ulcer model, and during the examination of rat's stomach, it was clearly shown, that *Trichosanthes dioica* has potent Anti-ulcer activity. The Research thus primarily focuses using the plant for further study and to bring an effective and efficient ulcer healing drug in the market.

Keywords: *Trichosanthes dioica* (Roxb.), Methanolic extract of *Trichosanthes dioica* (MeTD), Anti-ulcer activity, Histopathology study

*Corresponding Author Email: devanshmehta.pharma@gmail.com
Received 21 December 2015, Accepted 28 December 2015

Please cite this article as: Mehta D *et al*., Anti-ulcer activity in Trichosanthes Dioica (Roxb.): a Histopathology Report Analysis. American Journal of PharmTech Research 2016.

INTRODUCTION

World over the use of Traditional medicines and medicines obtained from plant source have been an area of interest both for Healthcare Practitioner's (HCP's) and by the patients due to the widespread applications and advantages over the allopathic medicines available for the mankind. Fossil records date back human use of plants as medicines to around 60, 000 years old to the Middle Paleolithic age.[1] Due to rich diversity of medicinal plants available for us, it holds great treasure for the medicinal community to come out with an effective and efficient treatments for the benefit of the patients. The main advantage the traditional medicines or medicines obtained from plant source, lies, in their low side effects as compared to the conventional medicines available for treatment and healing purpose. Considering the same, it is not hidden fact that, Indian subcontinent holds huge advantage in terms of availability of traditional medicines. In India, Traditional medicines are practiced widely by Ayurveda, & it holds a tradition which is over 5000 year's old.[2] *Trichosanthes* (family Cucurbitaceae) has around 80 species world over. Around 20 species have been recorded in India, of which, *Trichosanthes anguina* & *Trichosanthes dioica* are cultivated as vegetables. *Trichosanthes dioica* is perennial crop and is widely available for 8 months from February to September. It is in practice for cure in digestion & appetite. Trichosanthes dioica, has been found to possess numerous pharmacological activity or actions, which are mainly, Ameliorative effects, Anti-diabetic effects, Anti-oxidant, Anti-inflammatory, Anti-pyretic effects, Anti diarrheal effects, Anti-microbial activity, Cholestrol lowering activity, Hypoglycemic activity, Anti-nociceptive activity, Wound healing activity, Anti-worm activity & laxative action.[3] (Figure 1)

Figure 1: Trichosanthes dioica[4]

MATERIALS AND METHOD

Collection and Preparation of Plant:

The leaves of *Trichosanthes dioica* (Roxb.) were collected from farms of Hastinapur district & surrounding locations. Around 2 Kg of leaves were collected & kept in shade (Shade drying). Shade drying is required to help making powder form of dried leaves along with preserving the vital phyto-constitutes of the plant in leaves.

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. (Figure 2)

Figure 2: Soxhlet Apparatus, A- Water outlet, B- Condenser, C-Thimble, D-Siphone tube, E-Round bottom flask, F-Heating mental, G- Water inlet

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. Thus Methanol was chosen as Solvent for the present study.[5-6]

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 was done in three successive stages of changing the solvent with consecutive maceration for 48 hrs each. The drug was filtered at the first stage & then re-macerated with fresh petroleum ether, the same step was done in three consecutive stages.[6]

Soxhlet Apparatus Extraction:

Soxhlet apparatus invented by Franz von Soxhlet was used to extract the vital constituents. It helps in dissolving the vital constitutes in the packing of the apparatus having the plant powdered form in the solvent. Thus highly applicable for the research work. (Figure 2)

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. (Figure 3)

Figure 3: Drying of Extract

Phyto-chemical screening:

Extracts obtained after drying were subjected to Phyto-chemical screening using standard procedures.[6-7]

HISTOPATHOLOGY

A portion of stomach tissue was fixed in formalin diluted to 10% with normal saline and preceded for histopathology. After paraffin embedding and block making, serial sections of 5 μm thickness

were made, stained with hematoxylin and eosin (HE) and viewed and examined under microscope.[9]

Instruments used for Histopathology were,

i. Tissue processor- Shandon
ii. Embedding Station- Shandon
iii. Stainer-Shandon
iv. Hematoxylin and Eosin – Harris Hemotoxylin of Nice Company

ANTI-ULCER MODELS USED IN PRESENT STUDY

The present study was carried out in three ulcer models, mainly, Pylorus Ligation Ulcer Model, Ethanol Induced Ulcer Model and Indomethacin Induced Ulcer Model. Indomethacin Induced Ulcer Model is often called NSAID's induced ulcer model.[8] All the models were carried out for present study using standard protocol as mentioned in vogels guide for screening models.[8]

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 antiulcerogent 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 1: Group Protocols for study

Groups	**Experimental Protocol**			
		Ulcer Models		
		Ethanol-Induced Model	**Indomethacin Induced Model**	**Pylorus-ligation Induced Model**
Group 1	Control	6	6	6
Group 2	(-ve) Control	6	6	6
Group 3	Test 1 (100mg/kg)	6	6	6
Group 4	Test 2(200 mg/kg)	6	6	6
Group 5	Standard Control (Omeprazole)-- 20 mg/kg	6	6	6

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. (Table 2)

Table 2: Phytochemical screening

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

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.

Histopathology Studies Proving Anti-ulcer Activity in *Trichosanthes dioica* (Roxb.)

Histopathologies of rat tissues among untreated and treated groups were carried out to understand the gastroprotective manifestation more clearly. Microscopic examination reveals epithelial lining cells, submucosal layers, muscularis and adventia layer

The surface epithelial (Mucous) cells.

These consist of epithelial cell lining covering the inner surface of the stomach being in direct contact with the lumen. These cells are irregular shapes having pyramidal with ovoid nucleus surrounded by cytoplasmic mass. The apical portions of these cells are occupied by dense discrete granules.

Submucosal Cells.

This layer lies over the mucosa and consists of fibrous connective tissue, separating the mucosa from the next layer. The meissner's plexus (nerve bundles derived from the plexuses of parasympathetic nerves around the superior mesenteric artery. is in this layer

Parietal Cells

These cells are known as acid-forming cells and are the principle secretors of hydrochloric acid (HCL) in the stomach. They are scattered among the other cell types. The parietal cells are large in size with a spherical or pyramidal outline and with acidophilic cytoplasm and centrally spherical nucleus.[8] The histopathology test results are according to ulcer models covering each group in respective ulcer models.

Ethanol Induced Ulcer Models Histopathology Reports Results

Group I: Rats of this group were considered as control group where, neither pretreatment for five days nor ulcer induction was carried out in the rats of this group. Only normal distilled water was given to rats of this group. The photomicrograph shows a normal rat tissue with no ulceration. (Figure 4)

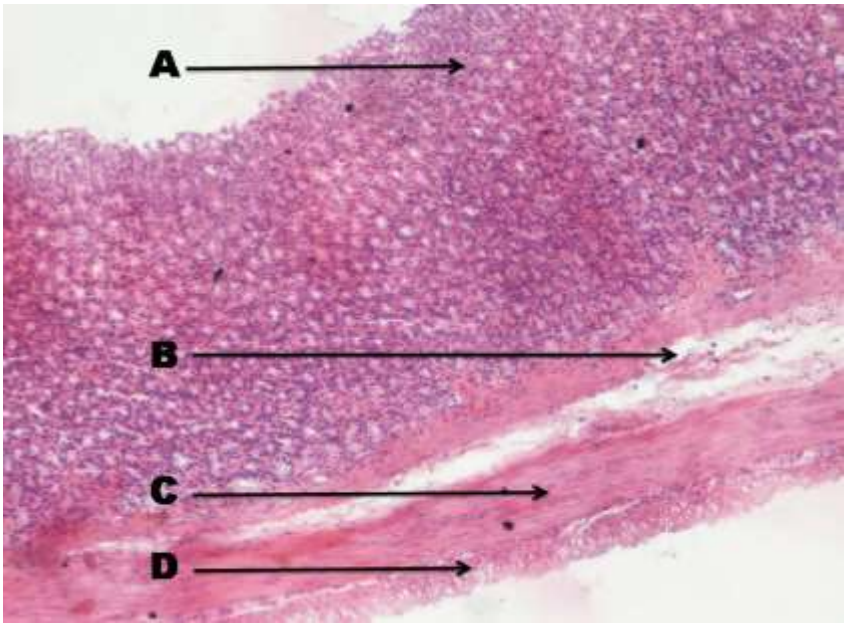

Figure 4: Photomicrograph of a 3×2.5×0.8 cm H & E paraffin stained cross-section from the stomach of Normal group (H/3006/12) of Ethanol Induced Model. Following microscopic view shows A) Mucosa, B) Submucosa, C) Muscularis and D) Adventia at X 100 magnification.

Group II: Rats of this group were considered as Negative control where, pretreatment with test or standard drug was not given for the period of five days, but on the last day ulcer induction was carried out using ethanol. Photomicrograph below shows clearly erosion of epithelial lining of cardiac end of stomach. (Figure 5 & Figure 6)

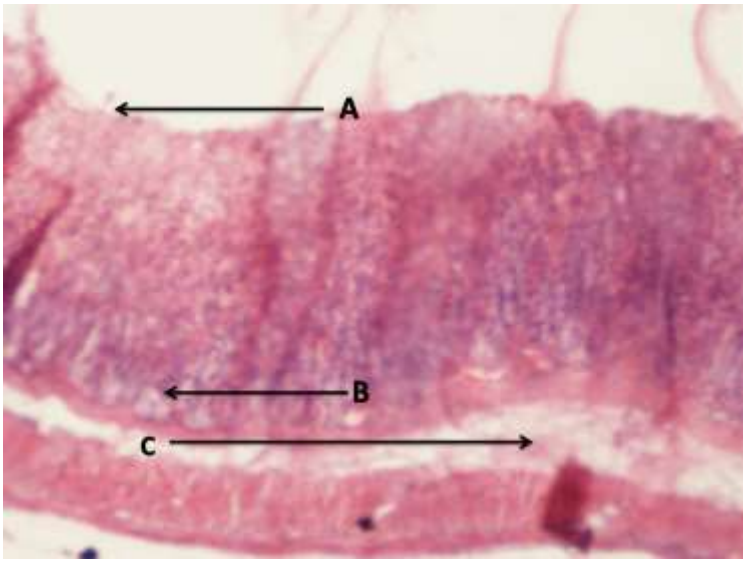

Figure 5: Photomicrograph of 4x2.5x0.8 cm H & E stained cross section from the stomach of Negative control group (H/3001/12 of Ethanol Induced Model. Following microscopic view

shows A) Erosion of Epithelial lining of mucosal layer having ulceration, B) Oedamatous section, C) Oedamatous Submucosal lining at X 100 magnification

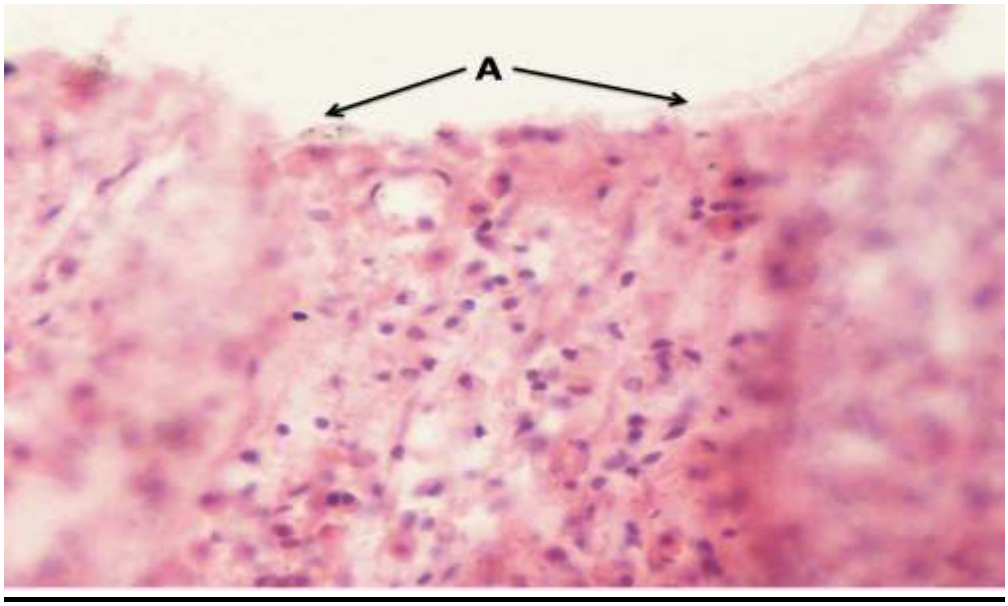

Figure 6: A photomicrograph with H & E stained microscopic view clearly shows the Ulceration of epithelial lining in negative control group (H/3001/12) from stomach section of Ethanol Induced Model at 4 X 100 magnification

Group III: Rats of this group were subject to pretreatment with omeprazole 20 mg/kg B.wt. for the period of five days followed by ulcer induction by ethanol on the last day. The photomicrograph below shows a cross section with parietal cell hyperplasia along-with increased eosinophils and inflammatory cells. (Figure 7)

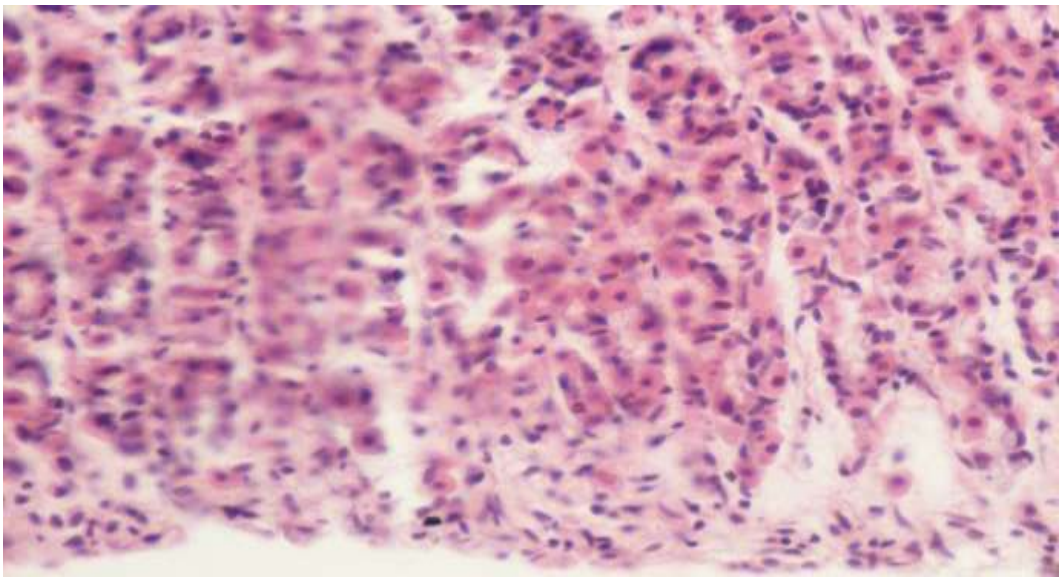

Figure 7: Photomicrograph of 4x2.5 cm H & E stained cross-section from the standard

control (Omeprazole - 20 mg/kg B.wt. treated) group (H/2960/12) of Ethanol Induced Model showing normal mucosa with reduction of eosinophils at 4 X 100 magnification.

Group IV: Rats of this group were subject to lower dose of *Trichosanthes dioica* leaves Methanolic extract (MeTD) at dose of 100 mg/kg B.wt. for five days followed by ulcer induction by ethanol on the last day. The photomicrograph below shows erosions of epithelial lining with Odematous submucosal and inflammatory cells. (Figure 8)

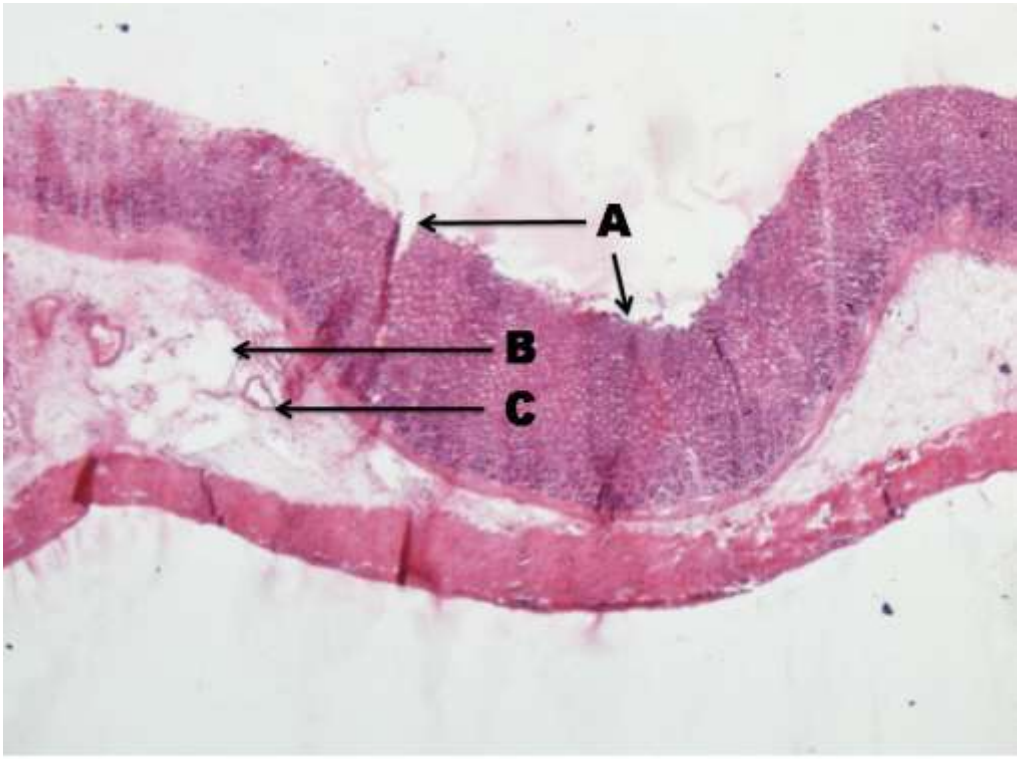

Figure 8: Photomicrograph of 4.5x3.2 cm H & E stained cross-section of stomach from Test 1- 100mg/kg B.wt. of Trichosanthes dioica extract group (H/2958/12) of Ethanol Induced Model showing A) Decrease in a bit of erosions of epithelial lining depicting ulceration, B) Odematous Submucosa, C) Inflammatory Cells at X 100 magnification.

Group V: Rats of this group were subject to pretreatment with *Trichosanthes dioica* Methanolic extract at dose of 200mg/ Kg B.wt. for period of five days followed by ulcer induction on the last day. The photomicrograph below shows normal epithelium with no ulceration thus confirming gastro-protective activity in the experimental drug under study. (Figure 9)

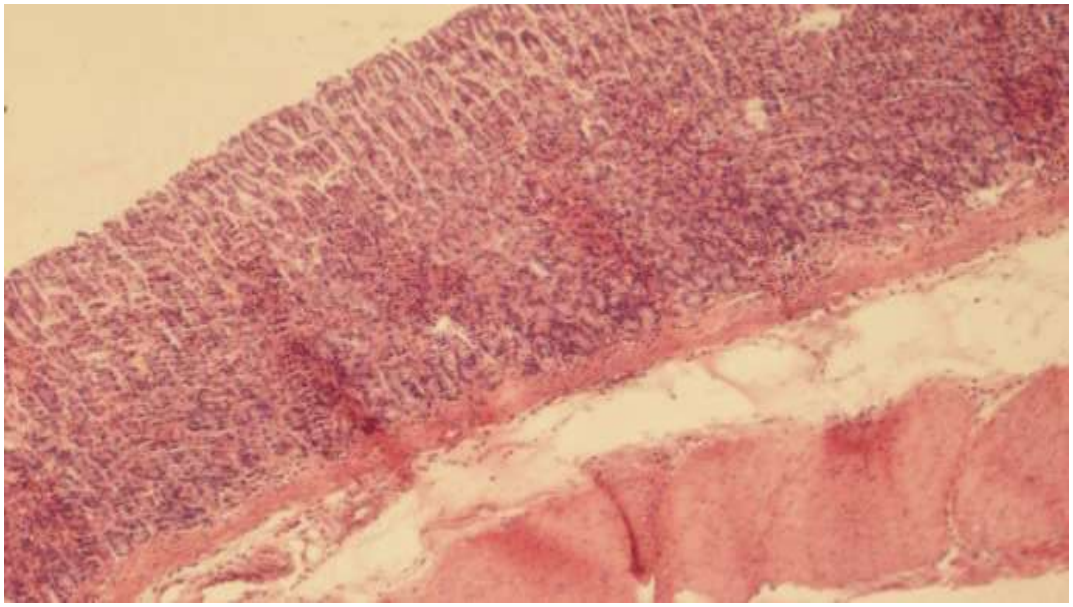

Figure 9: Photomicrograph of 5x2.5 cm H & E stained cross-section of stomach section from Test 2 – 200mg/kg B.wt. Trichosanthes dioica group (H/2959/12) of Ethanol Induced Model showing Normal Mucosa with prevention of epithelial erosions and prevention of damage to submucosal lining at X 100 magnification. This clearly shows the effectiveness of Trichosanthes dioica in protecting ulcer formation

The Results clearly showed the effectiveness of *Trichosanthes dioica* in ulcer healing.

Indomethacin induced Ulcer Model Histopathology Report Results

Group I: Like in ethanol induced model, similarly the rats in the control group were barred with pretreatment and ulcer induction. The photomicrograph below shows normal epithelial lining alongwith numerous eosinophils. (Figure 10)

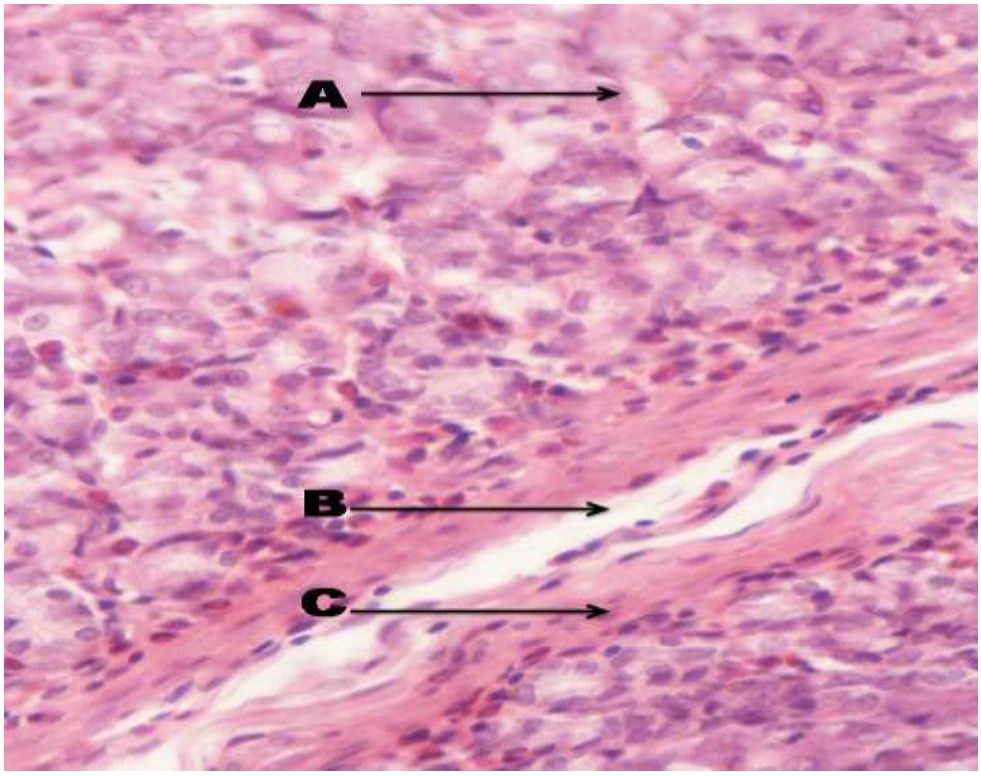

Figure 10: **Photomicrograph of 3x2x1.5 cm H & E stained cross-section from Control group (H/3010/12) group of Indomethacin Induced Model showing A) Normal Mucosal, B) Submucosa, and C) Muscularis at X 100 magnification**

Group II: The rats in this group were not subject to pretreatment with test drug or standard drug but given normal distilled water, followed by ulcer induction by administrating Indomethacin on the last day. The stomach were taken out and were filled with formalin for better staining under the photomicrographic view. The micrograph below shows clearly exfoliation of epithelial lining along with increase in patietal cell. Thus increased acid production causing ulceration. (Figure 11)

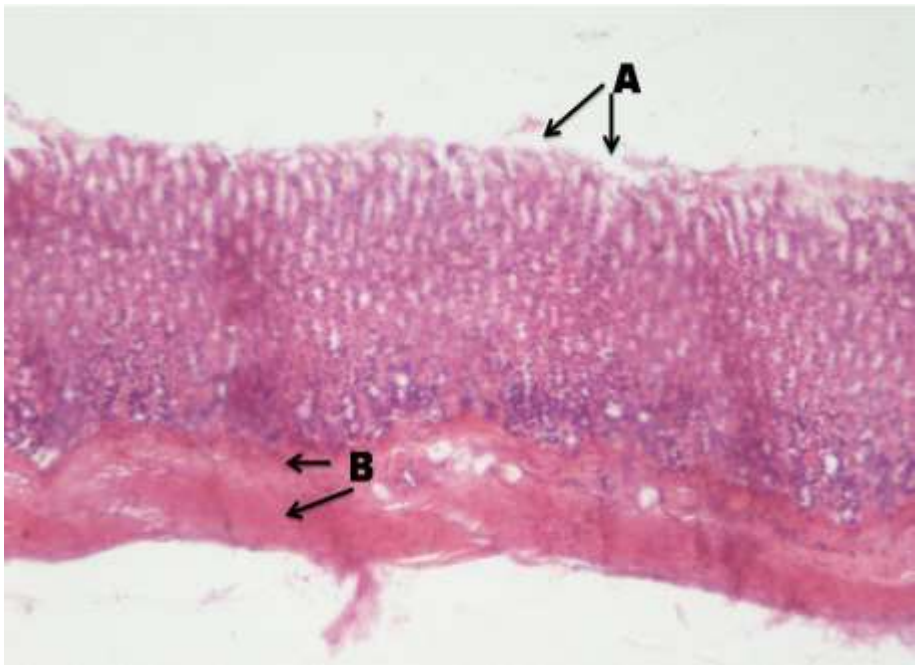

Figure 11: Photomicrograph of 3.5x2 cm H & E Cross section of Negative control group (H/3011/12) of Indomethacin Induced Model showing A) Epithelial Ulceration and Erosion of lining, B) Increased Parietal Cells and eosinophils at X 100 magnification.

Group III: The rats were subject to pretreatment with standard dose in present case it was omeprazole at dose of 20 mg/kg B.wt. for the period of five days. The ulcer was induced by administrating Indomethacin. The photomicrograph below shows submucosal with few eosinophils. (Figure 12)

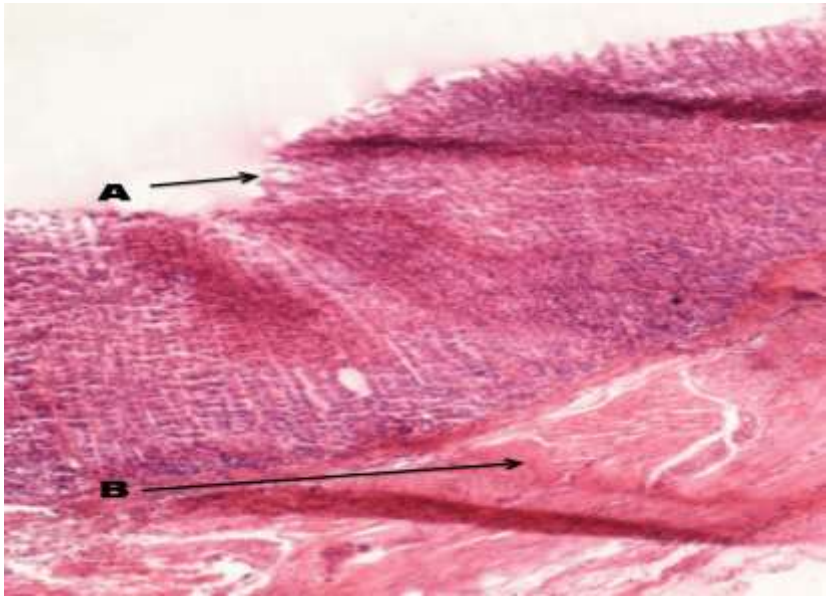

Figure 12: Photomicrograph of 2.5x5.7 cm H & E stained cross-section of stomach of Standard control (Omeprazole – 20 mg/kg B.wt.) group (H/3012/12) of Indomethacin

Induced Model showing A) Normal Epithelial lining, B) Near to normal Muscularis at X 100 magnification

Group IV: The rats in this group were subject to pretreatment with *Trichosanthes dioica* Methanolic extract at dose of 100 mg/kg B.wt. Followed by ulcer induction by Indomethacin. The stomach was taken out and without cutting it was filled with formalin and ligated at both ends of esophageal and duodenal opening for proper fixing of ulcer and better resolution. The photomicrograph below shows clearly atrophic mucosa alongwith erosions of epithelial lining. (Figure 13)

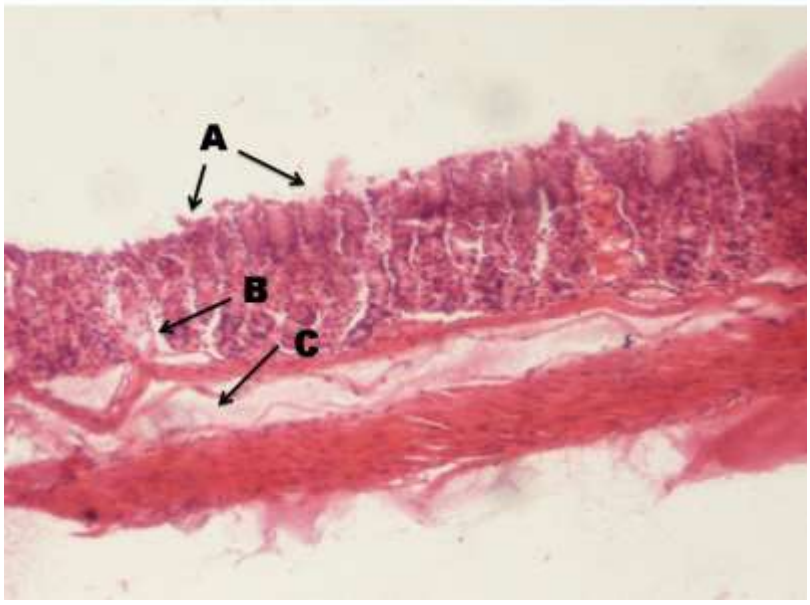

Figure 13: Photomicrograph of 2.5x4 cm H & E stained cross-section of stomach of Test 1 – 100 mg/kg B.wt. of Trichosanthes dioica group (H/3013/12) of Indomethacin Induced Model showing A) Ulceration of epithelial lining, alongwith B) Atrophic mucosa and C) Bit of Submucosal odematous.at X 100 magnification

Group V: The rats in this group were subject to pretreatment with *Trichosanthes dioica* Methanolic extract for the period of five days followed by ulcer induction with Indomethacin. The stomach section was kept in formalin a night before ulcer examination for better resolution and study of ulcers created. (Figure 14)

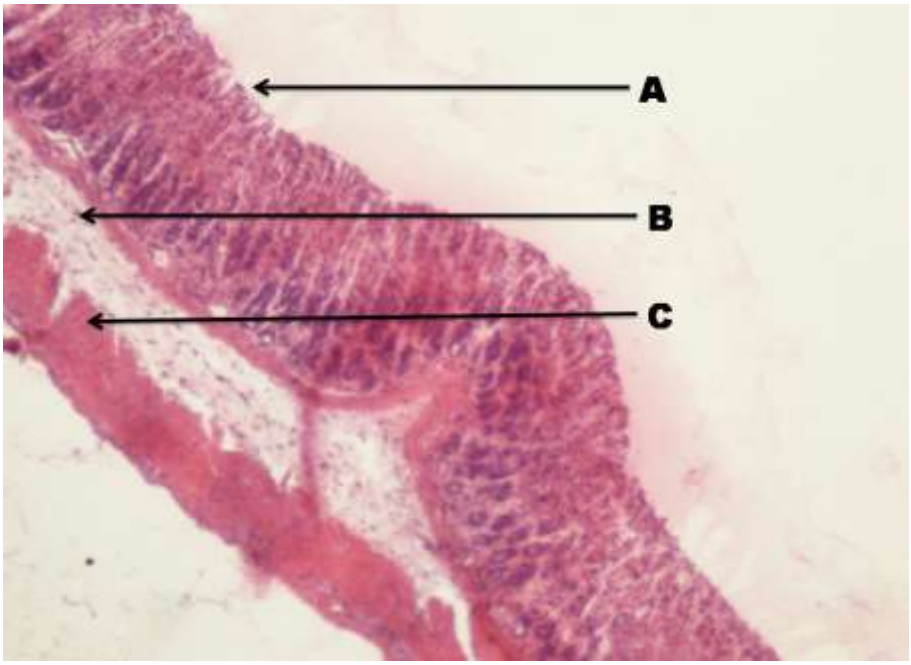

Figure 14: Photomicrograph of 3.7x4 cm H & E stained cross section of stomach from Test 2-200mg/kg B.wt. of Trichosanthes dioica Extract group (H/3014/12) of Indomethacin Induced Model showing A) Normal Mucosa, B) Normal Submucosa, C) Normal Mascularis at X 100 magnification

Pylorus-Ligation Ulcer Model Histopathology Reports

Group II: Negative control with ulcer induction for cross reference to study degree of gastro-protective effects of test 1 and test 2 groups. (Figure 15)

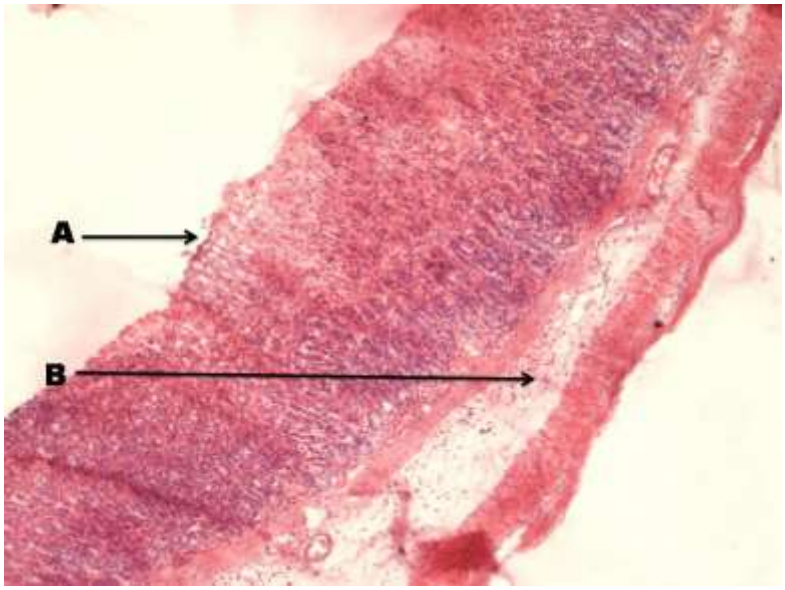

Figure 15: Photomicrograph of 3.5 x 5 cm H & E stained cross-section of rat stomach from Negative control group (H/3027/12) of Pylorus-ligation Induced Model showing A) Epithelial

erosions and B) Odematous submucosal layer at X 100 magnification

Group III: Standard drug was given for period of five days followed by ulcer induction through pylorus ligation. The photomicrograph clearly shows increased parietal cells and eosinophils. The omeprazole shows to play protective role by decreasing the ulcer forming factors. (Figure 16)

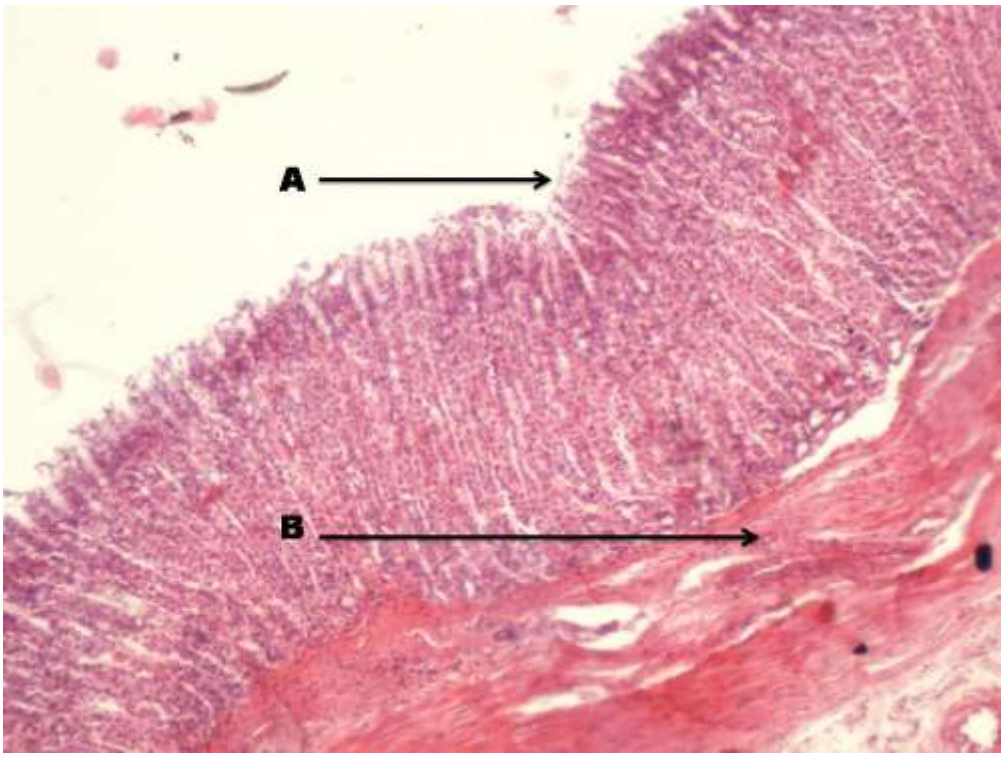

Figure 16: Photomicrograph of 5x3 cm H & E stained cross-section of rat stomach from standard control (Omeprazole – 20 mg/kg B.wt.) group (H/3029/12) of Pylorus-ligation Induced Model showing A) Normal Epithelial lining with reduction in eosinopils, B) Increased Parietal Cells at X 100 magnification.

Group IV: The rats in this group were subject to pretreatment with test 1 dose of 100 mg/kg B.wt. of *Trichosanthes dioica* Methanolic extract, followed by ulcer induction through pylorus ligation. The photomicrograph clearly shows the epithelial lining exfoliation and submucosal odematous. (Figure 17)

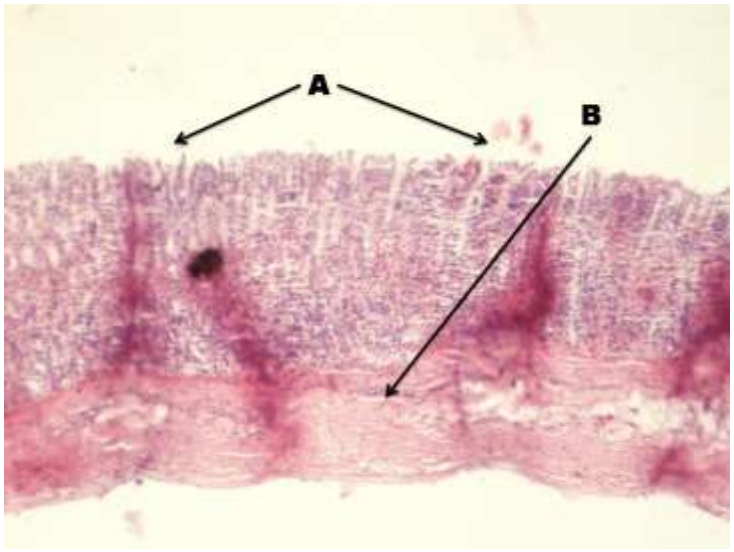

Figure 17: The photomicrograph of 5.7x3.2 cm H & E stained cross-section of stomach of rat from Test 1 – 100mg/kg B. wt. of Trichosanthes dioica Methanolic extract showing A) Epithelial lining erosion with traces of ulceration and B) traces of Oedematous submucosal lining at X 100 magnification.

Group V: The rats in this group were subject to pretreatment with higher dose of *Trichosanthes dioica* Methanolic extract at dose of 200 mg/kg B.wt. followed by ulcer induction through pylorus ligation. The photomicrograph shows normal epithelial, submucosal and muscularis lining with increased parietal cells, depicting ulcer healing via normalization of surrounding tissues. (Figure 18)

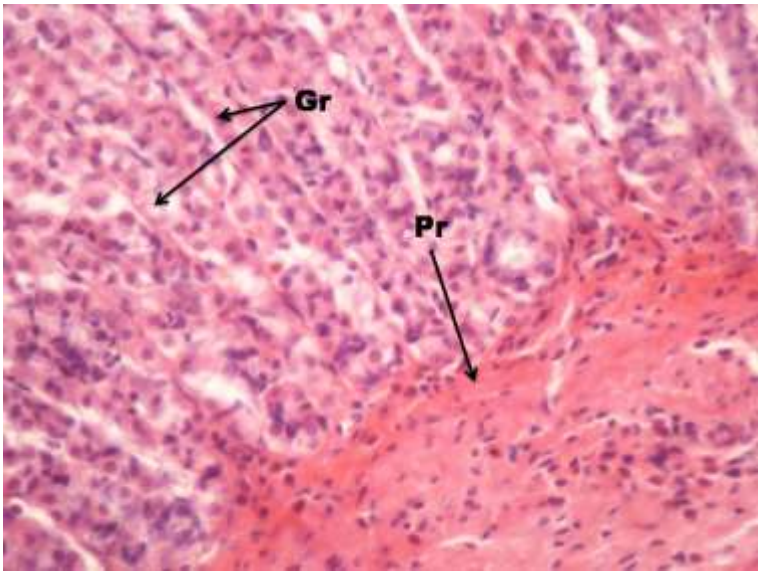

Figure 18: A photomicrograph of 4x3 cm of H & E stained cross section from stomach of test 2- 200mg/kg B.wt. group of Pylorus ligation Model showing A) Normal gastric pit lining and B) Submucosal lining at X 100 magnification.

CONCLUSION

The present Histopathology study of Rats stomach clearly shows the effects of doses of Methanolic extract of Trichosanthes dioica in prevention of ulcer. Moreover the reports sets an example in the ulcer healing drug research the effectiveness of Trichosanthes dioica in cure of ulcer, hence shall be explored to bring the drug in the market.

ACKNOWLEDGEMENT

I would like to thank the pathology department of Subharti Medical College for helping in histopathology study & to bring it to effectiveness.

REFERENCES

1. Solecki R. Shanidar IV, a Neanderthal flower burial in northern Iraq. Science 1975; 190:880–881.
2. Morgan K. Medicine of the Gods: Basic Principles of Ayurvedic Medicine, Available from: URL: [http: //www. compulink.co.uk/~mandrake/ayurveda.htm] 2002.
3. Mehta Devansh, Sharma AK. Trichosanthes dioica (Roxb.): A Review on Pharmacological update. Inventi Impact: Planta Activa 2012; 4S:187-194.
4. Trichosanthes dioica (Roxb.). Google Images 2015. Available from: URL: http://www.hygeiajournal.com/Downloads/1429479830untitled.JPG
5. Aberoumand, A., 2011. Screening of phytochemical and anti-nutrients compounds of eight food plants sources. W. J. Sci.Tech. 1(4): 49-53.
6. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., 2011. Phytochemical screening and extraction: A Review. Int.Pharm.Sci. 1(1): 98-106.
7. Singh, J., Maceration, Percolation and Infusion techniques of extraction of medicinal and aromatic plants[Online]. 2012 September 10[cited 2012]; Accessed from URL: http://www.ics.trieste.it/media/136911/df4318.pdf
8. Vogel GH, Vogel WH, Scholkens BA, Sandow J, Muller G, Vogel WF. Drug discovery and evaluation, Pharmclgcal assay 2002; (2): J.3.7- 3.7.5.
9. Khattab F, Khatta I. Histological and Ultrastructural studies on the gastric mucosa of rat after treatment with ethylene glycol. Aust J Basic & Appl Sci 2007; 1(3): 157-68.