CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1175794

Available online at: http://www.iajps.com Research Article

PHYTOCHEMICAL EVALUATION AND PHARMACOLOGICAL SCREENING OF ETHANOLIC EXTRACT OF AERIAL PARTS OF POLYGONUM GLABRUM FOR ANTIULCER ACTIVITY IN WISTAR RATS

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Abstract:

Polygonum glabrum has been used to treat peptic ulcer disease , but its efficacy has not been validated. The present study was therefore carried out to evaluate the anti-ulcer activity of 75% ethanolic aerial extract of polygonum glabrum in wistar rats. The Antiulcer Activity of 75% ethanolic aqueous extract of aerial parts of Polygonum glabrum at a dose of 200, 400 mg/kg b.w p.o was investigated in Diclofenac induced ulcers in Albino Wistar rats (180-230 gm). In this model parameters evaluated are gastric volume, total acidity, free acidity and ulcer index. There was no mortality up to a dose of 2000 mg/kg b.w p.o indicating the safety of the plant. Preliminary Phytochemical studies showed the presence of Carbohydrates, Flavanoid, Glycosides, Tannins, Protein, Terpenes and Volatile Oils. 75% ethanolic aqueous extract of Polygonum glabrum at a dose of 200,400 mg/kg b.w p.o produced significant reduction of ulcers in diclofenac induced ulcer Model as compared to control group by decreasing gastric volume, total acidity, free acidity and severity of ulcer. The ulcer protective effect of extract was comparable with that of the standard drugs. Results of study suggest that 75% ethanolic aqueous extract of Polygonum glabrum posses Mucoprotective effect which may be due to the presence of flavanoids in the extract as it has an astringent property.

Keywords: *Antiulcer activity, Polygonum glabrum, Diclofenac, Mucoprotective effect and Experimental rats.*

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Please cite this article in press as Mehnoor Farheen et al., ***Phytochemical Evaluation and Pharmacological Screening of Ethanolic Extract of Aerial Parts of Polygonum Glabrum for Antiulcer Activity in Wistar Rats****, Indo Am. J. P. Sci, 2018; 05(02).*

INTRODUCTION:

This study is intended to evaluate the Anti-Ulcer activity of 75% ethanolic aqueous extract of aerial parts of Plant by observing its action on the healing of gastric ulcers induced by the model diclofenac sodium Induced Ulcer Model in rats.

The antiulcer treatment of today's medicine is generally a triple therapy regimen i.e., it includes two antibiotics combined with one drug from either of two other classes of drugs – one designed to inhibit acid secretion and the other to protect gastric mucosa from chemical attack.

Now-a-days quadruple therapy regimen is in practice, but this treatment doesn't work in everyone. These triple and quadruple therapy regimen gladden the heart of pharmaceutical industry but not of Mother Nature who prefer natural remedies without side effets.

A peptic ulcer is an open crater or sore that develop in the inner lining (mucosa) of the stomach or the duodenum. A coating of mucus and other chemicals normally shields the stomach and duodenum from digesting themselves. When these protective mechanisms are disrupted, powerful digestive acids can erode into the lining of these organs and cause peptic ulcer.

Flavonoids are potent antioxidants and are of considerable interests recently because of their potential beneficial effects on human health in curing diseases. Catechin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activities.

The result of the present investigation that the formulation of Polygonum glabrum possesses antiulcer activity in Diclofenac induced acute ulcer model. It has shown a significant reduction in the gastric lesions in both the models. To regain the balance, different therapeutic agents including plant extracts are used (in experimental animals) to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells/or enhancing prostaglandin synthesis. Ranitidine the proton pump inhibitor play an important role in the reduction of gastric volume and total acidity and thus perform a cytoproective effect. The present results demonstrate that the formulation of Polygonum glabrum protect the rat gastric mucosa against hemorrhagic lesion produced by diclofenac.

Diclofenac induced gastric lesion development might be due to decrease in gastric blood flow, which ultimately leads to the progress of the hemorrhagic and necrotic aspect of tissue injury. It is of interest to note that administration of antioxidants inhibit diclofenac induced gastric injury in the rats. Polygonum glabrum possess significant antioxidant activity. In conclusion, the antiulcer effects of the above plant have been reported earlier, but there are no studies reporting the Polygonum glabrum and their activity in these models are quite impressive. The antiulcer activity of the formulation Polygonum glabrum can be compared to the activity of the standard drug Ranitidine.[1]

PATHOGENESIS

- It is not clear how the mucosal erosions occur in stress ulcers because actual hypersecretion of gastric acid is demonstrable in only Cushing's ulcers occurring from intracranial conditions such as due to brain trauma, intracranial surgery and brain tumours. In all other etiologic factors, gastric acid secretion is normal or below normal. In these conditions, the possible hypotheses for genesis of stress ulcers are as under:
- 1. Ischaemic hypoxic injury to the mucosal cells.
- 2. Depletion of the gastric mucus 'barrier' rendering the mucosa susceptible to attack by acid-peptic secretions.[2]

Causes

- The following ulcers causes severe stress. They are as follows:
- i) Psychological stress
- ii) Physiological stress as in the following:
- Shock, Severe trauma, Septicaemia, Extensive burns, Intracranial lesions., Intake of drug (ex., steroids, butazolidine, aspirin, indomethacin), Local irritants (e.g. alcohol, smoking, coffee etc).[3]

COMPLICATIONS

Perforated ulcer, Haemorrhage, Common signs of bleeding include vomiting bright red blood or passing blood or tarry black stools.Gastric Outlet Obstruction (GOO) which is due to edema or scarring.[4]

TREATMENT OF PEPTIC ULCER

Reduction of gastric acid secretion: H_2-antihistamines – Cimetidine, Ranitidine, Famotidine,[3,5]

DIAGNOSIS:

Esophagogastroduodenoscopy (EGS) a form of endoscopy, also known as a gastro copy in which a fiber optic tube with an attached camera is threaded

down the throat and esophagus into stomach and duodenum. With this sensitive method, the upper digestive tract is viewed, ulcer can be identified.[6]

SCREENING METHODOLOGIES FOR ANTIULCER ACTIVITY:

In Vivo Methods:

1. Pylorus Ligation In Rats (Shay model)
2. Stress Ulcer Through Immobilization Stress
3. Stress Ulcers By Cold Water Immersion
4. Indomethacin Induced Ulcers In Rats
5. Ethanol Induced Mucosal Damage In Rats (Cytoprotective Activity)
6. Subacute Gastric Ulcer In Rats
7. Gastric Ischemia-Reperfusion Injury In Rats.
8. NSAID's induced ulcer in wistar rats. [7]

Gastric and duodenal ulcers

- Thus, the etiology of peptic ulcers possibly may not be explained on the basis of a single factor but is multifactorial. These factors are discussed below but the first two—H. pylori gastritis and NSAIDs-induced injury are considered most important.[8]

DRUG UNDER STUDY

Polygonum glabrum (Family- Polygonaceae) known as thick flower knot weed or common marsh Buckwheat **[9]** is a perennial shrub commonly found in India.

Synonyms:

Persicaria glabra, Persicaria densiforium, Polygonum densiflora, Polygonum portoricense**.[9]**

Origin:

The name Polygonum is derived from the Greek words "poly", that means "many" and "gonu", that means "Knee or joint" hence "many joints" because of thickened joints on the stem. The species name glabrum is based on the smooth or hairless appearance of the leaves. [10]

Vernacular names:

LANGUAGES		**NAMES**
English	**:**	Common Marsh Buckwheat
Hindi	:	Sauriarak, Jioti, Balrampur
Tamil	:	Attalaree, Sivappu kumbakodaali
Marathi	:	Sherad, Rakta rohida
Sanskrit	:	Rakta rohidaa[11]

Similar species:

Polgonum aviculare L., Polgonum accuminatum L. , Polygonum capitatum, Polygonum lapthifolium.[12]

Distribution:

Polygonum glabrum is generally distributed in India, it widely grows in Karnataka, Gujrat, Maharashtra, Madhya Pradesh, Uttar Pradesh, West Bengal, Orissa, Punjab, Rajasthan and Sikkim.[13]

Habitat:

Habit: Annual herb.

Habitat: Generally grows near river banks, stream sides, marshy areas, sea level to 100 meters. [14]

Phytochemical constituents:

The various phytochemical constituents present in polygonum glabrum are:

Flavonoids were identified as 7,4'-dimethyl quercetin,3'-methylquercetin and quercetin, rutin, rhamnetin, avicularin and the flavonoid glucoside was isoquercitrin. Carbohydrates like mono and sesquiterpenes, tannins like syringic and vanillic acid, apart from this proteins, glycosides, proteins and volatile oils are also present.[15]

Pharmalogical Activities:

1) **Anti-Depressant Activity:** The action produced by ***Polygonum glabrum***(Ethanolic extract) was more than that of diazepam in open field explanatory behavior to see the anti depressant activity. Observations confirm that ***Polygonum glabrum*** posses significant antidepressant activity.[16]
2) **Hepatoprotective Activity:** The ethanolic leaf extract of ***Polygonum glabrum*** exhibited significant hepatoprotective effect of methanolic extract of ***Polygonum glabrum*** was studied against CCl4 induced hepatic injury in albino rats.[17]
3) **Anti-Inflammatory:** Anti-Inflammatory studies were conducted on a hot water decoction and on ethanolic extracts of stem of ***Polygonum glabrum***. Effective Anti-Inflammatory activity was demonstrated against acute carragagean induced paw oedema, exudate and granuloma formation in granuloma pouch test.[18]
4) **Anthelmintic Activity:** A pure Anti-Elmintic substance (PGA) has been separated from the methanol-aqueous extract of leaf of ***Polygonum glabrum*** PGA demonstrated mulluscidial activity against *Biomphalaria glabrata* and *limnea turncutata*. It also showed antiparasitic in

vitro activity of several fractions isolated from the plant.[19]

5) **Larvicidal Activity:** This study is on the evaluation of larvicidal activity to determine the efficacies of ethanolic extracts using ***Polygonum glabrum.*** The larvicidal activity of ***Polygonum glabrum*** extract against instar larvae of *Culex quinque fasciatus* was evolved.[20]
6) **Anti-Fungal Activity:** ***Polygonum glabrum*** significantly inhibited the growth of *Colletotrichum truncatum.*[21]
7) **Anti-Bacterial:** The presence of Anti-Bacterial activity was tested by Agar well diffusion method against three grams positive *Bacillus subtilis*, *Bacillus cereus* and *Staphylococus aureus* bacterial strains ***Polygonum glabrum*** showed Anti-Bacterial activity.[22]
8) **Anti-Oxidant Activity:** In Ethanolic extract of ***Polygonum glabrum*****,** Antioxidant status in liver was determined by measuring the activity of malonaldehyde (MDA), glutathione (GSH), total thiol and catalase (CAT). Total wet weight and histopathological study of isolated liver specimen was also carried out.[23]
9) **In vitro Cytotoxic, Membrane Stabilizing and Thrombolytic Activity:** The crude methanol extract of leaves of *Polygonum glabrum* Willd and its Kupchan fractions were screened for cytotoxic, membrane stabilizing and thrombolytic activities. Among all fractions, the crude methanol extract showed significant cytotoxic activity having LC 50 value 0.74 ± 0.023 µg/ml.[24]

TRADITIONAL USES:

Polygonum glabrum is extensively used in traditional system of medicine in the treatment of numerous diseases. Various parts of the plant were extensively used in Ayurveda, Siddha, Unani and Homeopathic medicines.

REGION	PLANT PART USED	MEDICINAL USES
Omdurman, Sudan	Leaves	Anthelmintic, antimalarial[25]
Mangalore, Karnataka	Leaves and Roots	As colic, febrifuge, piles, jaundice
Gondia District(M.S.) India	Boiled Plant paste	on cuts and wounds[26]
Dir Kohistan Valley Pakistan	Whole plant	Fresh plants are powdered and then Stirred in the standing water for hunting fishes
Pakistan	Peels from stems	To treat rheumatism[25]
Gujarat	Plant juice and root stock	Used in pneumonia, jaundice, fevers
Madugga tribes of Siruvani forest, South India	Leaf	The leaf extract (about 5 ml) is mixed with common salts (1-2gm); boiled and used in desentry

MATERIALS AND METHODS:

Chemicals required:

- Diclofenac sodium - 400mg/kg (Asha analytical Pvt. Ltd ,Uppal,Hyd)
- Ranitidine- 13.5 mg/kg (Vinal labs,Yadgirigutta)
- NaOH (Asha analytical Ltd,Uppal,Hyd)
- Topfers reagent (Nice chemicals Pvt Ltd,Cochin)
- Formalin 10% (Asha analytical Pvt Ltd,Uppal,Hyd)
- Tween 80 2%(Asha analytical Pvt Ltd,Uppal,Hyd)
- Distilled water

Collection of plant material:

The Aerial parts of Polygonum glabrum used for the present studies was collected from Chitoor district of Andhra pradesh. The plant had been recognised, established and validated by differentiating with the voucher of specimen accessible at Survey of medicinal plants & collection unit, Botany Department, Sri Venkateswara University, Tirupathi by Field Botanist Dr. Madhav shetty. The bark was cut into small pieces and shade dried. The dried material was pulverized into coarse powder separately by a mechanical grinder. The obtained powder was then utilised for extraction purpose.

Preparation of ethanolic Extract:

In Soxhlet apparatus, the disintegrated drug was dabbed and packed fairly and extracted with 1500 ml of ethanol for 7 days. Using Rotary flash evaporator the extract was condensed and dabbed and kept in dessicator til it was used.

Experimental animals:

Swiss Albino adult rats were obtained from Mahaveer enterprises, Hyd(169/CPCSEA/1999). The rats were allocated casually into 5 groups comprising of 6 rats in each group. Each rat weighs between 180-200 gms were housed in four different groups (six rats per cage). The animals were left for 2 days to adapt to the animal room conditions. They were preserved in the standard laboratory conditions in temperature about 22±2°c, humidity, 12 hours light and dark cycles fed with standard pellet diet (Hindustan lever, Bangalore) and sufficient tap water.

Methods:

The rats are divided into different groups each comprising of minimum of five rats as detailed below

Group I – Control rats (Diclofenac +tween 80)

Group II – Diclofenac induced ulcer rats orally treated with Ranitidine (13.5 mg/kg b.w)

Group III – Diclofenac induced ulcer rats orally treated with formulation (*Polygonum glabrum*) at the dose of 200 mg/kg b.w (SINGLE DOSE)

Group IV – Diclofenac induced ulcer rats orally treated with formulation (DOUBLE DOSE)

Animals in every groups were kept for 1 day fasting after receiving diclofenac sodium (NSAIDs, 20 mg/kg) by all the animals. The oral feeding of water and diclofenac sodium was continued for 3 days, the animal of II, III and IV were administered with ranitidine (13.5mg/kg), extract (200mg/kg), extract (400mg/kg) respectively after 3 h. of diclofenac.administration.

On 4th day the animals were sacrificed, stomach were removed & cut along the greater curvature.

EVALUATION PARAMETERS:

Collection of Gastric Juice:

The stomach was removed carefully by closing esophagus, opened along the greater curvature and the gastric contents were removed out. The gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5 min; the volume of the supernatant was expressed as ml/100 gm body weight. The mucosa was passed with saline and examined for gastric lesions using a dissecting microscope, ulcer score was determined.

Ulcer Scoring:

After sacrificing the rat, stomach was removed and opened along the greater curvature, and washed it slowly under running tap water. Put it on the glass slide and observe under 10X magnification for ulcer. Score the ulcers as below.

0 = normal coloured stomach
0.5 = red colouration
1 = spot ulcers
1.5 = haemorrhagic streaks
2 = Ulcers ≥ 3 but ≤ 5
3 = Ulcers >5

Mean ulcer score for each animal is expressed as Ulcer Index.

Free acidity and Total acidity:

Note the total volume of NaOH which corresponds to the total acidity. Acidity (mEq/1/100 g) can be expressed as :

$$\textbf{Acidity} = \frac{\text{Vol.of NaOH} \times \text{Normality} \times 100}{0.1} \textbf{ mEq/l/100 g}$$

Statistical analysis of data:
Results were expressed as mean ± S.E.M. The statistical difference between the groups in the term of the mean rate of wound healing was calculated in terms of ANOVA mean ± S.E.M. The difference was considered significant if P< 0.05.

RESULTS:

Table 1: Phytochemical Screening

S. No.	Test	Pet ether Extract	Chloroform Extract	ethanolic Extract
1	**Carbohydrates**			
	Mohlish's test	++	++	++
	Fehling's test	++	++	++
2	**Proteins and amino Acids**			
	Ninhydrin test	+	+	+
	Biuret test	+	+	+
3	**Alkaloids**			
	Mayer's test	++	++	++
	Wagner's test	++	++	++
4	**Fixed oils and fats**			
	Spot test	+	-	-
5	**Glycosides**			
	Borntrager's test	+	+	+
	Legals test	++	+	+
6	**Triterpenoids**			
	Tin + thionyl chloride	+	-	-
7	**PhenoliPG and tannins**			
	Ferric chloride test	++	++	++
	Gelatin test	++	++	++
	Lead acetate test	++	++	++
8	Flavonoids	+	+	+

(+) = Presence, (++) = present in excess amount, (-) = Absence

Pharmacological studies:

In diclofenac sodium induced ulcers

- **Effect of Gastric Volume :**

Administration of the extract significantly decreased the gastric volume when compared with the rats treated with Ranitidine. Comparing the gastric volume and gastric acidity, the gastric volume gets decreased, at the same time the gastric acidity also reduced significantly.

- **Effect of Free Acidity and Total Acidity :**

Based on the titre values, free acidity and total acidity were determined. The free acidity and total acidity of extract on albino rats reduced significantly in contrast with the standard group treated with Ranitidine.

- **Ulcer index :**

By taking the mean ulcer score of each groups, the ulcer index was calculated. Then the graph of mean ulcer score was plotted by taking groups on x-axis and ulcer index on y-axis. The histograms of different groups were then introduced by comparing the ulcer index of group II with group III, IV and V. It was observed that the ulcer index of Dose group V was notably nearly equal when compared to the standard group (Group-III) treated with Ranitidine.

Table 2: Effect Of Formulation on Gastric Volume Free Acidity Total Acidity and Ulcer Index

Groups	Body wt. of rats	Drugs given	Gastric volume	Free Acidity	Total Acidity	Ulcer index
Group I	177.2 ± 1.15	Diclofenac sodium + 2% Tween 80	1 ± 0.04	14.70 ± 0.29	29.6 ± 0.69	3.7 ± 0.14
Group II	161.2 ± 2.15	Ranitidine + Diclofenac sodium	0.8 ± 0.05	8.8 ± 0.31	15.56 ± 0.69	2.2 ± 0.10
Group III	172.5 ± 4.45	Herbal formulation + Diclofenac sodium	0.6 ± 0.03*	7.3 ± 0.32*	12.56 ± 0.68*	2 ± 0.18*
Group **I**V	164.4 ± 1.16	Herbal formulation(double dose)+ Diclofenac sodium	0.5 ± 0.04**	4.6 ± 0.42**	9.5 ± 0.59**	1.5 ± 0.9**

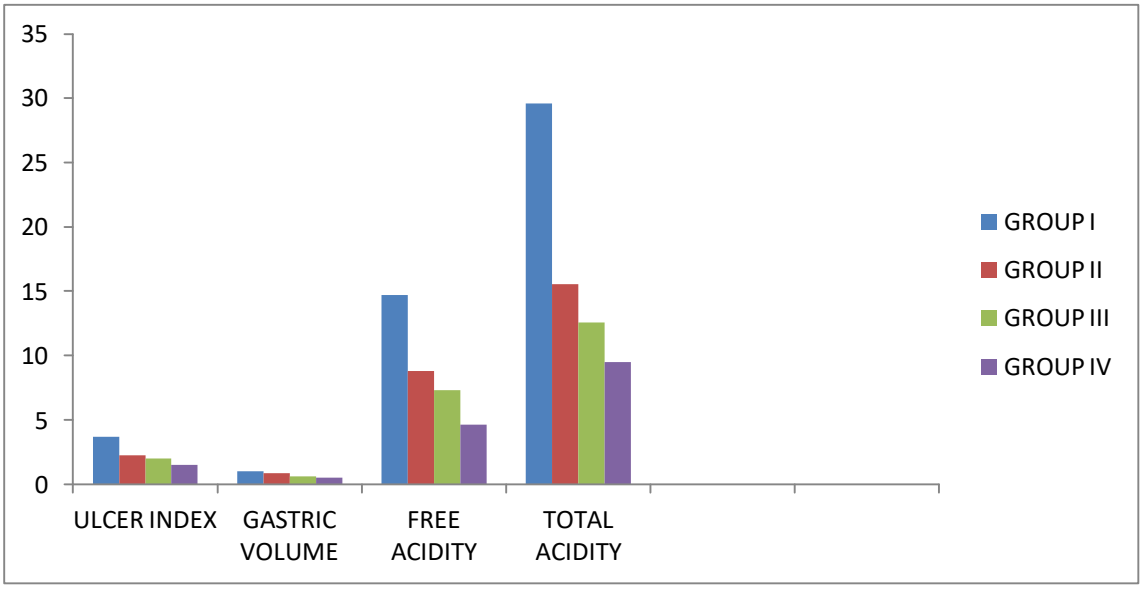
Values are expressed in terms of mean ± SEM of 5 rats (ANOVA)

P values: **< 0.001 - Highly significant

* <0.05 - Significant

N S: Non Significant

COMPARISION OF PARAMETERS



Graph 1: Comparision of Parameters

Group I: Control received only Diclofenac sodium (20 mg/kg)
Group II: Standard received Ranitidine (13.5 mg/kg)
Group III: Dose I received Formulation PG (200 mg/kg) for 3 days + Diclofenac sodium (20 mg/kg)
Group IV: Dose II received Formulation PG (400 mg/kg) for 3 days + Diclofenac sodium (20 mg/kg)

MACROSCOPICAL VIEW OF RAT STOMACH
After mounting the rat stomach on glass slide and observed in 10x magnification, different scores were noted in each groups. The mean ulcer score represents the ulcer index. The scores on each group were compared, i.e., group I with group II, III and IV. It was noticed that Group III and IV shows similarity in the score as that of group II.

- **IN DICLOFENAC SODIUM INDUCED ULCERS :**

Oral administration of 20mg/kg b.w. of diclofenac sodium caused a significant ($p< 0.05$) increase in the degree of ulceration in the rats.

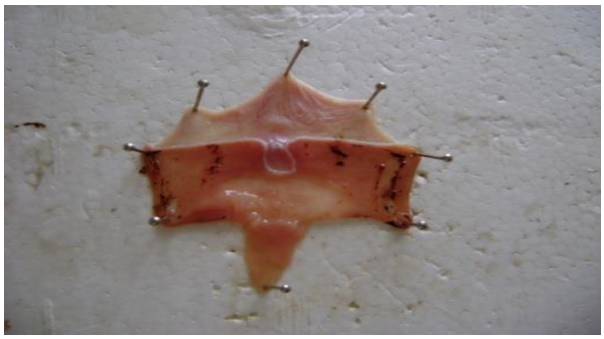


Fig. 1: Group I (CONTROL)

Fig. 2: Group II (STANDARD)

A significant reduction was observed in the activity of standard drug Ranitidine in the Diclofenac sodium induced animals.

Extracts at 200mg/kg b.w. and 400mg/kg b.w. regimen resulted in significant improvement in these parameters and the observable effects compared well with both normal control group and standard drug (Ranitidine) employed in the study.

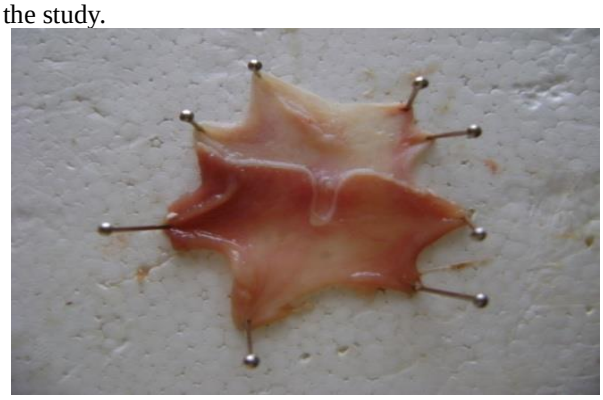


Fig.3: Group III (SINGLE DOSE)

Fig. 4: Group IV (DOUBLE DOSE)

DISCUSSION:
Before screening the test extract for antiulcer activity, the extract was undergone to the acute toxicity studies as per OECD guide lines no. 420 (fixed dose method).The extract was established to be nontoxic at 2000mg/kg as indicated by the mortality in the treated group. Hence, the 2000 mg/kg was treated as cut off tolerable dose, $1/10^{th}$ (200mg/kg), and $1/5^{th}$ (400mg/kg) of this dose were adopted for the further study.

It is evident from the result of the current investigation that the *Polygonum glabrum* formulation possesses antiulcer activity in Diclofenac induced acute ulcer model. It has shown a notable depletion in the lesions of gastric in both the models.

Diclofenac induced gastric lesion development may be because of decrease in gastric blood flow, which ultimately leads to the progress of the hemorrhagic and necrotic feature of tissue injury. It is to be note that the applications of antioxidants obstruct diclofenac causes gastric injury in the rats. *Polygonum glabrum* possess significant antioxidant activity.

In conclusion, the antiulcer effects of the above plant have been reported earlier, but there are no studies reporting the *Polygonum glabrum* and their activity in these models are quite impressive. The antiulcer activity of the formulation *Polygonum glabrum* can be differentiated to the standard drug Ranitidine activity.

SUMMARY AND CONCLUSION:

From the results discussed above it can be summarized that the *Polygonum glabrum* formulation shows the antiulcer activity against the Diclofenac sodium induced gastric ulceration animal model of rats. At the dose level tested, the signs of toxic effects were not shown in treated mice as well as rats.

Peptic ulcer disease is most common one. Many drugs are there in market to treat the ulcer, but they are having lot of adverse effects. In the present theory, using combination of herbal drugs have proved that these are the effective alternatives for chemical drugs.

The Anti-ulcer Herbal formulation *Polygonum glabrum* is having significant activity in animals models used, equated with the drug Ranitidine which is standard.

In the current study the 70 % Polygonum glabrum herb ethanolic extract shown better Anti-ulcer activity in experimental rat models, because of the existence of flavonoids and other poly phenolic compounds. Hence, the research justifies that the herbal extract could be effectively utilised in the treatment of Ulcer. Further studies are required to quarantine and characterize the active component(s) accountable for the anti- ulcer property of the test extract and findings should be confirmed by performing clinical studies.

ACKNOWLEDGEMENT:

Behind every success there are lot many efforts, but efforts are fruitful due to hands making the passage smoother. This thesis of m. pharmacy is the result of a challenging journey, upon which many people have contributed and given their support. It is our privilege to thank Mrs. Mehnoor farheen, without whose guidance I could not have completed my project work. I derive great pleasure in expressing our sincere gratitude to our principal Dr.Mrs. Sunita, for her timely suggestions, which helped me to complete this work successfully. The satisfaction and euphoria that accompany the successful completion of the task would be great but incomplete without the mention of the people who made it possible with their constant guidance and encouragement crowns all the efforts with success. In the context, I would like to thank my parents, for showing their unlimited support and encouragement. I would like to thank all the other staff members, both teaching and non-teaching, who have extended their timely help and eased my task.

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