GASTROPROTECTIVE EFFECT OF *SWERTIA CHIRAYITA* – A STUDY WITH ULCER INDUCED RATS

Selvaperumal Munis Karthikeyan Narmadha Selvamathy[1], Arumugam Geetha[1*], Panerselvam Saranya[1],

[1]Department of Biochemistry, Bharathi Women,s College, North Chennai, Tamil Nadu, India.

Summary

The aim of the present study was to evaluate the gastroprotective effect of hydro alcoholic extract of *Swertia chirayita* (SCHAE) on experimental ulcers in rats.Male albino Wistar rats were induced for gastric ulcer by ethanol, pyloric ligation (PL) and cold restraint stress (CRS) after pretreatment with SCHAE (100/200mg/ kg b.wt) for 30 days. The gastro protective effect of SCHAE was assessed by lesion index, pH, titrable acidity, level of pepsin, lipid peroxides (LPO), reduced glutathione (GSH), myeloperoxidase activity and by the levels of enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), nucleic acid and protein bound carbohydrates. The gastric tissues were used for the determination of adherent mucus content and also for the histological examination. A significant reduction in lesion index were observed in ulcer induced animals pretreated with 200mg/kg b.wt of SCHAE. The mucin content was found to be restored significantly in ulcer induced SCHAE pre-treated animals. The alterations observed in the level of LPO, pH, titrable acidity were found to be minimized in SCHAE treated animals. Histological observations on gastric mucosa also confirmed the gastro protective activity of SCHAE. Normal rats treated with SCHAE did not show any toxicity.From the results of this study, it could be concluded that SCHAE acts as an antiulcer agent probably by its free radical scavenging activity and mucin protective nature.

Key words: *Swertia chrayita*; Gastric ulcer; Mucin; lipid peroxides; antioxidants; H+K+ATPase.

Introduction

Peptic ulcer is a disease of multifactorial etiology including genetic factors, life-style factors and infection with *Helicobacter pylori* (*H. pylori*). It is a benign lesion of the gastric or duodenal mucosa occurring at sites where the mucosal epithelium is exposed to acid and pepsin. Peptic disease is caused by, and its persistence is due to the breakdown of a supposed equilibrium between aggressive and defensive factors [1, 2].

Defensive factors mainly involve mucus-bicarbonate secretion and prostaglandins[3]. The putative pathogenic properties of *H. pylori* may be produced by disease-inducing factors that cause adverse pathological effects in the mucosa, such as pepsinogen stimulation, reduced mucus secretion, and increased gastric secretion [4].

Gastric ulcer is a common disorder where inflammation in the gastric mucosa is observed[5]. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products and drugs [6]. These agents have been implicated in the pathogenesis of gastric ulcer, including increased gastric acid and pepsin secretion, decreased gastric blood flow, suppressed endogenous generation of prostaglandins, inhibited mucosal growth and cell proliferation, and altered gastric motility [7]. Overproduction of gastric acid was considered to be the most important factor in the pathogenesis of peptic ulcer and the treatment was based mainly on the inhibition of gastric acid secretion [8].

Most of the antisecretory drugs such as proton pump inhibitors (omeprazole, lansoprazole etc.) and histamine H_2 - receptor blockers (ranitidine, famotidine etc.) are extensively used to control increased acid secretion and related disorders caused by stress, non steroidal anti-inflammatory drugs (NSAIDS) and *H. pylori,* but there are reports of adverse effects and relapse in the long run [9,10]. On the contrary, most of the herbal drugs reduce the effect of offensive factors and proved to be safe, clinically effective, better patient tolerance, relatively less expensive and globally competitive (Goel and Sairam 2002). Plant extracts, however, are some of the attractive sources of new drugs and used traditionally for the treatment of gastric ulcers. One such plant is *Swertia chirayita* (Roxb. ex Flem.) H. karst (Gentianacea) and is a native of temperate Himalayas found at an altitude of 1300 - 3000 mts from Kashmir to Bhutan and in the Kasi hills at 1200 – 1500 mts. It can also grow in sub-temperate region such as southern hilly areas. It has antihelmintic, antimalarial, cardiac stimulant, hepatoprotective, hypoglycemic and anti-inflammatory activities [11]. Traditional consumption of *Swertia chirayita* for gastric complications has been followed in many parts of India [12] by concoction and infusing the whole plant in water. But it lacks experimental proof.

Based on the details available in the literature, the present study was directed towards the assessment of gastroprotective efficacy of *Swertia chirayita*, against ethanol (EtOH), cold restraint stress (CRS), aspirin (ASA) and pyloric ligation (PL) induced gastric ulcer models. Lesion index, volume of gastric juice, pH, titrable acidity and concentrations of pepsin, myeloperoxidase (MPO), lipid peroxides (LPO), reduced glutathione (GSH), enzymatic antioxidants- glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT),nucleic acid, protein bound carbohydrates and adherent mucus content were determined to evaluate the ulcer protective efficacy of hydroalcoholic extract of aerial parts of *Swertia chirayita* (SCHAE).

Materials and Methods

Plant material

The aerial parts of *Swertia chirayita* was obtained from local drug market, Chennai, Tamilnadu and authenticated by the Taxonomist Dr. Jayaraman (Retired Professor), Plant Anatomy Research Centre, Chennai. (Voucher No. of the specimen PARC/2008/149).

Preparation of Plant Extract

The aerial parts of *Swertia chirayita* were air dried and coarsely ground into homogenous powder. The powder was soaked in alcohol – water mixture in the ratio 7:3 for 4 days. The extract was concentrated and lyophilized by freeze drying. The dried extract (SCHAE) was stored in vacuum desiccator for further use.

Animal used

Male albino rats of Wistar strain weighing about 150-200g were obtained from the Tamilnadu Veterinary and Animal Sciences University, Chennai, India. They were acclimatized to animal house conditions, fed commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. This study was conducted according to the guidelines approved by the Institutional Animal Ethics Committee (IAEC No.290/CPCSEA/12/12/08-06).

Preventive effect of Swertia chirayita on ulcerogen induced gastric ulcer

Rats were treated with 50/100/200 and 1000mg/kg b.wt of hydroalcoholic extract of *Swertia chirayita* orally for 30 days and on day 31, all the animals were subjected to ulcerogenesis. The ulcerogen treated rats were sacrificed and the ulcer score was determined in gastric mucosa. It was observed that 200mg/kg b.wt has produced optimum reduction in ulcer score. Thus 100 and 200mg/kg b.wt have been chosen as the dosages for the study.

Groupings

Group I : Control rats without any treatment (n=6).

Group II (a-d) : Ulcerogens treated rats: a-ethanol; b-CRS; c-PL and d-ASA (n=6).

Group III (a-d): Rats pre-treated with 100mg/kg b.wt of hydroalcoholic extract once daily for 30 days + ulcerogen (n=6).

Group IV (a-d): Rats pre-treated with 200mg/kg b.wt of hydrolcoholic extract for 30 days + ulcerogen (n=6).

Group V : Rats treated with 200mg/kg b.wt of Swertia chirayita for 30 days (n=6).

Group VI : Rats pre-treated with 30mg/kg b.wt of Ranitidine for 30 days.

Group VII (a-d): Rats pre-treated with 30mg/kg b.wt of Ranitidine for 30 days + ulcerogen (n=6).

Ulcer induction by (a) EtoH (b) CRS (c) ASA and (d) PL were done after the last dose of drug.

Ethanol induced ulcer (a)

The gastric ulcer was induced in rats by administrating 80% EtOH (1 ml/200g/1h) and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers. After identification of ulcer areas, the length of the ulcer was measured along the greater curvature. Number of hemorrhagic spots was converted to mm^2 of ulcer [13]. The mean ulcer size was calculated by dividing the total length (in mm) of ulcers for all the animals divided by total number of animals.

Cold restraint induced ulcer (CRS) (b)

On day 30, after 30 min of *Swertia chirayita* or ranitidine treatment, rats were immobilized in a stress cage containing water at 4-6°C 14. The animals were sacrificed 2h later and ulcer index was calculated following the method as described earlier.

Pyloric ligation (PL) - induced ulcer (c)

Drugs were administered for a period of 30 days. On 30th day after the last dose, the rats were kept for 18h fasting and care was taken to avoid coprophagy. Animals were anaesthetized using diethyl ether, the abdomen was opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post - operative period 15. After 4h, stomach was dissected out and the contents collected. The ulcer score was done by the method as described earlier.

Aspirin – (ASA) – induced ulcer

ASA at the dose of 200mg/kg was administered to the animals and ulcers were scored after 4h. The stomach was taken out and cut open along the greater curvature and the ulcer index was scored by a person unaware of the experimental protocol in the glandular portion of the stomach [16].

Determination of acid secretory parameters

The animals were sacrificed, stomach was dissected out and the gastric juice collected was centrifuged for 5 min at 2000 x g and the volume of the supernatant was expressed as ml/120 g b.wt and pepsin was assayed using hemoglobin as substrate and results were expressed as µM of tyrosine liberated/ml [17]. pH was measured using pH meter and total acid output was determined by titrating with 0.01N NaOH, using phenolphthalein as indicator and was expressed as mEq/l/120 g. The absorbance of the solution was read at 650 nm. Results were expressed as µM of tyrosine liberated/ml.

Assay of H^+K^+ ATPase (EC no: 3.6.3.10)

The parietal cells were isolated from mucosal scrapings of experimental rats [18]. The scrapped contents were homogenized in 20 mM Tris- HCl, pH 7.4, centrifuged for 10 min at 5000 x g and the remaining supernatant was subsequently centrifuged at 5000 x g for 20 min. The protein concentration was determined by the method of Bradford with bovine serum albumin as standard [19] and the prepared parietal cell extract was employed to determine the H^+K^+-ATPase inhibition.

The H^+K^+-ATPase activity was assayed by the method of Ricardo et al [20]. One ml of the reaction mixture contained an aliquot of enzyme in 20mM Tris-HCl, pH 7.4, 2 mM $MgCl_2$ and 2mM KCl. Reaction was started with the addition of 2mM ATP and incubated for 30 min at 37°C and the reaction was terminated by the addition of ammonium molybdate and trichloroacetic acid followed by centrifugation at 2000 x g. The amount of inorganic phosphorus released from ATP was measured at 640 nm. The enzyme activity was expressed as nM of pyruvate liberated/min/mg protein.

Determination of gastric wall mucin and myeloperoxidase activity (EC no: 1.11.1)

Gastric wall mucin content was determined by the method of Corne et al [21]. The glandular segment of the stomach was separated from lumen of the stomach, weighed and transferred immediately to 10 ml of 0.1 % w/v Alcian blue solution (in 0.16 mM sucrose solution buffered with 0.05 ml sodium acetate at pH 5. Tissue was stained for 2h in Alcian blue, and excess dye was removed by successive rinses with 10 ml of 0.25mM sucrose, first for 15 min and then for 45 min. Dye complexed with gastric wall mucus was extracted with 10 ml of 0.5 mM MgCl2 which was intermittently shaken for 2h. Four ml of the blue extract with equal volume of diethyl ether was shaken vigorously. The resulting emulsion was centrifuged at 4000 x g for 10 min and the aqueous layer was measured at 580 nm. The quantity of Alcian blue extracted per g of wet glandular tissue was then calculated.

MPO activity in the gastric mucosa was measured according to the method of Bradley et al[22]. Pre-weighed tissue was homogenized (1:10 wt/vol) in 0.5% hexadecyltrimethyl ammonium bromide in 50 mM potassium phosphate buffer (pH 6.0) before sonication in an ice bath for 20s. Three freeze/thaw cycles were performed followed by sonication (20s in ice bath).

The sample was centrifuged at 17000 x g (5 min, 4°C) and MPO was assayed by mixing of 0.1ml of supernatant and 2.9ml of 50mM/L potassium phosphate buffer (pH 6.0) containing 0.167 g/L o-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. The change in absorbance at 460nm was measured for 4 min using an UV Elico spectrophotometer.

Estimation of protein and nucleic acids

Protein was estimated by the method of Bradford 19 using alkaline copper reagent and folin's reagent. The colour developed was read at 640 nm. The level of protein was expressed as mg/g tissue. DNA was estimated according to the method[23]. The nucleic acid extract was treated with diphenylamine reagent and the blue color developed was read at 640 nm. Values were expressed as mg/g tissue. RNA was determined by the method of Rawal et al[24]. The nucleic acid extract was treated with orcinol and the green color developed was read at 675 nm. Values were expressed as mg/g tissue.

Determination of glycoprotein components in gastric mucosa

Glycoproteins in gastric mucosal tissues were precipitated, hydrolysed and the protein bound hexose, hexosamine, fucose and sialic acid were estimated. The known amount of defatted tissue was hydrolysed with 1.0 ml of 2N HCl and 1% phosphotunstic acid at 100° C for 4 h to liberate the protein – bound compounds. The hydrolysate was neutralized with 4N sodium hydroxide and was used for the estimation. An aliquot of gastric juice was divided into two fractions and to each of these fractions was added 95% ethanol in 1:10 ratio. The precipitate of one of the tube was used to estimate the sialic acid and the precipitate of other tube was utilized to estimate hexosamine, hexose, fucose and protein.

Estimation of hexose was done by the method Niebes [25] using orcinol reagent. The colour developed was read at 540nm. The values were expressed as mg/g tissue. Hexosamine was estimated by the method of Wagner [26] using acetyl acetone reagent and Ehrlich's reagent and read at 540 nm. The content of hexosamine was expressed as mg/g tissue. Analysis of sialic acid was carried out by the method of Warren[27]. Periodate solution and tiobarbituric acid was added and the absorbance was read at 540nm. The levels of sialic acid was exprssed as mg/g tissue. Fucose was estimated by the method of Winzler [28] using H_2SO_4 reagent and cysteine- HCl. The absorbance was read at 396 nm. The fucose content was expressed as mg/g tissue.

Estimation of lipid peroxides, reduced glutathione and antioxidant enzymes

Lipid peroxides in terms of malondialdehyde (MDA) was estimated using 1,1',3,3'- tetra methoxypropane as the standard and was expressed as nM/mg protein [29]. GSH content of gastric tissue and serum was determined by the method of Flohe and Gunzler [30]. Aliquots of homogenate or serum were mixed with equal volume of ice cold 5 % TCA and the precipitated proteins were removed by centrifugation.

The supernatant was added to equal volumes of 0.5 M Tris-HCl, pH 9.0 containing 20mM DTNB to yield yellow chromophore of thionitrobenzoic acid, which was measured at 412 nm. GSH was used as a reference standard. The activity of GPx (EC no: 1.11.1.9) was expressed as nM of GSH oxidized/min/mg protein [31]. Superoxide dismutase (SOD, 1.15.1.1) activity was also estimated [32]. The inhibition of reduction of nitroblue tetrazolium to blue colored formazan in presence of phenazine metho sulphate and NADH was measured at 560nm using *n*- butanol as blank. The activity was expressed as units/mg protein. Decomposition of H_2O_2 by CAT (EC no: 1.11.1.6) was followed at 240nm [33]. One unit of CAT is defined as the amount of enzyme required to decompose 1 µM of H_2O_2/min, at 25 ° C. The activity was expressed as units/mg protein.

Histhopathology

For histological examination, the stomach tissues were excised and rinsed with ice – cold solution (0.9 % sodium chloride) to remove blood and debris of adhering tissues. The tissues were then fixed in 10% formalin for 24 h. The fixative was removed by washing through running tap water overnight, after dehydration through a graded series of alcohols; the tissues were cleaned in methyl benzoate and embedded in paraffin wax. Sections were cut into 5µM thickness and stained with hematoxylin and eosin. After dehydration and cleaning, the sections were mounted and observed under light microscope for details.

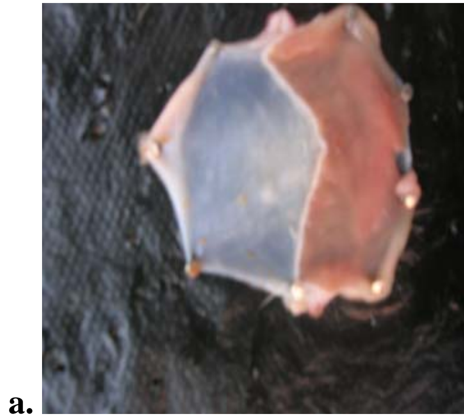
Statistical analysis

The results are expressed as mean ± S.D. for six animals in each group. The data were analyzed by using a commercially available statistic software package (SPSS for Windows V.10.5). ANOVA was performed to arrive at the level of significance of alterations.

Results & Discussion

Ulcer is a recurrent disease affecting large populations in all geographical regions. Reactive oxygen species have been implicated in the pathogenesis of a wide variety of clinical disorders associated with gastric functions. Ulcer caused by necrotizing agents due to superficial damage to mucosal cells [34] and gastric mucosal damage by NSAIDS due to decrease in PG synthesis and increases in acid secretion [35] have been reported. Ulcers due to stress are associated with physiological and psychological factors. Though the causative factors may be different, the net imbalance in offensive and defensive factors is thought to be the cause for ulcerogenesis.

The ulcerogen induced gastric damage was evidenced by marked gross mucosal lesion, including hemorrhage bands, spots and lesions. On gross examination, these bands of hemorrhage were characterized by different sizes of the glandular part of stomach. (Figure 1 a - k). Ulcerated rats pretreated with *Swertia chirayita* and ranitidine showed very mild lesions. Whereas rats that received test drug shows normal glandular part of the stomach.

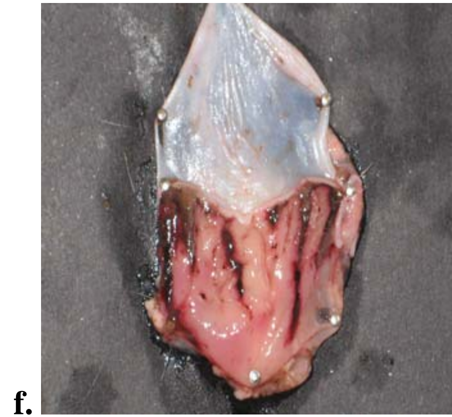
a. 

b.

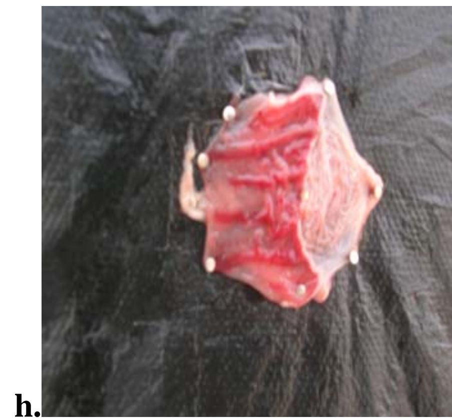
c.

d.

e.

f. 

g.

h. 

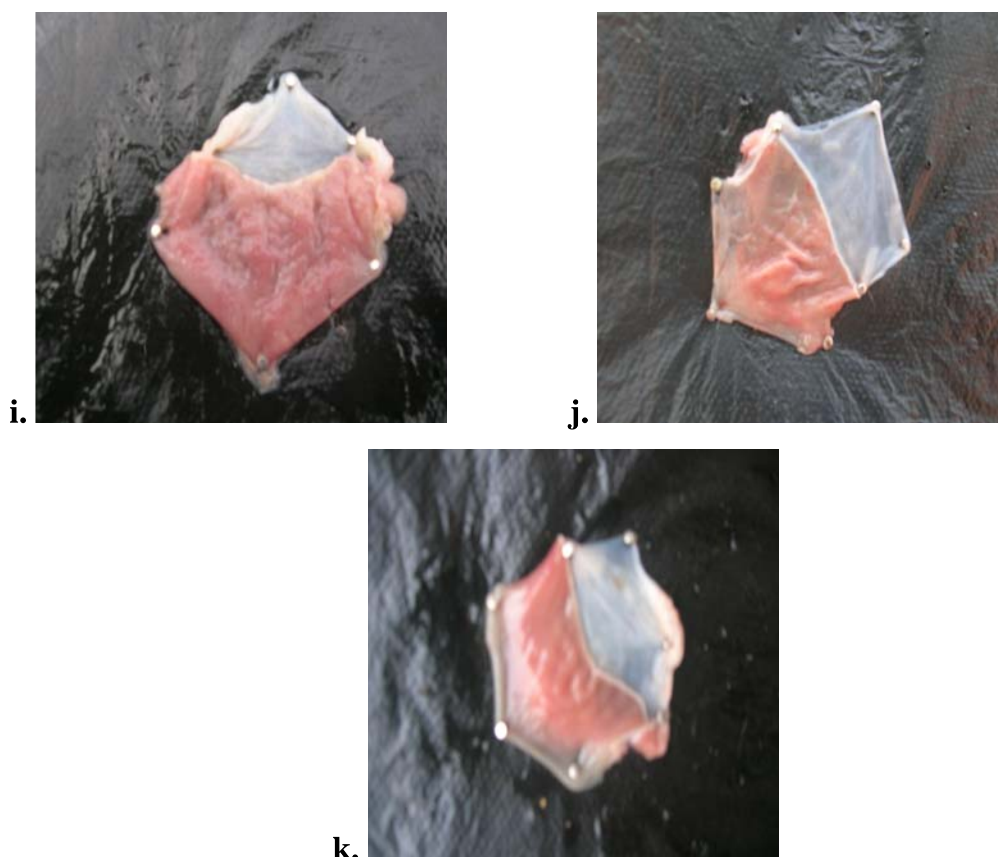


Figure no.1: Macroscopic assessment of gastric mucosal injury induced by ulcerogen and its prevention by hydro alcoholic extract of *Swertia chirayita*: **a)** Normal rat **b)** Ethanol rat (1ml/200g/ b.wt) **c)** Hydro alcoholic extract of *Swertia chirayita* (200mg/kg/b.wt)+ Ethanol **d)** Pylorus ligation **e)** Hydro alcoholic extract of *Swertia chirayita* (200mg/kg/b.wt) + Pylorus ligation **f)** Cold restraint stress **g)** Hydro alcoholic extract of *Swertia chirayita* (200mg/kg/b.wt) +Cold restraint stress **h)** Aspirin (200mg/kg/b.wt) **i)** Hydro alcoholic extract of *Swertia chirayita* (200mg/kg/b.wt) + Aspirin **j)** 200mg of hydro alcoholic extract of *Swertia chirayita* (200mg/kg/b.wt) **k)** 30mg of ranitidine + Ethanol.

The ulcer score was minimized significantly and bloody streaks, oozing of blood into the lumen of the stomach observed in ulcerogen treated animals and that were significantly minimum in *Swertia chirayita* pre treated rats. 100mg of hydroalcoholic extract of *Swertia chirayita* shows slight depletion in the hemorrhagic bands in the glandular part of the stomach when compared to that of ulcerogen induced rats. Those rats that received 200mg of drug shows no ulceration as normal mucosa with intact epithelium basal of hyperplasia of gland when compared to that of rats treated with 100mg/kg b.wt of test drug. Further, we assessed the protective effect by investigating the biochemical parameters such as alterations in the gastric mucin, GSH, nuclei acid, glycoprotein, $H^{+}K^{+}$-ATPase and myeloperoxidase activities and antioxidant enzyme levels such as CAT, SOD, GPx etc., in the ulcerated stomach as well as in the serum of

ulcerated and hydrolcoholic extract of *Swertia chirayita* treated rats. The results obtained were comparable with those of the standard drug ranitidine.

In ulcer induced rats pretreated with *Swertia chirayita*, there was a significant decrease in the volume of gastric fluid when compared to that which did not receive any drug treatment. CRS, PL and ASA models showed significant alteration with 200 mg SCHAE /kg b.wt. The pH of gastric fluid collected at the time of sacrifice showed decrease in pH and increase in titrable acidity in ulcerated rats without drug treatment. Ulcer induced rats pretreated with *Swertia chirayita* showed significant increase in pH and decrease in acidity. Pepsin, the proteolytic enzyme present in gastric lumen that digest the food protein was found to be elevated significantly in ulcer induced animals without any drug treatment in such an extent to digest the mucosal protein to cause inflammation. Pretreatment with the drug was found to reduce pepsin concentration to near normal level and the effect was comparable to that of ranitidine (Table 1).

Another important gastroprotective factor is the inhibition of acid secretion, since when levels of acid overwhelm the mucosal defense mechanism it leads to ulcer formation [36]. Acid reducing property has been reported with many other anti-ulcer drugs [37]. In our study, oral administration of hydroalcoholic extract of *Swertia chirayita* was found to reduce the acidity in the stomach.

Pepsin plays a key role in the development of reflux esophagitis by producing an early irreversible lesion, due to the damage of junctional complex that results in an increase in paracellular permeability. The irreversibility of the increase in paracellular permeability is likely to aid conversion of non-erosive to erosive damage of the epithelium by permitting luminal acid greater access to the basolateral membrane of esophageal epithelial cells, which is known to be acid permeable.

Pepsin activity is dependent on gastric acidity. At pH 6.0 or below, pepsinogen is auto catalytically converted to pepsin which is optimally active at pH 1-3.3. If pepsin were to penetrate below the surface epithelium, a possible target of proteolysis would be collagen, especially type IV, which is hydrolyzed by pepsin at the non-helical ends of the molecules releasing monomers [38]. This would disrupt mucosa, since cross-linking of collagen by carbohydrate moieties occur at the non-helical spacing [39]. Once the surface epithelium is partly disrupted, the process would allow further access of acidified proteases. Another possible pathogenic role for pepsin via digestion of collagen might occur during the repair of chronic ulceration. Since new collagen is essential to the structural repair of the mucosa, including new blood vessels the high susceptibility of both collagen III and IV to peptic digestion [38, 39] might delay ulcer healing. When pepsin is rendered inactive, the mechanism that promotes healing might involve removal of proteolysis rather than pH perse. Therefore, greater suppression is necessary to ensure healing of gastric ulcer. The pepsin activity is significantly reduced in hydroalcoholic extract of *Swertia chirayita* pretreated ulcer induced rats. The reduced inflammation and ulcer score observed in the gastric mucosa of *Swertia chirayita* treated rats might be due to its suppressive action on pepsin activity.

Table no. 1 Effect of SCHAE on volume, pH, titrable acidity and pepsin concentration of gastric juice from experimental animals

Rats pretreated with SCHAE/Ranitidine for 30 days (dose in mg/kg b.wt)	Volume (ml/120g)	pH	Titrable acidity mEq/l/120 g	Pepsin concentration (µmol of tyrosine liberated/ml)
Control (I)	3.42 ± 0.37	3.19 ± 0.39	95.50 ± 9.8	420.0 ± 45.78
EtOH (II a)	4.95 ± 0.54*	1.38 ± 0.17*	143.25 ± 17.19*	675.9 ± 74.34*
SCHAE (100) + EtOH (III a)	4.30 ± 0.49*	1.65 ± 0.19*	167.27 ± 16.03***	550.1 ± 56.71***
SCHAE (200) + EtOH (IV a)	3.13 ± 0.33**	2.91 ± 0.32*	99.67 ± 10.86*	443.67 ± 47.02*
Ranitidine (30) + EtOH (VII a)	3.09 ± 0.30*	2.89 ± 0.30*	98.32 ± 10.73*	430.0 ± 45.58*
CRS (II b)	4.72 ± 0.48***	1.27 ± 0.16*	120.01 ± 13.21***	629.11 ± 58.20*
SCHAE (100) + CRS (III b)	4.22 ± 0.44**	1.99 ± 0.34*	140.71 ± 14.18***	520.62 ± 53.73***
SCHAE (200) + CRS (IV b)	3.17 ± 0.32**	2.96 ± 0.33*	100.53 ± 10.95**	432.13 ± 49.03*
Ranitidine (30) + CRS (VII b)	3.11 ± 0.31**	2.88 ± 0.30*	90.40 ± 9.4***	428.27 ± 45.17*
PL (II c)	4. 44± 0.40***	1.21 ± 0.15*	130.11 ± 13.71*	670.99 ± 68.51*
SCHAE (100) + PL (III c)	3.95 ± 0.39**	1.50 ± 0.33**	157.03 ± 15.73**	538.37 ± 48.22***
SCHAE (200)+ PL (IV c)	3.15 ± 0.35**	2.92 ± 0.31*	101.72 ± 13.61***	429.15 ± 43.77*
Ranitidine (30) + PL (VII c)	3.13 ± 0.33**	2.86 ± 0.29*	100.62 ± 10.98***	427.01 ± 43.12*
ASA (II c)	4.63± 0.42***	1.15 ± 0.13*	124.12 ± 13.52***	600.01 ± 62.0*
SCHAE (100) + ASA (III c)	4.07 ± 0.40**	1.83 ± 0.33*	143.16 ± 14.97***	536.31 ± 54.07***
SCHAE (200)+ ASA (IV c)	3.18 ± 0.32***	2.94 ± 0.33*	100.01 ± 10.92**	441.16 ± 45.43*
Ranitidine (30) + ASA (VII c)	3.17 ± 0.32**	2.91 ± 0.31*	87.91 ± 9.0***	439.23 ± 45.1*
SCHAE (200) (V)	3.47 ± 0.42NS	3.21 ± 0.44NS	91.67 ± 9.2NS	449.63 ± 46.31NS
Ranitidine (30) (VI)	3.85 ± 0.49NS	3.27 ± 0.48NS	96.42 ± 10.60NS	454.2 ± 49.96NS

Groups are compared as: Control vs ulcer (EtOH, CRS, PL and ASA), SCHAE (100/200 mg/kg b.wt) and ranitidine treatment (with EtOH, CRS, PL and ASA – induced ulcer) vs respective ulcer, control vs ranitidine & SCHAE (200mg/kg b.wt.) alone. Values are expressed as mean ± S.D. for six animals in each group. *$p < 0.001$, **$p < 0.01$, ***$p < 0.05$ and NS - non significant

Table no. 2 Effect of SCHAE on Myeloperoxidase and H^+K^+-ATPase activities in gastric mucosa

Rats pretreated with SCHAE/Ranitidine for 30 days (dose in mg/kg b.wt)	**Myeloperoxidase (U/g tissue)**	**H^+K^+ATPase (nMol of phosphorus liberated/min/mg protein)**
Control (I)	3.18 ± 0.33	1.85 ± 0.20
EtOH (II a)	11.8 ± 1.40*	2.77 ± 0.29*
SCHAE (100)+EtOH (III a)	3.42 ± 0.35*	1.93 ± 0.20*
SCHAE (200)+EtOH (IV a)	3.39 ± 0.35*	1.89 ± 0.19*
Ranitidine (30) + EtOH (VII a)	3.37 ± 0.35*	1.86 ± 0.21*
CRS (II b)	14.5 ± 1.49*	2.61 ± 0.27*
SCHAE (100)+ CRS (III b)	3.41 ± 0.38*	1.82 ± 0.22*
SCHAE (200)+ CRS (IV b)	3.38 ± 0.35*	1.79 ± 0.19*
Ranitidine (30) + CRS (VII b)	3.36 ± 0.36*	1.73 ± 0.19*
PL (II c)	9.61 ± 0.98*	2.27 ± 0.24*
SCHAE (100)+ PL (III c)	3.39 ± 0.36*	1.72 ± 0.19*
SCHAE (200)+ PL (IV c)	3.37 ± 0.37*	1.69 ± 0.19*
Ranitidine (30) + PL (VII c)	3.29 ± 0.37*	1.58 ± 0.18*
ASA (II c)	14.12 ± 1.65*	2.52 ± 0.26*
SCHAE (100)+ ASA (III c)	3.41 ± 0.37*	1.88 ± 0.21*
SCHAE (200)+ ASA (IV c)	3.37 ± 0.37*	1.86 ± 0.20*
Ranitidine (30) + ASA (VII c)	3.31 ± 0.38*	1.79 ± 0.19*
SCHAE (200) (V)	3.02 ± 0.36^{NS}	1.87 ± 0.22^{NS}
Ranitidine (30) (VI)	3.22 ± 0.33^{NS}	1.92 ± 0.23^{NS}

Groups are compared as: Control vs ulcer (EtOH, CRS, PL and ASA), SCHAE (100/200 mg/kg b.wt) and ranitidine treatment (with EtOH, CRS, PL and ASA – induced ulcer) vs respective ulcer, control vs ranitidine & SCHAE (200mg/kg b.wt.) alone. Values are expressed as mean ± S.D. for six animals in each group. *$p < 0.001$ and NS - non significant

The activities of myeloperoxidase and H^+K^+ATPase in gastric mucosa of control and experimental rats are shown in (Table 2). In ulcerogen treated rats without drug treatment, the activity of MPO was increased significantly when compared to control rats, whereas in *Swertia chirayita* + ulcerogen received rats the activity was significantly decreased. Rats administered with SCHAE alone recorded no significant alteration in the MPO activity when compared to that of control rats. The activity of H^+K^+ATPase in ulcerated rats was found to be elevated and it was significantly decreased in *Swertia chirayita* treated rats ($P<0.01$).

The proton pump is the main component responsible for the acidic environment of the stomach, and drug that inhibits this enzyme represent the principal pharmacological treatment for peptic ulcer. The hydroalcoholic extract of *Swertia chirayita* was found to inhibit the acid secreting enzyme H^+K^+-ATPase. The H^+K^+-ATPase is selectively inhibited by acid blockers and so used to treat gastric ulcers [40]. Activation of cAMP pathway stimulates the H^+K^+-ATPase on parietal cells, a high capacity proton pump, with its insertion into the apical membrane leading to the formation of secretory canaliculi [41]. In recent years, the drugs that reduce the acid secretion and H^+K^+-ATPase activity are the preferred therapeutic choice due to their clinical efficacy [41].

We have previously reported that *Swertia chirayita* inhibited the activity of gastric mucosal H^+K^+ATPase, the proton pump *in vitro*, similar to omeprazole [42]. Considerable attention has been focused on the plants which contain natural antioxidant components because of their modulatory role on physiological function and biotransformation action involved in the detoxification process.

MPO activity is closely associated with neutrophil dependent inflammatory response in experimental ulcer. MPO and other tissue damaging substance are released from the cells to gastric lumen during ulcerogenesis. MPO mediates LPO and increased MPO activity has been reported in diclofenac sodium induced ulcer [43]. In all the ulcer models investigated the MPO activity was found to be increased concomitantly with the gastric lesion. Pretreatment with hydroalcoholic extract of *Swertia chirayita* reduced the alteration in enzyme activity suggesting the effect of hydroalcoholic extract of *Swertia chirayita* in preventing a neutrophil associated infiltration and inflammatory response induced by ulcerogens.

Gastric adherent mucin content of ulcerogen received rats was significantly reduced when compared to that of control rats, but shown an increase in *Swertia chirayita* treated groups when compared to rats that did not receive the drug treatment. No significant alteration in mucin content was observed in *Swertia chirayita* and ranitidine treated control rats. In ulcerogen treated rats, a significant decrease in the level of nucleic acids (DNA and RNA) in gastric mucosa was observed when compared to control animals. Ulcerogen treated rats caused a significant increase in DNA ($p<0.001$) and RNA ($p<0.01$) levels when compared to ulcerogen treated rats (Table 3).

Table no: 3 Effect of SCHAE on Mucin content, levels of DNA and RNA in gastric mucosa

Rats pretreated with SCHAE/Ranitidine for 30 days (dose in mg/kg b.wt)	Mucin content (µg Alcian blue /g of glandular tissue)	DNA (mg/g/tissue)	RNA (mg/g/tissue)
Control (I)	482.12 ± 53.03	2.62 ± 0.31	1.55 ± 0.17
EtOH (II a)	245.00 ± 26.95*	1.80 ± 0.20*	1.17 ± 0.12**
SCHAE (100)+EtOH (III a)	298.5 ± 29.37**	2.07 ± 0.27***	1.29 ± 0.14***
SCHAE (200)+EtOH (IV a)	515.11 ± 59.75*	2.44 ± 0.31*	1.44 ± 0.17**
Ranitidine (30) + EtOH (VII a)	521.30 ± 60.47*	2.47 ± 0.34*	1.46 ± 0.22**
CRS (II b)	260.12 ± 28.61*	1.62 ± 0.17*	1.11 ± 0.12**
SCHAE (100)+ CRS (III b)	303.15 ± 30.63***	1.99 ± 0.21**	1.26 ± 0.13***
SCHAE (200)+ CRS (IV b)	490.31 ± 55.54*	2.39 ± 0.27*	1.45 ± 0.18**
Ranitidine (30) + CRS (VII b)	494.00 ± 57.79*	2.42 ± 0.29*	1.47 ± 0.23*
PL (II c)	254.32 ± 27.97*	1.79 ± 0.19*	1.13 ± 0.14**
SCHAE (100)+ PL (III c)	293.76 ± 29.61***	2.03 ± 0.20***	1.27 ± 0.13***
SCHAE (200)+ PL (IV c)	482.29 ± 48.96*	2.42 ± 0.29*	1.43 ± 0.16**
Ranitidine (30) + PL (VII c)	476.31 ± 47.92*	2.45 ± 0.30*	1.46 ± 0.22**
ASA (II c)	258.11 ± 27.99*	1.53 ± 0.16*	1.09 ± 0.11**
SCHAE (100)+ ASA (III c)	289.62 ± 28.63***	1.82 ± 0.19***	1.23 ± 0.12***
SCHAE (200)+ ASA (IV c)	487.22 ± 51.63*	2.43 ± 0.29*	1.47 ± 0.23*
Ranitidine (30) + ASA (VII c)	522.31 ± 62.67*	2.47 ± 0.31*	1.49 ± 0.24*
SCHAE (200) (V)	494.00 ± 56.90NS	2.63 ± 0.30NS	1.56 ± 0.18NS
Ranitidine (30) (VI)	490.00 ± 53.90NS	2.65 ± 0.33NS	1.57 ± 0.20NS

Groups are compared as: Control vs ulcer (EtOH, CRS, PL and ASA), SCHAE (100/200 mg/kg b.wt) and ranitidine treatment (with EtOH, CRS, PL and ASA – induced ulcer) vs respective ulcer, control vs ranitidine & SCHAE (200mg/kg b.wt.) alone. Values are expressed as mean ± S.D. for six animals in each group. *p < 0.001, **p < 0.01, ***p < 0.05 and NS - non significant

The strengthening of the mucin barrier, lead to decrease in DNA content of gastric juice, indicating a decrease in shell shedding. In the present study the DNA content in mucosa was increased in *Swertia chirayita* treated animal, which act as reliable index for cell exfoliation and represent the increase in the life span of mucosal cells. Cell shedding is an indication of integrity of the gastric mucosa. The enhanced level of cell shedding denotes the loss of integrity and decrease life span of cell, whereas decreased shedding could indicate enhanced life span of cells and promotion of defensive mechanism. Thus *Swertia chirayita* administration could have reduced cell shedding, there by maintaining the integrity of mucosa and its defensive mechanism. The increase in DNA content in gastric mucosa indicated gastric mucosal renewal.

The importance of mucus secretion as a response to gastric mucosal trauma has long being recognized. Mucosal barriers are the most significant factors for gastric protection. Mucus also protects the mucosa and sub-mucosa from inflammatory reaction and higher the mucin content lower is the free acidity 44. In the stomach, the integrity of the mucus layer is continuously being challenged by the corrosive action of acid and pepsin and often by bile salts present in the refluxed duodenal contents. Mucin content was found to be preserved significantly in rats preadministered with 200mg of test drug/ kg b.wt. Rats received 100 mg of test drug showed a less significant increase in the mucin content when compared to ulcerogen treated rats without prior drug treatment. So, *Swertia chirayita* has possible role in enhancing mucosal resistance to acid and this could have offered gastroprotection against experimental ulcer.

Table 4 illustrate the levels of total protein bound carbohydrate complexes in the gastric mucosa of control and experimental group of rats. The levels of protein, total protein bound carbohydrate - hexose, hexosamine, sialic acid, fucose, total protein bound carbohydrate and total protein bound carbohydrate/protein ratio were significantly decreased in ulcerogen treated rats when compared to control rats, whereas in drug treated rats, these levels were significantly maintained.

Glycoproteins are proteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide side-chains. The carbohydrate is attached to the protein in a cotranslational or posttranslational modification. This process is known as glycosylation. In proteins that have segments extending extracellularly are often glycosylated.

Gastric mucosal hexosamine increased significantly in the drug treated group. Hexosamine, derivatives of mucoproteins are found in the joints and cartilages. Due to their physico chemical properties they are chief viscosity raising agents. *Swertia chirayita* exhibited ulcer healing activity by increasing hexosamine and carbohydrate/protein ratio and adherent mucus content in ulcerogen treated rats.

Table no: 4 Level of hexose, hexosamine, sialic acid, fucose, protein, total protein bound carbohydrate (TPBC) and total protein bound carbohydrate/protein ratio in the gastric mucosa of control and experimental group of rats

Rats pretreated with SCHAE/Ranitidine for 30 days (dose in mg/kg b.wt)	Hexose (mg/g tissue)	Hexosamine (mg/g tissue)	Sialic Acid (mg/g tissue)	Fucose (mg/g tissue)	Protein (mg/g tissue)	TPBC (mg/g tissue)	TPBC:P
Control (I)	14.62 ± 1.72	8.72 ± 1.13	1.81 ± 0.22	3.29 ± 0.39	19.62 ± 2.42	28.63 ± 3.42	1.46 ± 0.17
EtOH (II a)	8.32 ± 0.87*	4.05 ± 0.48*	0.59 ± 0.10*	2.09 ± 0.22*	14.00 ± 1.71**	15.00 ± 1.55*	1.11 ± 0.12*
SCHAE (100) + EtOH (III a)	10.21± 1.15**	5.87 ± 0.68*	0.73 ± 0.08**	2.47 ± 0.27***	16.03 ± 1.69***	17.17 ± 1.99***	1.29 ± 0.14***
SCHAE (200) + EtOH (IV a)	13.42 ± 1.38*	8.20 ± 0.84*	1.68 ± 0.17*	3.12 ± 0.32*	18.50 ± 1.91*	25.86 ± 2.66*	1.42 ± 0.15**
Ranitidine (30) + EtOH (VII a)	13.51 ± 1.42*	8.92 ± 0.94*	1.74 ± 0.18*	3.21 ± 0.34*	18.75 ± 1.97*	25.89 ± 2.72*	1.49 ± 0.16*
CRS (II b)	8.29 ± 0.85*	4.01 ± 0.41*	0.53 ± 0.05*	2.11 ± 0.22*	13.92 ± 1.43**	14.91 ± 1.53*	1.01 ± 0.10*
SCHAE (100) + CRS (III b)	10.13 ± 1.20**	5.73 ± 0.62*	0.69 ± 0.07*	2.57 ± 0.30**	15.97 ± 1.91***	17.03 ± 1.87***	1.17 ± 0.12***
SCHAE (200) + CRS (IV b)	13.46 ± 1.41*	8.18 ± 0.85*	1.72 ± 0.18*	3.15 ± 0.33*	18.77 ± 1.95*	25.72 ± 2.67*	1.30 ± 0.14*
Ranitidine (30) + CRS (VII b)	13.51 ± 1.45*	8.91 ± 0.95*	1.77 ± 0.19*	3.18 ± 0.34*	18.82 ± 2.01*	25.77 ± 2.76*	1.33 ± 0.14*
PL (II c)	8.37 ± 0.85*	4.04 ± 0.41*	0.56 ± 0.06*	2.07 ± 0.21*	13.84 ± 1.41**	14.86 ± 1.52*	1.04 ± 0.11**
SCHAE (100) + PL (III c)	10.72 ± 1.26**	5.98 ± 0.71*	0.71 ± 0.07**	2.43 ± 0.26***	15.73 ± 1.88***	17.01 ± 1.73***	1.19 ± 0.13***
SCHAE (200)+ PL (IV c)	13.49 ± 1.48*	8.17 ± 0.91*	1.67 ± 0.18*	3.11 ± 0.34*	18.72 ± 2.06*	25.79 ± 2.84*	1.34 ± 0.15**
Ranitidine (30) + PL (VII c)	13.54 ± 1.52*	8.87 ± 0.99*	1.72 ± 0.19*	3.14 ± 0.35*	18.84 ± 2.11*	25.83 ± 2.89*	1.36 ± 0.15*
ASA (II c)	10.97 ± 1.32**	3.99 ± 0.47*	0.6 ± 0.07*	2.00 ± 0.23*	13.77 ± 1.61**	14.77 ± 1.73*	1.07 ± 0.13**
SCHAE (100) + ASA (III c)	13.27 ± 1.43*	4.92 ± 0.54**	0.79 ± 0.09*	2.41 ± 0.25**	15.62 ± 1.81***	16.93 ± 1.71***	1.21 ± 0.14***
SCHAE (200)+ ASA (IV c)	13.52 ± 1.51*	8.16 ± 0.91*	1.73 ± 0.19*	3.16 ± 0.35*	18.79 ± 2.09*	25.82 ± 2.87*	1.37 ± 0.15*
Ranitidine (30) + ASA (VII c)	13.56 ± 1.55*	8.89 ± 1.02*	1.79 ± 0.20*	3.19 ± 0.36*	18.85 ± 2.15*	25.87 ± 2.95*	1.38 ± 0.16*
SCHAE (200) (V)	14.64 ± 1.74^{NS}	8.92 ± 1.06^{NS}	1.84 ± 0.22^{NS}	3.32 ± 0.41^{NS}	19.54 ± 2.33^{NS}	28.52 ± 3.39^{NS}	1.41± 0.17^{NS}
Ranitidine (30) (VI)	14.67 ± 1.53^{NS}	9.01 ± 0.94^{NS}	1.87 ± 0.19^{NS}	3.35 ± 0.35^{NS}	19.79 ± 2.06^{NS}	28.57 ± 2.97^{NS}	1.44 ± 0.15^{NS}

Groups are compared as: Control vs ulcer (EtOH, CRS, PL and ASA), SCHAE (100/200 mg/kg b.wt) and ranitidine treatment (with EtOH, CRS, PL and ASA – induced ulcer) vs respective ulcer, control vs ranitidine & SCHAE (200mg/kg b.wt.) alone. Values are expressed as mean ± S.D. for six animals in each group. $^{*}p < 0.001$, $^{**}p < 0.01$ and NS - non significant

The increase in carbohydrate protein ratio indicates that there was an increase in glycoprotein content of the gastric mucosa in *Swertia chirayita* treated rats. The mechanism of action of *Swertia chirayita* on gastric mucosa may be due to its mucin preserving effect. Repair of gastric mucosa is both by restitution and cell proliferation. Recently, some polyphenolics have been found to have a preventive action on gastric injury in rats. Some research has focused on the antiulcer activity of polyphenols [45]. This activity was mainly explained by strong antioxidant power and/or by some other factors, such as strong protein-binding capacity, modulation of leukocyte function [46], mucus production and restoration etc., [45] The strong protein-binding capacity has been reported to be characteristic of highly polymerized procyanidins [47]. It is believed that the antioxidant activity of polyphenols is an important factor because reactive oxygen and/or free radicals are related to the occurrence of ulcers. Some saponins have been found to promote ulcer healing by forming protective mucus barrier on the gastric mucosa [48].

Table 5 present the concentrations of lipid peroxides and the compromised levels of antioxidants such as GSH and GPx in the serum and gastric mucosa of experimental animals. In all the ulcer induced animals without any drug treatment, the malondialdehyde concentration was found to be elevated significantly ($P<0.001$). A significant alteration in the levels of above mentioned parameters were observed in ulcer induced rats pretreated with *Swertia chirayita*. A significant restoration in the levels of antioxidant enzymes were observed with 200 mg of SCHAE /kg b.wt in all the ulcer models.

Antioxidant offer protection from cytotoxic and genotoxic action against environmental toxins. The study reported by Prosenjit et al [49]. Revealed that amarogentin present in *Swertia chirayita* activate antioxidant enzymes such as GPx, SOD and CAT and reduce the formation of lipid peroxide during dimethylbenz(a)anthracene (DMBA) induced papillomas. This report is parallel to our results which state that the anti-ulcer property of *Swertia chirayita* is attributed to its antioxidant components which scavenge the free radicals during chemical induced ulcerogenesis. The high scavenging ability of the extracts may be due to high phenolic contents, flavonoid constituents and considerable amount of reducing equivalents as we reported earlier [42].

Gastric mucosa and serum levels of enzymatic antioxidants such as SOD and CAT are shown in (Table 6). We have observed a significant ($P<0.001$) decrease in serum and gastric mucosa of SOD and CAT in ulcer induced rats that did not receive the drug treatment. *Swertia chirayita* pretreatment was found to restore the activities of SOD and CAT in CRS, ASA and PL induced ulcer models. The effects were comparable to those rats received the standard drug ranitidine.

Table no: 5 Effect of SCHAE on TBARS, GSH and GPx in the gastric mucosa and serum

Rats pretreated with SCHAE/Ranitidine for 30 days (dose in mg/kg b.wt)	TBARS (nmol/mg protein)		GSH		GPx (nmol of GSH oxidized/min/mg protein)	
	Gastric mucosa	Serum	Gastric mucosa (nmol/g tissue)	Serum (μmol/dl)	Gastric mucosa	Serum
Control (I)	0.91 ± 0.10	0.42 ± 0.04	7.21 ± 0.70	7.19 ± 0.92	270.12 ± 28.09	320.11 ± 38.39
EtOH (II a)	2.41 ± 0.28**	2.30 ± 0.27*	3.71 ± 0.44	3.92 ± 0.47	112.71 ± 13.52	180.56 ± 18.01
SCHAE (100) + EtOH (III a)	0.98 ± 0.13**	0.46 ± 0.05*	6.53 ± 0.67*	6.46 ± 0.66*	247.90 ± 25.53*	288.46 ± 29.42*
SCHAE (200) + EtOH (IV a)	0.89 ± 0.10**	0.43 ± 0.04*	6.65 ± 0.68*	6.49 ± 0.67*	253.90 ± 25.90*	291.87 ± 30.06*
Ranitidine (30) + EtOH (VII a)	0.83 ± 0.08**	0.39 ± 0.03*	6.72 ± 0.71*	6.62 ± 0.71*	256.90 ± 26.97*	300.08 ± 31.51*
CRS (II b)	1.61 ± 0.17**	1.41 ± 0.15*	3.50 ± 0.36*	3.87 ± 0.39*	115.30 ± 11.76*	167.00 ± 17.03*
SCHAE (100) + CRS (III b)	0.96 ± 0.12**	0.44 ± 0.04*	6.45 ± 0.66*	6.45 ± 0.66*	244.10 ± 25.39*	286.01 ± 29.46*
SCHAE (200) + CRS (IV b)	0.89 ± 0.09**	0.41 ± 0.04*	6.60 ± 0.69*	6.55 ± 0.68*	250.00 ± 25.75*	297.02 ± 30.89*
Ranitidine (30) + CRS (VII b)	0.87 ± 0.09**	0.38 ± 0.03*	6.66 ± 0.71*	6.57 ± 0.73*	253.90 ± 27.17*	309.12 ± 33.08*
PL (II c)	1.81 ± 0.19**	1.90 ± 0.22*	3.54 ± 0.36*	3.89 ± 0.42*	112.01 ± 11.43*	143.11 ± 14.65*
SCHAE (100) + PL (III c)	0.94 ± 0.11**	0.41 ± 0.04*	6.50 ± 0.68*	6.44 ± 0.68*	245.11 ± 25.74*	286.99 ± 30.13*
SCHAE (200)+ PL (IV c)	0.84 ± 0.08**	0.40 ± 0.04*	6.67 ± 0.73*	6.47 ± 0.71*	252.01 ± 28.23*	295.06 ± 32.46*
Ranitidine (30) + PL (VII c)	0.82 ± 0.08**	0.39 ± 0.13*	6.70 ± 0.75*	6.52 ± 0.73*	258.21 ± 28.4*	300.03 ± 33.62*
ASA (II c)	1.49 ± 0.15**	1.32 ± 0.04*	3.52 ± 0.41*	3.84 ± 0.45*	114.00 ± 13.34*	158.22 ± 18.51*
SCHAE (100) + ASA (III c)	0.97 ± 0.13**	0.43 ± 0.04*	6.48 ± 0.67*	6.49 ± 0.67*	249.00 ± 25.65*	289.22 ± 29.79*
SCHAE (200)+ ASA (IV c)	0.91 ± 0.10**	0.41 ± 0.04*	6.62 ± 0.73*	6.58 ± 0.72*	250.00 ± 29.58*	293.01 ± 32.23*
Ranitidine (30) + ASA (VII c)	0.89 ± 0.09**	0.40 ± 0.04*	6.71 ± 0.78*	6.65 ± 0.77*	250.06 ± 27.51*	297.10 ± 34.45*
SCHAE (200) (V)	0.99 ± 0.14NS	0.43 ± 0.04NS	7.91 ± 0.94NS	6.75 ±	267.11 ± 31.79NS	311.01 ± 37.01NS
Ranitidine (30) (VI)	0.97 ± 0.13NS	0.45 ± 0.05NS	7.99 ± 0.96NS	6.89 ±	269.11 ± 32.29NS	319.12 ± 38.29NS

Groups are compared as: Control vs ulcer (EtOH, CRS, PL and ASA), SCHAE (100/200 mg/kg b.wt) and ranitidine treatment (with EtOH, CRS, PL and ASA – induced ulcer) vs respective ulcer, control vs ranitidine & SCHAE (200mg/kg b.wt.) alone. Values are expressed as mean ± S.D. for six animals in each group. *p < 0.001, **p < 0.01 and NS - non significant

Table no: 6 Effect of SCHAE on SOD and CAT in the gastric mucosa and serum

Rats pretreated with SCHAE/Ranitidine for 30 days (dose in mg/kg b.wt)	SOD (U/mg protein)		CAT (µmol of H_2O_2 Consumed/min/mg protein)	
	Gastric mucosa	Serum	Gastric mucosa	Serum
Control (I)	62.17 ± 7.32	62.11 ± 6.92	5.41 ± 0.64	5.03 ± 0.79
EtOH (II a)	42.24 ± 4.22*	44.11 ± 5.21**	4.11 ± 0.49**	4.12 ± 0.49***
SCHAE (100) + EtOH (III a)	48.01 ± 4.82***	49.92 ± 5.01***	4.59 ± 0.51***	4.56 ± 0.52***
SCHAE (200) + EtOH (IV a)	57.11 ± 6.08*	57.17 ± 5.89*	5.15 ± 0.53**	4.87 ± 0.54***
Ranitidine (30) + EtOH (VII a)	57.24 ± 5.91*	58.25 ± 6.12*	5.23 ± 0.55**	4.93 ± 0.56***
CRS (II b)	41.15 ± 4.22*	41.18 ± 4.24*	3.91 ± 0.42*	4.09 ± 0.41***
SCHAE (100) + CRS (III b)	48.56 ± 4.93***	47.06 ± 4.73***	4.48 ± 0.49***	4.53 ± 0.51***
SCHAE (200)+ CRS (IV b)	57.11 ± 5.94*	57.14 ± 5.94*	5.29 ± 0.55*	4.91 ± 0.55***
Ranitidine (30) + CRS (VII b)	58.21 ± 6.23*	58.23 ± 6.23*	5.35 ± 0.57*	4.96 ± 0.58**
PL (II c)	42.26 ± 4.32*	41.11 ± 4.19*	3.51 ± 0.36*	4.01 ± 0.40**
SCHAE (100)+ PL (III c)	49.06 ± 5.39***	46.98 ± 4.67***	3.98 ± 0.43***	4.47 ± 0.48***
SCHAE (200)+ PL (IV c)	57.17 ± 6.29*	57.14 ± 6.29*	5.03 ± 0.55*	4.98± 0.60**
Ranitidine (30) + PL (VII c)	57.21 ± 6.33*	58.08 ± 6.52*	5.17 ± 0.58*	5.01 ± 0.67**
ASA (II c)	42.09 ± 4.92*	41.13 ± 4.81*	4.08 ± 0.48*	4.03 ± 0.40**
SCHAE (100)+ ASA (III c)	48.92 ± 5.17***	47.03 ± 4.71***	4.53 ± 0.50***	4.49 ± 0.49***
SCHAE (200)+ ASA (IV c)	57.09 ± 6.28*	57.11 ± 6.34*	5.18 ± 0.57*	4.93 ± 0.56***
Ranitidine (30) + ASA (VII c)	58.19 ± 6.75*	58.17 ± 6.64*	5.27 ± 0.63*	4.97 ± 0.59**
SCHAE (200) (V)	61.15 ± 7.28NS	62.22 ± 7.41NS	5.67 ± 0.67NS	4.92 ± 0.58NS
Ranitidine (30) (VI)	61.55 ± 7.39NS	62.47 ± 6.45NS	5.73 ± 0.61NS	4.95 ± 0.51NS

Groups are compared as: Control vs ulcer (EtOH, CRS, PL and ASA), SCHAE (100/200 mg/kg b.wt) and ranitidine treatment (with EtOH, CRS, PL and ASA – induced ulcer) vs respective ulcer, control vs ranitidine & SCHAE (200mg/kg b.wt.) alone. Values are expressed as mean ± S.D. for six animals in each group. *p< 0.001, **p<0.01 and NS - non significant.

Reactive oxygen species especially .OH plays a major role in oxidative damage of gastric mucosa in almost all forms of gastric ulcer [50, 51]. Normally the damage caused by superoxide anions is counteracted by dismutation with SOD [52]. SOD converts the reactive O2 to H2O2, which if not scavenged by CAT, can by itself cause lipid peroxidation by increasing the generation of hydroxyl radicals [51]. Hence decrease in CAT level could have led to increase in accumulation of these reactive products and thus, might have caused lipid peroxidation and tissue damage in ulcerogen treated rats. The results of this study showed that SCHAE treatment restore the activities of antioxidant enzymes SOD and CAT in gastric tissue. The potent free radical scavenging activity of *Swertia chirayita* reported 42 could have been accounted for ulcer preventing action.

The observations by the histological examination of stomach excised from the experimental rats were presented in (Figure 2 a-f). Gastric picture of an ethanol treated control rat shows erosion of mucosal epithelium and inflammation. In rats pretreated with hydroalcoholic extract of *Swertia chirayita* and ranitidine, gastric mucosa shows normal basal layers, lining epithelium and lamina.

Rats received SCHAE upto 1000mg/kg b.wt did not show any alteration in the liver and heart marker enzymes in serum showing the non toxic nature. Also results of our study proved that the *Swertia chirayita* possess antiulcer activity against experimentally induced various gastric ulcer models. Rats treated with 200mg/kg b.wt. of hydroalcohollic extract of *Swertia chirayita* did not show any abnormal alteration in the parameters analyzed showing the non-toxic nature of drug.

Conclusion

This study concludes that *Swertia chirayita* can be used in anti-ulcer drug formulations which act as both cytoprotective and anti-secretory agent. The antiulcer activity of *Swertia chirayita* may be attributed to its antisecretory and antioxidant activities. The ulcer preventing action of the test drug might probably by preserving the gastric mucin content. The gastro protective activity of SCHAE was comparable to that of ranitidine. Rats treated with 200mg/kg b.wt of SCHAE did not show any abnormal alteration in the parameters analyzed, showing the non-toxic nature of drug.

Acknowledgement

The author S.M.K. Narmatha Selvamathy as senior research fellowship thank Indian council of Medical Research for the financial support (file ref: D.O. No. 45/36/2007/BMS/TRM).

References

1. Goel RK, Sairam K. Antiulcer drugs from indigenous sources with emphasis on *Musa sapientum*, *Tamrabhasma*, *Asparagus racemosus* and *Zingiber officinale*. Indian J Pharmacol 2002; 34: 100-110.

2. Jain M, Devi CS. Antiulcerogenic and ulcer healing effects of *solanum nigrum* (L) on experimental ulcer models: possible mechanism for the inhibition of acid formation. J Ethanopharmacol 2006; 104: 156-163.
3. Hoogerwerf WA, Pasricha PJ. In: Hardman JG, Limbrid LE, Goodman CS, Gilman BT, (Ed.) Goodman and Gilman's: the pharmacological basis of therapeutics Agents used for the control of gastric acidity and treatment of peptic ulcers and gastroesophageal refux diseases. 10th ed. Mcgraw–hill, New york, 2001; 1005-1019.
4. Huttermann W. Short-term treatment of gastric ulcer with once daily omeprazole. Scand J Gastroenterol1986; 21:179.
5. Manomani S, Vishwanahtan VP, Subramanian S, Govindasamy S. Biochemical studies on the antiulcerogenic activity of cauvery 100, an ayurvedic formulation in experimental ulcers. Indian J Pharmacol 1995; 27: 101-105.
6. Peskar BM, Maricic N. Role of prostaglandins in gastroprotection. Dig Dis Sci 1998; 43: 23s – 29s.
7. Konturek PC, Brzowski T, Sliwowski Z, Pajido R, Stachura J, Hahn EG, *et al*. Involvement of nitric oxide and prostaglandins in gastroprotection induced by bacterial lipopolysaccharide. Scand J Gastroenterol 1998; 33: 691-700.
8. Bandhopadhyay U, Biswas K, Chatterjee R, Bandyopadhyay P, Chattopadhyay I, Ganguly CK, *et al*. Gastroprotective effect of Neem (*Azadiracta indica*) bark extract: possible involvement of H^+K^+ ATPase inhibition and scavenging of hydroxyl radical. Life Sci 2002; 71: 2845- 2865.
9. Martelli A, Mattlioli F, Mereto E, Brambilla CG, Sini D, Bergamaschi R, *et al*. Eavluation of omeprazole genotoxicity in a battery of *in vitro* and *in vivo* assays. Toxicology 1998; 30: 19-41.
10. Wolfe MM, Sachs G. Acid suppression: optimizing therapy for gastroduodenal disease and stress- related erosive syndrome. Gastroenterology. 2000; 118: s9- s31.
11. Joshi P, Dhawan V. *Swertia chirayita*- an overview. Curr Sci 2005; 89: 635-639.
12. Rafatullah S, Tari M, Mossa JS, Al-yahya MA, Al-said MS, Ageel AM. Protective effect of *Swertia chirata* against indomethacin and other ulcerogenic agent induced gastric ulcer. Drugs Exp Clin Res 1993; 19: 69-73.
13. Hollander D, Tarnawski A, Krause WJ, Gergely H. Protective effect of sucalfate against alcohol-induced gastric mucosal injury in the rat. Macroscopic, histologic, ultrastructural, and funtional time sequence analysis. Gasteroenterology 1985; 88: 366-374.
14. Senay EC, Levine RJ. Synergism between cold and restraint for rapid production of stress ulcer in rats. Proc Soc Exp Biol Med 1967; 124: 1221-1223.
15. Sanyal AK, Pandey BL, Goel RK (1971) In: Pfeiffer CJ, (Ed.). The effect of cyproheptadine on gastric activity, an experimental study. Peptic ulcer. Munksguard, Copenhagen, 1971; 312-318.
16. Goel RK, Das DG, Sanyal AK. Effect of vegetable banana powder on changes induced by ulcerogenic agents in dissolved mucosubstances in gastric juices. J Gastroenterol 1985; 4: 249 – 251.
17. Shay H, Komarov SA, Fels SS, Mreanze D, Gruenstein M, Siplet HI. A simple method for the uniform production of gastric ulceration in the rat. Gasteroenterology 1947; 5: 43-61.

18. Cheon HG, Lim H, Lee DH. Biochemical properties of a newly synthesized H^+K^+ ATPase inhibitor, 1-(2-methyl-4-methoxyphenyl)-4-[(3-hydroxypropyl)amino]-6-methyl-2,3-dihydropyrrolo[3,2-c] quinoline. Eur J Pharmacol 2001; 41: 181-186.
19. Bradford MM. A rapid and sensitive method for the quantitaitn of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-254.
20. Ricardo RC, Cristiane HB, Dagoberto S, Elizabeth EM, Frederick CK, Rosa IS, *et al*. Inhibition of gastric H^+K^+-ATPase activity by flavanoids, coumarins and xanthones from Mexican medicinal plants. J Ethanopharmacol 2006; 105: 167-172.
21. Corne SJ, Morrissey SM, Wooda RJ. Proceedings: A method for the quantitative estimation of gastric barrier mucus J Physiol 1974; 242: 116p-117p.
22. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 1982; 78: 206- 209.
23. Burton K. The conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956; 62: 315 – 323
24. Rawal VM, Patel US, Rao GN, Desai RR. Clinical and biochemical studies on cataractous human lenses III. Quantitative study of protein and RNA. Arogya J Health sci 1967; 3: 69 – 72.
25. Niebes P. Determination of enzymes and degradation products of glycosaminoglycan metabolism in healthy and various subjects. Clin chim Acta 1972; 42: 399 – 408.
26. Wagner WD. A more sensitive assay for discriminating galactosamine and glucosamine in mixtures. Anal Biochem 1979; 94: 349 – 396.
27. Warren L. Thiobarbituric acid assay of sialic acids. J Biol Chem 1959; 234: 1971 – 1975.
28. Winzler RJ. Determination of serum glycoproteins. Methods Biochem Anal 1955; 2: 279 – 311.
29. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186: 421- 431.
30. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol 1984; 105: 114 - 121.
31. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979; 582: 67-78.
32. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21: 130-132.
33. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47: 389-94.
34. Miller TA. Mechanisms of stress – related mucosal damage. Am J Med 1987; 83: 8-14.
35. Goel RK, Bhattacharya SK. Gastroduodenal mucosal defense and mucosal protective agents. Indian J Exp Biol 1991; 29: 701-704.
36. Schubert ML. Gastric secretion. Curr Opin Gastroenterol 2007; 20: 519-525.
37. Naik Y, Smitha J, Harish Nayaka MA, Lakshman Shylaja MD. Gastroprotective effect of swallow root (*Decalpepsis Hamiltonii*) extract: possible involvement of

H^+K^+ ATPase inhibition and antioxidative mechanism. J Ethanopharmacol 2007; 112: 173-179.
38. Kresina TF, Miller EJ. Isolation and characterization of basement membrane collagen from human placental tissue. Evidence for the presence of two genetically distinct collagen chains. Biochem 1979; 18: 3089-3097.
39. Dolz A, Engel J, Kubn H. Folding of collagen. Eur J Biochem 1988; 178: 357-366.
40. Nagaya H, Satoh H, Maki Y. Actions of antisecretory agents on proton transports in hog gastric microsomes. Biochem Pharmacol 1987; 36: 513-519.
41. David Perlin S. Ion pumps as targets for therapeutics intervention. Old and new paradigms. Elect J Biotechnol 1988; 1: 55-64.
42. Narmadha Selvamathy SMK, Saranya P, Annie Jeyachristy S, Geetha A. Antioxidant and H^+K^+ATPase inhibitory activity of various extracts of *Swertia chirayita*. Inter J Pharm Biol Sci 2009; 3:1-10.
43. Matsui H, Murata Y, Kobayashi F, Shiba R, Momo K, Kondo Y, Nakahara A, *et al.* Diclofenac- induced gastric mucosal fluresecence in rats. Dig Dig Sci 2001; 46: 338-344.
44. Tse SK, Chadee K. The interaction between intestinal mucus glycoproteins and enteric infections. Parasitol Today 1991; 7:163-172.
45. Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, Pergolizzi S, *et al*. Chemical characterization and biological effects of *Sicilian Opuntia ficus indica* (L.) Mill. fruit juice: antioxidant and antiulcerogenic activity. J Agric Food Chem 2003; 51: 4903–4908.
46. Osakabe N, Sanbongi C, Yamagishi M, Takizawa T, Osawa T. (1998) Effects of polyphenol substances derived from *Theobroma cacao* on gastric mucosal lesion induced by ethanol. Biosci Biotechnol Biochem 1998; 62: 1535–1538.
47. Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaji N. Antiulceractivity of grape seed extract and procyanidins. J Agri Food Chem 1998; 46: 1460–1464.
48. Trease EG, Evans WE. Saponins and cardioactive drugs, in: Textbook of Pharmacognosy, second ed., Bailiere Tindal, London, 1978; 475–506.
49. Prosenjit S, Suvra M, Ashes D, Prabhas C, Das Sukta D. Evaluation of the anticarcinogenic activity of *Swertia chirata* Buch.Ham, an Indian medicinal plant, on DMBA-induced mouse skin carcinogenesis model. Phytotherapy Research 2004; 5: 373 – 378.
50. Phull PS, Jacyna CJ, Jacyna MR. A radical view of stomach: the role of oxygen-derived free radicals in gastroduodenal disease. Am J Gastroenterol Hepatol 1995; 7: 265-274.
51. Das D, Bandyopadhyay D, Bhattacharjee M, Banerjee RK. Hydroxyl radical is the major causative factor on stress- induced gastric ulceration. Free Radic Biol Med 1997; 23: 8-18.
52. Fridovich I. Biological effects of superoxide radical. Arch Biochem Biophys 1986; 247: 941-948.

*Corresponding author present address: Dr. A. Geetha, Associate Professor, Department of Biochemistry, Bharathi Women's College, Chennai – 600 108, Tamil Nadu, India
Email address: drgeetha08@yahoo.in