Research Article

Evaluation of *Gymnospora montana* (Celastraceae) fruit extract for anti-ulcer activity in rats

Kiran B. Kotade[*1], Shivanand N. Hiremath[2]

[1]*Shri J.J.T. University, Jhunjhunu, Rajasthan, India -333001*

[2]*P.R.E.S.'s, Pravara College of D Pharmacy, Loni, Ahmednagar, Maharashtra, India-413736*

Received: 28 August 2018 Revised: 12 September 2018 Accepted: 29 September 2018

Abstract

Objective: The objective of present study is to systematically investigate and confirm the folklore use of *Gymnospora Montana* fruits in different models of ulcer. **Materials & methods:** The ethanolic extract of *Gymnospora montana* fruit were selected and equipped in doses of 100 and 500 mg/kg. The antiulcer activity of *Gymnospora montana* was evaluated by aspirin induced ulcers, pyloric ligation models and Cysteamine induced ulcers. **Results:** It was revealed that ethanolic extract had marked antiulcer activity in all these models at a dose of 500 mg/kg. In addition, it was also found to increase the mucin content of the gastric mucosa. The observed results were attributed to the rich content of flavonoids in the ethanolic extract. **Conclusion:** The present study affords a significant contribution in confirmation of the forklore effects of a traditional remedy.

Keywords: *Gymnospora montana,* Shay rats, duodenal ulcer, peptic ulcer, flavanoids

Introduction

The term peptic ulcer refers to acid peptic injury of the digestive tract, resulting in mucosal break reaching the submucosa. Peptic ulcers are usually located in the stomach or proximal duodenum, but they can also be found in the oesophagus or Meckel's diverticulum. (Del Valle, 2014). Traditionally, a hypersecretory acidic environment together with dietary factors or stress were thought to cause most peptic ulcer diseases, but the discovery of Helicobacter pylori (*H. pylori*) infection and the widespread use of non-steroidal anti-inflammatory drugs (NSAIDs) in the second half of the 20th century have changed this perception. NSAIDs including low-dose aspirin are some of the most commonly used drugs. They have good efficacy and a long history of clinical use, but can cause peptic ulcers which may have fatal complications. As widespread use of NSAIDs and aspirin is common in various contries, the associated gastrointestinal toxicities have substantial implications for the healthcare system (Drini, 2017). The occurrence of idiopathic ulcers seems to be increasing and is associated with high mortality (Huang et al., 2002; Wong et al., 2009). In addition, the modern world is encapsulated in the ever-increasing balloon of different stressers that contribute to the ever-increasing incidence of stress-ulcers. Modern lifestyle with increasing dependence on the fast-food also aggravates that problem of peptic ulcer diseases.

The global decline of peptic ulcer disease during the past century has occurred most rapidly in the past two decades (Sonneberg, 2013). This decreasing trend could be related to a cohort effect that occurred before the introduction of potent anti-secretory agents and *H. pylori* treatment. In the treatment of peptic ulcer, polypharmacy induced side effects, non-compliance, and unhealthy eating habbits seem to be the hurdles. Therefore, the use of probiotics, vaccines, and herbal medicines in combination with antibiotics or as monotherapy seems to be particularly promising. It is reported that some natural food products, like broccoli, garlic, green tea, licorice, honey, and curcumin, could be used as adjuvant therapies (Cerella et al., 2011). Now-a-days plants are being looked upon by the pharmaceutical industry as most important source for active leads.

One such promising plant can be *Gymnospora montana*, which is a traditional herbaceous plant of Celastraceous family found in different regions of India. In vernacular language it is called as Vikalo. It is a large, much branched, spinescent, woody shrub or a small tree. It is a shrub found in

Address for Corresponding Author:
Kiran B. Kotade
Shri J.J.T. University, Jhunjhunu, Rajasthan, India -333001
Email: kirankotade@gmail.com

DOI: https://doi.org/10.31024/ajpp.2019.5.1.11

Southeast Asia. The stems when young are prickly. Leaves: drying yellowish, thick, and 3 cm–5 cm × 2 cm–3.8 cm. The fruits are 5mm long globose capsules ripening into purple. The seeds are glabrous, chestnut brown, rugose and embedded in an aril.

In Indian forklore it is used to purify the blood; cure peptic ulcer and haemorrhoids. It is reported to relieve kapha, inflammation, burning sensation, thirst and corneal opacity. It is also used in treatment of snake bite and pediculosis. The pulverized leaves when given in milk to children act as vermifuge and decoction of the leafy twig is used in mouth wash to relieve toothache. In certain parts of Saurashtra (Gujarat, India) its leaves are extensively used as a folklore cure for jaundice. In the Philippines, a decoction of the leaves of *Gymnosporia spinosa* (Blco.) Merr. & Rolfe is drunk to assuage headache. Methanolic extract of leaves of *Gymnosporia montana* is reported to lower the enzymatic activity of transaminases, the level of lipid constituents and the level of orosumucoid in the serum, and the level of glycogen in the liver of animals poisoned with carbon tetrachloride (De et al., 1994).

In view of this it was thought worthy to systematically investigate the antiulcer potential of ethanolic extract of the fruits of *Gymnospora montana*. In the present investigation, ethanolic extract was used as our preliminary investigations revealed that the ethanolic extract contained rich amount of flavonoids, which are usually reported to have antiulcer activity.

Material and methods

Collection and authentication of plant material

The fruits of *Gymnosporia montana* (Roth) Bemth were collected from forest of District Indore, Madhya Pradesh and authenticated by Department of Botany, PVP College, Pravaranagar. The fruits were plucked, air-dried in shade, powdered and stored in air-tight containers.

Preparation of plant extract

About 1 kilogram of fruit was thoroughly washed under running water to remove adherent soil and impurities. The cut chips were shade dried by making into chips and finally powdered to mesh 60#. The air dried powder was exhaustively extracted by hot percolation method (soxhalation) with different solvents of increasing order of polarity, starting with a highly nonpolar solvents viz., Petroleum ether followed by Chloroform, and Ethanol (95%). Initially about 200 g of powder was extracted with 600 ml of Pet. Ether. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and the solvent was distilled off using rotary vacuum flash evaporator. The obtained residue was dried in a desiccator over anhydrous sodium sulphate. The average yield, colour, odour and constituency were recorded. The left over mark was air dried at room temperature and was similarly extracted with chloroform and Ethanol respectively.

Quantitative test for flavonoids

The total flavonoid content was determined by aluminum chloride colorimetric method (Chang et al., 2002; Pal et al., 2009). In brief, 0.5 ml of ethanolic extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UV spectrophotometer (Shimadzu UV-1601, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Total flavonoid contents were calculated as quercetin equivalent from calibration curve prepared using quercetin. The results reveal that total flavonoid content in the ethanolic extract was found to be 12.15 mg quercetin equivalents/g respectively.

Animals

Adult Sprague-Dawley rats of either sex born and reared in Animal House of P.R.E.S's. College of Pharmacy (For Women), Nashik MS were employed for the present studies. These were group housed (three per cage; all males/all females to avoid breeding) in opaque (white) polypropylene cages. The animal house temperature was maintained at 25 ± 2 ◦C under 12:12 h light (0700-1900 h): dark cycle. All animals had free access to rodent chow (Amrut Feeds, Maharashtra, India) and water. All studies were approved by Institutional Animal Ethics Committee of the Department, and the guidelines given by CPCSEA, New Delhi were strictly followed.

Study design

Rats were group housed (5-6 per cage) and divided into groups; Control, Ulcer control, Ranitidine (50 mg/kg) orally, ethanolic extract treated (E100 and E 500 mg/kg), for the paradigm of aspirin induced ulcers. All solutions were prepared fresh and administered in a dose volume of 10 ml/kg.

Aspirin-induced ulcer

All animals were fasted for at least 15 hr. prior to administration of the aspirin or dose medium. During the fast, they were allowed water ad libitum. Water was withdrawn at the time of administration of the drug or dose medium. Eighty-five milligrams of pure aspirin was dissolved in 5.95 ml. of 0.15 M citrate buffer, pH 5.60. The pH of this mixture after solution of the aspirin was 4.60. A similar buffer solution for control animals was adjusted to

pH 4.60 with the aid of the glass electrode and careful addition of 1N HC1. Enough aspirin solution was administered to each experimental animal to give a dose of 150 mg/kg body weight. Corresponding volumes of buffer solution were administered to control animals. The positive control group received ranitidine (50 mg/kg) in 1% CMC. These solutions were administered orally to the animals by means of a No. 16 curved steel oral catheter which was dipped in mineral oil for lubrication.

Exactly 24 hr. after administration of the dose solution, each animal was killed by etherization. The stomach was removed, opened along the line of lesser curvature, stretched, and pinned on a large rubber stopper. The mucosal surface was washed with 0.9% saline to remove any mucous, ingested feces, or other debris. Each stomach was then examined grossly and graded as follows: Ulcer index (UI) was calculated as a product of the ulcer numbers and ulcer severity score. The number of ulcers was noted and the severity recorded with the following scores: 0 = no ulcer; 1 = superficial ulcers (< 3 mm diameter); 2 = deep ulcers (> 10 ulcers with 3 mm diameter); 3 = 1 or more ulcers (4–6 mm diameter); 4 = 1 or more ulcers (≥ 7 mm diameter); 5 = Perforation.

The following parameters were recorded and calculated: Gastric juice volume (GJV) in mL; Free acidity (FA) at pH 3.8 and total acidity (TA) at pH 8.3 in mEq/L were measured by titrating against 0.5 N NaOH, with Toepfer's reagent and phenolphthalein, respectively, as indicators . Gastric wall mucus content was determined by the method described by Corne et al. (1974) with slight modifications. The stomach was opened along the greater curvature, weighed, and immersed in 10 ml of 0.1% Alcian Blue in 0.16 M sucrose/0.05 M sodium acetate, pH 5.8 for 2 h. The excessive dye was then removed by two successive rinses in 0.25 M sucrose solution (15 min each). The remaining dye complexes with the gastric mucus were extracted with 0.5 M $MgCl_2$ for 2 h and shaken intermittently for 1 min in every 30 min interval. The blue extract was then shaken vigorously with an equal volume of diethyl ether and the emulsion was centrifuged at 3600 rpm for 10 min. The OD of Alcian Blue in the aqueous layer was read at 580 nm using a spectrophotometer. The quantity of Alcian Blue extract per gram wet stomach was then determined from a standard curve. The stomach tissues biopsies of the rats were fixed in 10% formalin, dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. The tissues were then cut into 2–3 µm thick sections by a microtome, fixed on the slides and stained with hematoxylin-eosin (H&E). The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken with a Leica DM 750 camera at x 100 magnification (Angelo et al., 2010; Hemmati et al., 1973; Marya, 2002; Malagelada 2007).

Pylorus ligation induced ulcer

Rats weighing 150–170 g are starved for 48 h having access to drinking water ad libitum. During this time, they are housed single in cages with raised bottoms of wide wire mesh in order to avoid cannibalism and coprophagy. Seven animals are used per dose and as controls. Under anesthesia, a midline abdominal incision is made. The pylorus was ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. The abdominal wall was closed by sutures. The test compounds are given either orally by gavage. After 19h the animals were sacrificed. The abdomen is opened, and a ligature was placed around the esophagus close to the diaphragm. The stomach was removed, and the contents are drained in a centrifuge tube. Along the greater curvature, the stomach was opened and pinned on a cork plate. The mucosa was examined with a microscope. The parameters analysed as index of ulcer are same as above (Shay et al., 1945; Kulkarni, 1999).

Cysteamine induced ulcers

Rats weighing 150–170 g are starved for 48 h having access to drinking water ad libitum. During this time, they are housed single in cages with raised bottoms of wide wire mesh in order to avoid cannibalism and coprophagy. Five-Seven animals are used per dose and as controls. Cysteamine HCL was dissolved in distilled water to get a concentration of 10 % w/v. Several dosage regimens and routes are employed by various researchers. Our aim was to study the effect of therapeutic agents on ulcer healing hence we administered cysteamine HCL at a dose of 28 mg/100g p.o. 3 times daily (4-5 h gap) in fed rats as it is reported to reduce the mortality (20 %) and increase the incidence of ulcers. After 24 h of the last dose of cysteamine the rats were euthanized, the isolated duodenum was gently opened and rinsed with ice-cold saline. Photographs of specific areas of damage under a dissection microscope with magnification were taken. To investigate the degree of gross mucosal damage, the mucosal sides of the duodenums were photographed using a digital camera. The area of damage was then fixed in 10% formalin for histologic evaluation. In this paradigm, only ulcer index was calculated as mentioned above (Desai et al., 1999)

Statistical analysis

All the means are presented with their standard error of mean (mean ± S.E.M.). Wherever necessary the data were analyzed by one-way ANOVA followed by Tukey-Kramer post hoc test. $P < 0.05$ was considered statistically significant.

Results

Aspirin induced ulcers

One way ANOVA revealed a significant influence of all treatments on the parameters investigated viz., gastric volume, free acidity, total acidity, total acid output, mucin content and the ulcer index ($P < 0.0001$). Aspirin administration at a dose of 150 mg/kg had produced significant increase ($P<0.001$) in the gastric volume, free acidity, total acidity, total acid output whereas the mucin content was significantly reduced. Overall the ulcer index was significantly higher than that of control. All these changes observed in the ulcer control group were significantly decreased in *G. montana* group that had received the dose of 500 mg/kg, whereas the groups receiving 100 mg/kg did not affect these parameters. All the results are depicted in figure 1.

Pylorus ligation induced ulcers

The mean ulcer index was found to be 17.7 ± 2.0 mL in pylorus-ligated or SHAY rats. The total acidity in sham operated rats was found to be 10.0 ± 0.8 mmol. The free acidity in sham operated rats was found to be 6.0 ± 1.0 mEq/L. All these changes were significantly reversed by the treatment with *G. montana* at a dose of 500 mg/kg. These effects were comparable to the effects of standard ranitidine (50 mg/kg). The results are depicted in figure 2.

Cystemaine-induced ulcers

The mean ulcer index was found to be 3.2 ± 1.3 mL in cysteamine treated rats. One way ANOVA revealed that all the treatments has a significant influence on the ulcer index [F (4, 26) = 10.5, P<0.0001]. Post-hoc Tukey's test further revealed that the ulcer index was significantly increased in the Cysteamine treated rats (P<0.001). This increase was significantly reduced by ranitidine treatment (P<0.05) and also by the treatment with ethanolic extract of *G. montana* at a dose of 500 mg/kg (P<0.01). 100 mg/kg dose was found to be ineffective. The results are depicted in figure 3.

Discussion

The objective of the present investigation was to systematize the reports regarding antiulcer activity of fruits of *G. montana*. The results revealed that the ethanolic extract of G. Montana possesses very good antiulcer activity and that was consistent in all the models used in the present investigation.

All non-steroidal anti-inflammatory drugs (NSAIDs)

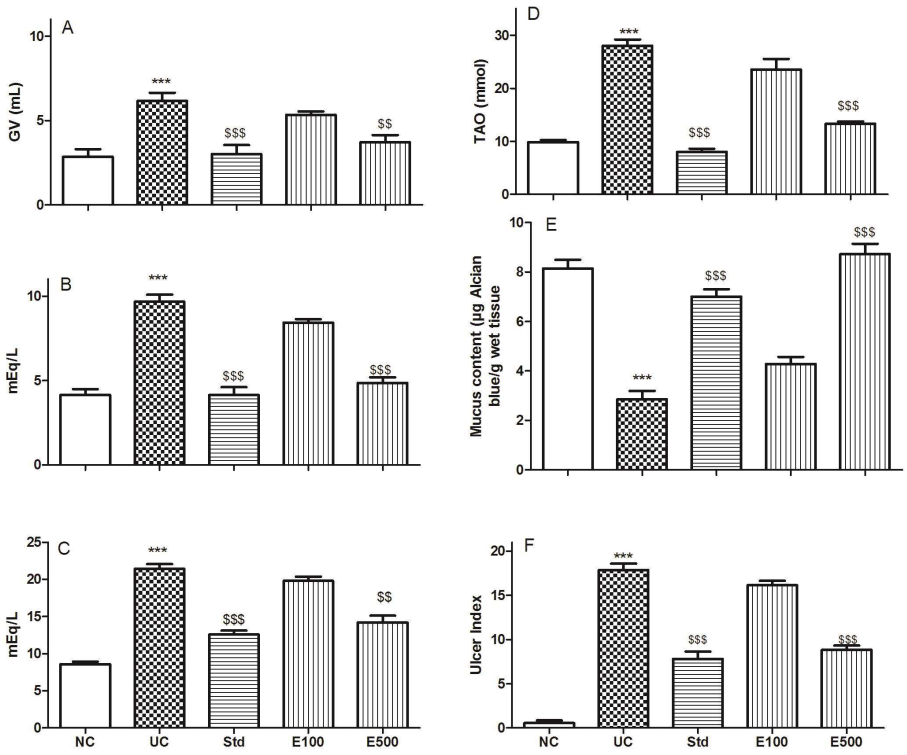

Figure 1. Effect of ethanolic extract of *G. montana* on the ulcers induced by aspirin in rats: **(**A) Effect on gastric volume, (B) Effect on free acidity, (C) Effect on total acidity, (D) Effect on total acid output, (E) Effect on gastric mucus content, (F) Effect on ulcer index. ***P<0.001 vs. sham control; $$$P<0.001, $$P<0.01, $P<0.05 vs. Ulcer control, One-way ANOVA followed by Tukey's post-hoc test.

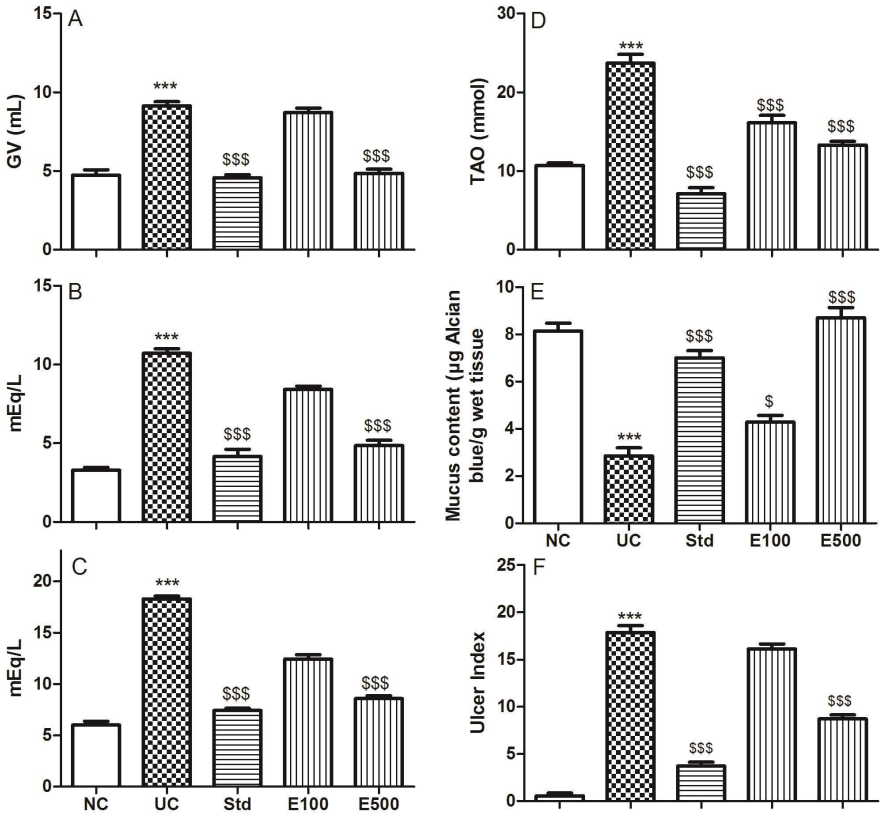

Figure 2. Effect of ethanolic extract of *G. montana* on the ulcers induced by pylorus ligation in rats: (A) Effect on gastric volume, (B) Effect on free acidity, (C) Effect on total acidity, (D) Effect on total acid output, (E) Effect on gastric mucus content, (F) Effect on ulcer index. ***P<0.001 vs. sham control; $$$P<0.001, $$P<0.01, $P<0.05 vs. Ulcer control, One-way ANOVA followed by Tukey's post-hoc test.

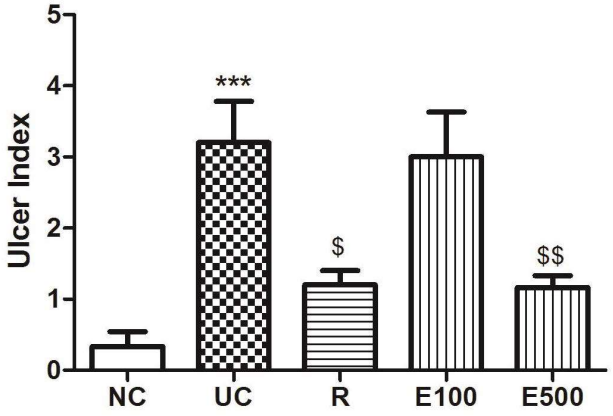

Figure 3. Effect of ethanolic extract of *G. montana* on the duodenal ulcers induced by cysteamine in rats: Data is presented as mean± S.E.M. of n=5-7 observations. After 24 h of the last dose of cysteamine the rats were euthanized, the isolated duodenum was gently opened and rinsed with ice-cold saline. Photographs of specific areas of damage under a dissection microscope with magnification were taken. The number of ulcers was noted and the severity recorded with the following scores: 0 = no ulcer, 1 = superficial ulcers (< 3 mm diameter), 2 = deep ulcers (> 10 ulcers with 3 mm diameter), 3 = 1 or more ulcers (4–6 mm diameter), 4 = 1 or more ulcers (≥ 7 mm diameter), 5 = Perforation. ***P<0.001 vs. sham control; $^{\$\$}P$<0.01, $^{\$}P$<0.05 vs. Ulcer control, One-way ANOVA followed by Tukey's post-hoc test.

damage gastric mucosa. Anyone who takes two tablets of aspirin with a glass of water will suffer some gastric bleeding. If the person continues to take aspirin over a 24-h period, gastric erosions generally will develop. Therefore, aspirin-induced ulcer paradigm was the first choice to screen the antiulcer activity. The results revealed a potent antiulcer action at a dose of 500 mg/kg which was comparable to that of the standard ranitidine. The non-selective inhibition of cyclooxygenase by aspirin via its acetylation is thought to be responsible for the pleotropic actions that include reduced mucus secretion and bicarbonate secretion in stomach along with changes in the microcirculation that ultimately result in the precipitation of ulcers. Thus the antiulcer activity of the extract seen might be attributed to antagonism of these actions of aspirin via a mechanism other than that of cyclooxygenase resulting in increased mucus secretion and protection of the gastric mucosa. In fact, flavonoids have been reported to have several such action resulting in antiulcer effects. Thus, the antiulcer activity observed in the present investigation might be attributed to the presence of flavonoids in the extract.

Similarly, another model that was used in the present

investigation was that proposed by Shay et al. (1945). The pylorus ligated rat (Shay rat) was described by Shay et al. for the production of acute ulcers in the fore stomach, and it has been also employed as a means of studying gastric secretion. This technique is used extensively as a screening method of gastric secretory depressants and antipeptic ulcer agents because of the following advantages: 1) the simplicity of surgical procedure, 2) the high rate and rapid occurrence of ulceration, and 3) the possibility of measurements of gastric secretory rate and severity of ulceration in the same animals; These factors make up for the shortcoming that ulcers are present only in the fore stomach. With regard to the mechanism, it is reported that 19 h of pyloric ligation causes gastric juice accumulation. Hence, a similar protocol was employed in the present investigation. Similar, effects to that of aspirin model were noted in this protocol too. It cleary indicated the potent antisecretory effect of the extract which again can be attributed to the flavonoid induced suppression of acid secretion due to their action on the hydrogen-potassium ATPase in gastric lumen.

Finally the results were confirmed in the third model of cysteamine induce ulcers as duodenal ulcer (DU) is a serious public health problem that affects approximately 10% of the global population and is 2 to 3 times more prevalent than gastric ulcer. If DU is not treated or only partially treated, several complications such as perforation, stenosis, and hemorrhage can develop, and even more serious clinical conditions that necessitate surgery or mortality in the elderly can occur. Cysteamine is a low-molecular weight aminothiol that is broadly distributed in organisms and is a natural product of coenzyme A catabolism. Levels of free cysteamine in rodent tissues and human plasma are generally very low. It has been demonstrated that cysteamine levels are elevated in different brain regions, plasma and red blood cells after cysteamine administration in adult rats. Recently, it was found that cysteamine-induced duodenal ulceration in rats was exacerbated by elevated levels of endogenous iron in the proximal duodenum, and cysteamine increased the expression of divalent metal transporter-1 [DMT-1]. Thus, it can be said that the effects of cyteamine can be attributed to the abnormal handling of iron by the duodenal cells results in increased oxidative stress that ultimately manifests as duodenal ulcer. In this model too, the G. Montana extract was found to possess marked antiulcer activity, which again can be attributed to the presence of flavonoids as these are reported very good antioxidants. It appears that the enhancement of the mucosal defense system with phytoceuticals is very important in preventing ulcerogenesis caused by irritants in duodenum. Inflammation of duodenal mucosa is associated with ulcerogenesis, and the resistance of duodenal mucosa to irritants depends on the balance between defensive and aggressive factors. Although most DUs are curable by alleviating the effects of offensive damaging factors, there are still many cases experiencing persistent dyspeptic symptoms and recurrences of the ulcer even when continuously treated with acid suppressant. Herein, the benefit observed with the herbal agents can be put to use to alleviate the ulcer incidence.

Conclusion

The fruits of *Gymnospora montanna* possess antiulcer activity. It may be attributed due to antisecretory, cytoprotective and antioxidant properties. To conclude the ethanolic extract of *G. Montana* was found to possess very good antiulcer activity in three models of peptic ulcer and it can be attributed to the presence of flavonoids in it.

Conflict of Interest

The authors declare no conflict of interest.

References

Angelo AA, Hasan MA, Nagva MN, Khalifa HM, Ghany SA. 2010. A possible role of gastroprotectives on aspirin-induced gastric ulcers in rats. Bulletin of Alexandria Faculty of Medicine 46: 75–82.

Cerella C, Dicato M, Jacob C, Diederich M. 2011. Chemical properties and mechanisms determining the anti-cancer action of garlic derived organic sulfur compounds. Anti-Cancer Agents in Medicinal Chemistry 1: 267–271.

Chang C, Yang M, Wen H, Chern J. 2002. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of Food & Drug Analysis 10(3): 178-182.

De S, Ravishankar B, Bhavsar G. 1994. An Investigation on the Hepatoprotective Activity of Gymnosporia Montana. Planta Medica 60: 301-304.

Del Valle. 2014. Peptic ulcer diseases and related disorders. Harrisons principles of internal medicine, Eighteenth edition, New York: Mc-Graw Hill.

Desai JK, Goyal RK, Parmar NS. 1999. Characterization of dopamine receptor subtypes involved in experimentally induced gastric and duodenal ulcers in rats. Journal of Pharmacy and Pharmacology 51: 187-192.

Drini M. 2017. Peptic ulcer disease and non-steroidal anti-inflammatory drugs. Australian Prescriber 40(3): 91-93.

Hemmati H, Rezvani A, Djahanguiri B. 1973. Prevention of aspirin-induced ulceration in rats with alphamethyl dopa and disulphiram. Pharmacology 9: 374–8.

Huang J, Sridhar S, Hunt R. 2002. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory

drugs in peptic-ulcer disease: a meta-analysis. Lancet 359: 14–22.

Kulkarni SK. 1999. Hand book of experimental pharmacology, 3rd edn, pp. 148-150, New Delhi, Vallabh prakashan.

Malagelada J-R, KuipersMartin EJ, Blaser J. 2007. Acid Peptic Disease: Clinical manifestations, Diagnosis, Treatment, and Prognosis. In: Goldman: Cecil Medicine, 23rd ed. Philadelphia, PA: WB Saunders.

Marya RK. 2002. Text book of pathophysiology, pp. 52-54, New Delhi, CBS Publishers & Distributors.

Shay H, Komarov SA, Fele SS, Meranze D, Gruenstein H, Siplet H. 1945. A simple method for uniform production of gastric ulceration in rat. Gastroenterology 5: 43-61.

Sonnenberg A. 2013. Review article: historic changes of Helicobacter pylori-associated diseases. Alimentary Pharmacology and Therapeutics 38: 329–342.

Wong G, Wong V, Chan Y, Ching JY, Au K, Hui AJ, Lai LH, Chow DK, Siu DK, Lui YN, Wu JC, To KF, Hung LC, Chan HL, Sung JJ, Chan FK. 2009. High incidence of mortality and recurrent bleeding in patients with Helicobacter pylori-negative idiopathic bleeding ulcers. Gastroenterology 137: 525–531.