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Kritika Garg
(1)Department of Pharmacology, ROFEL Shri G. M. Bilakhia College of Pharmacy, Namdha Road, P. B. No. 11. Vapi, Gujarat, India
(2)Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, P. O. Limda, Tal. Waghodia, Dist. Vadodara, Gujarat, India

Arindam Paul
Department of Pharmacology, ROFEL Shri G. M. Bilakhia College of Pharmacy, Namdha Road, P. B. No. 11. Vapi, Gujarat, India

Mittal Dalal
Department of Pharmacology, ROFEL Shri G. M. Bilakhia College of Pharmacy, Namdha Road, P. B. No. 11. Vapi, Gujarat, India

Hardik Soni
Vasu Research Center (A Division of Vasu Healthcare Pvt. Ltd.), A2/624-625/2, G. I. D. C., Makarpura, Vadodara, Gujarat, India

Corresponding Author:
Kritika Garg
(1)Department of Pharmacology, ROFEL Shri G. M. Bilakhia College of Pharmacy, Namdha Road, P. B. No. 11. Vapi, Gujarat, India
(2)Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, P. O. Limda, Tal. Waghodia, Dist. Vadodara, Gujarat, India

Evaluation of acid neutralizing and antiulcer potential of a poly-herbal formulation

Kritika Garg, Arindam Paul, Mittal Dalal and Hardik Soni

Abstract
The present study was planned to evaluate the acid neutralizing and antiulcer potential of a polyherbal formulation by using various models like neutralization of 0.1N hydrochloric acid, acid neutralization capacity using artificial stomach model, asprin plus pylorus ligation induced gastric ulcer model, protein precipitation assay and prokinetic effect using isolated rat ileum. The toxicity studies were conducted and it was safe at a dose of 5000mg/kg. The *in-vitro* study revealed its significance statistically in acid neutralizing when compared to control group. The formulation also showed potential results with aspirin ligation method when compared to control with respect to ulcerative index, total acidity, total acid output at a dose of 200mg/kg. Thus, it can be said that the polyherbal formulation is considered to be safe and effective in treating patients with high acidity as compared to marketed formulation.

Keywords: Herbomineral drugs, antiulcer, acid neutralizing, artificial stomach model, ulcer index, gastric wall mucus content, gastro-protective effect.

1. Introduction
Acid neutralization and peptic ulcer therapy has undergone many progresses over the past few years and a number of drugs are now available for its treatment. These drugs are broadly classified into two, those that act against acid-pepsin secretion and those which afford cytoprotection. These drugs act by different mechanisms. Most of the commonly used drugs such as H_2-blockers (Ranitidine, Famotidine etc), M_1-blockers (Pirenzepine, Telenzepine etc), proton pump inhibitors (Omeprazole, Lansoprazole etc), decrease secretion of acid while, drugs like Sucralfate and Carbenoxolone promotes mucosal defenses. Of late the role of these drugs on the defensive factors is gaining importance. It is now assumed that these drugs ultimately balance the aggressive factors (acid, pepsin, *H. pylori*, bile salts) and defensive factors (mucin secretion, cellular mucus, bicarbonate secretion, mucosal blood flow and cell turnover). Although these drugs have brought about remarkable changes in ulcer therapy still the efficacy of these drugs is debatable. Reports on clinical evaluation of these drugs showed that there are incidences of relapses and adverse effects and danger of drug interactions during ulcer therapy. Hence, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules, which afford better protection and decrease the incidence of relapse. Peptic ulcer is classified into acute peptic ulcer are also known as stress ulcers that cause multiple or small mucosal erosions mainly found in stomach and rarely in duodenum and its other type is chronic peptic ulcer known as gastric ulcer or duodenal ulcer in which pathological changes may result due to implication of acid-pepsin, this types of ulcers are common due to life style and industrialization. Since from decades many indigenous drugs have been used in treatment of peptic ulcer for its effectiveness but still a need arises. In Ayurveda, peptic ulcer mostly refers to Amlapitta or Parinamasula. Amlapitta is a disease of the gastrointestinal tract, especially of the stomach. It has not been described as an independent disease in major Ayurvedic texts, but has been mentioned in short in Kashyapa Samhita. Amlapitta literally means, pitta leading to sour taste. Although, sour taste is one of the physiological properties of pitta, nevertheless, it is not realized in healthy condition, it is realized only when it aggravates. Apart from the stress laid on food habits and personal hygiene, some herbal drugs have also been mentioned. Modern medicine has not adequately evaluated the usefulness of these herbal drugs in ulcer therapy, although studies have been reported. VRC/AS/014 syrup, polyherbal formulations (provided by Vasu Research Centre, Vadodara) was designed to be used clinically as gastric acid neutralizer. It was also indicated for reduction in gastric ulcers. Most of the ingredients of this polyherbal formulation have shown gastroprotection and antacid properties. The VRC/AS/014 syrup consist of following ingredients prepared in flavoured syrup base: *Emblica officinalis* (Amalaki) [1–5] Fruit; *Asparagus racemosus* (Shatavari) Root tuber [6–10]; *Glycyrrhiza glabra* (Yashthimadhu)

Root [11]; *Hemidesmus indicus* (Sariva) Root [12, 13]; *Centella asiatica* (Mandukparni) Whole Plant [14-17]; *Terminalia chebula* (Haritaki) Fruit [18]; *Terminalia belerica* (Bibhitak) Fruit [19–26]; *Ipomoea turpethum* (Nishoth) Root [27]; Powder of: *Sodii carbonas* (Sarjakshar) Salt; and Black salt (Kala namak) Salt.

Therefore, this study was planned to provide with scientific preclinical data to support its clinical use for acid neutralizing and gastroprotective indication. The study was aimed to evaluate VRC/AS/014 syrup for its acid neutralizing and gastroprotective property in various *in-vitro* and *in-vivo* experimental models.

2. Materials and methods

2.1. Experimental animals

Adult wistar albino rats of either sex weighing 200-250 g were used and acclimatized to the experimental room having ambient temperature (22 ± 2ºC), humidity (30 - 70%) conditions, and 12 h light and dark cycle. Animals were caged in polypropylene cages with maximum of three animals per cages. The rats were fed with food pellets and water *ad libitum.* Study was conducted after obtaining approval by Institutional Animal Ethical Committee of ROFEL Shri G. M. Bilakhia College of Pharmacy as pr the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The protocol number was ROFEL/IAEC/2017/1.

2.2. Administration of test drugs and dosage

The test formulation (VRC/AS/014) a polyherbal formulation in form of syrup were received from Vasu Healthcare Pvt. Ltd., Vadodara, and Gujarat, India. VRC/AS/014 syrup was administered orally. Dose of the test formulation were fixed by extrapolating the human dose to laboratory animals. For acute toxicity study, the doses to be used in the step-up, step down procedure are 5, 50, 300 and 2000 mg/kg as per OECD guidelines no. 423 [28]. Since the formulation was expected to be non toxic, a limit test at dose of syrup equivalent to 2000 mg/kg of the dry mixture has been tested at the first step. Various *in-vitro* and *in-vivo* experimental models enlisted below were being used for the evaluation purpose of this acid neutralizing and antiulcer potential of test formulation.

In-vitro models was evaluated based on acid neutralizing activity by using - Neutralization of 0.1 N Hydrochloric acid and acid neutralizing capacity using artificial stomach model also prokinetic activity and astringent potential was also evaluated to determine the motility and astringent potential of test formulation.

2.3. Neutralization of 0.1N hydrochloric acid (*in vitro*): [29]

Neutralization of 0.1N hydrochloric acid was added to VRC/AS/014 test syrup under continuous stirring for 15 min so that proper mixing and agitation of the ingredient/material occurs. After 15 min acid base titration was carried out by 0.1N sodium hydroxide for neutralizing the excess of hydrochloric acid to maintain a stable pH.

2.4. Antacid capacity using artificial stomach model (*in vitro*): [30]

2.4.1. Apparatus

1. It consists of three parts: S1, S2 and S3. Where, S1 was the gastric reservoir, S2 was the influx for poly-herbal formulation i.e VRC/AS/014 and S3 was the output for gastric emptying. Albumin as protein was also added
2. The artificial duodenum also consists of three parts: D1, D2 and D3, where; D1 was the input for gastric reflux, D2 was the reservoir for bicarbonates secretory reflux and D3 was the influx for bile.

All the influx was controlled by a pump using tubes of different caliber. Two glass electrodes were immersed in both the gastric and duodenal reservoirs which were connected to pH recording system.

2.4.2. Experimental procedure

To assess the antacid characteristics, S1 was filled with 100 ml of (0.1 N) HCl solution (10 mmol) at time t_0. The influx was set constant to 3 ml/min and consist of (0.1 N) HCl solution, corresponding to an hourly acid output of 18 mmol/h, the output was adjusted to 3 ml/min. These refluxes were compared with the normal subjects.

At the same time the duodenal reservoir D1 was filled at t_0 with 30 ml of (0.1 N) hydrochloride acid solution, thus simulating the acidity in the pylorus. Stimulate bicarbonate secretion was kept constant at 3ml/min and consist of 0.1 N Sodium bicarbonate solution, corresponding to the duodenal secretion observed in normal subject. The glass electrode was plugged into the duodenal content.

The experiment solutions to be tested were added to the gastric content and pump was started by bringing S2, S3, D2 and D3 into action. The pH of the duodenal and stomach was recorded continuously throughout the experiment until the pH in S1 fell to its initial value of 1.0.

2.4.3. Parameters to be measured

a) The amount of acid (mmol H^+) consumed to reached pH 1.0 was measured.
b) Characterization of the relative contribution of neutralization and buffering capacity to the activity of the antacids and the pH of artificial duodenal was noted at 1min intervals and the mean duodenal pH was calculated.

2.5. Prokinetic activity (*in vitro*): [31]

2.5.1. Effect of VRC/AS/014 syrup on mortality using isolated Rat ileum

The rat ileum was placed in the assembly and was allowed to stabilize. Two equipotent response of acetylcholine of 1µg/ml was given and response was noted down further the dose of test syrup was administered and the response was recorded.

2.6. Antiulcer activity (*in vivo*): [32]

2.6.1. Aspirin plus pylorus ligation (PL) method

Animals were divided in 5 groups (group-I to group-V) consisting of 6 animals each in group.

Group-I served as a control were 1% CMC was administered as vehicle.

Group-II served as disease control at a dose of 200mg/kg body weight and was administered orally with aspirin and PL (pylorus ligation).

Groups-III and IV served as tested drug with a dose of 2 ml/kg and 4 ml/kg body weight. *

Group-V served as standard where Sucralfate will be orally administered at a dose of 300 mg/kg body weight.

*The intended dose of test drug for adult human is equivalent to 10 ml twice daily.

All the drugs were administered daily for 5 days and on day 6 after 12 hours of fasting pylorus were ligated under anesthesia by using ether.

Six hours after ligation, the animals were sacrificed and the

stomach were removed and opened along the great curvature. The antiulcer activity was evaluated by using various parameter- Ulcer index, total acidity, gastric wall mucus secretion.

2.6.2. Calculation of ulcer index

The ulcers were examined under a magnified lens. The ulcer area was measured with the help of vernier calliper. Each lesion of stomach was measured along its greatest length and breadth. For circular lesion

Ulcer index will be calculated by using following formula: UI= 10/X.

Where, X= total glandular area of stomach/total ulcerated areas.

The area of ulcerated portions was calculated as per the following formula:

Area of circular lesion= $\pi D^2/4$ (D= diameter of lesion)

Area of linear lesion= l × b (length and breadth of lesion respectively)

Area of stomach mucous= $\pi D^2/8$ (D= diameter of glandular part of stomach)

2.6.3. Volume of gastric secretion: [33]

The total gastric secretion was collected and measured. It was then centrifuge for 10 min at 1000xg. The volume of gastric content was expressed in ml/100 g body weight.

2.6.4. Total acidity

Gastric contents were assayed for total acidity by titration against 0.01 N sodium hydroxide to pH 8.0 using phenolphthalein as indicator. The change in colour indicates the reaction is complete. The amount of HCl in gastric content was calculated and expressed as mEq/L liberated per unit time of gastric juice and calculated as

Total acid output= Total acidity × Volume of gastric content per 100g of body wt.

2.6.5. Gastric Wall Mucus Content: [34]

The glandular segments of stomach after opening along the greater curvature was removed and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v Alcian blue solution for 2 hours. Excess dye was removed by rinsing the tissue twice with 10 ml of 0.25 M sucrose. Dye complex with the gastric wall mucus was extracted from the glandular tissue with 10 ml of 0.5 M magnesium chloride that was intermittently shaken for 1 min every 30 min for 2 h. Total volume of this blue colored extract solution was shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuge at 3600 rpm for 10 min and aqueous layer separated. Concentration of Alcian blue was determined in this layer. Absorbance will be recorded by using an UV-VIS spectrophotometer at 610 nm. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated from standard curve prepared with obeyed the Beer-Lambert law at the concentration of dye used.

2.7. Astringent potential (*in vitro*): [35, 36]

2.7.1. Effect of VRC/AS/014 syrup for determining its astringent potential

2ml albumin was prepared by using 1mg/ml of 0.2M acetate buffer containing 0.17M sodium chloride which is adjusted to pH 5 with sodium hydroxide/hydrochloric acid. The material was placed in centrifuged tubes and 3 ml of tannic acid solution was added to it. It was kept for 15 min and centrifuged at 5000 rpm for 15 min. the supernatant was discarded and the precipitate was rinsed with acetate buffer at pH 5. The supernatant was further discarded and 4 ml of SDS-triethanolamine was added finally to the precipitate with the addition of 1 ml of ferric chloride and absorbance was measured at 510 nm.

2.8. Statistical analysis

All continuous data was analysed using bartlett's test followed by one-way ANOVA and tukey's test. $P < 0.05$ was considered as statistically significant. In case of heterogenous data t-test was applied.

3. Results and discussion

3.1. Evaluation of acute toxicity (OECD 423 guidelines)

Acute toxicity study was carried out on the formulation by adopting OECD 423 guidelines. During the study no mortality was observed at a dose of 5000mg/kg and was found to be safe. There was no significant change with respect to body weight, clinical sign, behavioral change, autonomic symptoms, general awareness and gross pathological studies.

3.2. Acid Neutralizing Potential

The *in vitro* acid neutralizing potential of formulation was determined by treating the syrup with 0.1 N hydrochloric acid against 0.1 N sodium hydroxide. The acid neutralizing capacity was determined for flavoured syrup (flavour and syrup base without active ingredients), VRC/AS/014 syrup (i.e. poly-herbal formulation with active ingredients) and standard (milk of magnesia). The amount of acid neutralizing capacity was determined by comparing all the groups with each other and with blank (using distilled water instead of test).

3.2.1. pH of formulation

The flavoured syrup, VRC/AS/014 and standard were diluted with distilled water in a ratio of (1:10) and the pH of resulting solution was determined.

Table 1: pH of formulation

Solution	pH
Flavoured syrup	7.09
VRC/AS/014	8.02
Standard- Milk of Magnesia	8.88

3.2.2 *In vitro* Neutralization of acid

As mentioned above the excess acid was titrated against 0.1 N sodium hydroxide, using pH meter and the graph of change in pH vs ml of sodium hydroxide (Fig 2) was plotted. The end point was calculated from this graph and is shown in Table 4.

Table 2: *In vitro* Neutralization of acid

Series	ml of NaOH for neutralization
Blank	10.45 ± 0.13
Flavoured syrup	10.10 ± 0.15
VRC/AS/014	$7.25 \pm 0.11^{*\#}$
Standard- Milk of Magnesia	$4.73 \pm 0.48^{*\#\$}$

All values are expressed as Mean ± SEM (n=3), and analysed by one way ANOVA followed by Tukey's test. [Fcal (3, 8) = 102.2]

* denotes $p<0.05$ when compared with blank.

\# denotes $p<0.05$ when compared with flavoured syrup.

$ denotes $p<0.05$ when compared with VRC/AS/014.

As expected, the pH of flavoured syrup was close to Neutral (7.09) and accordingly the volume of NaOH required to

neutralize the excess acid was similar to that of blank. Thus, we can conclude that the flavoured syrup base does not contribute much to the acid neutralizing property of VRC/AS/014. The formulation under study was found to have an alkaline pH though the alkalinity which was less than that of marketed preparation of Milk of Magnesia. Further, the volume of NaOH required for neutralization was less for VRC/AS/014 (p<0.05) compared to flavoured syrup and the volume of NaOH consumed by standard was even less compared to flavoured syrup as well as VRC/AS/014 (p< 0.05).

3.3 Antacid capacity using artificial stomach model

Through this model the dynamic flow of fluid and pH condition of the upper GIT can be determined.

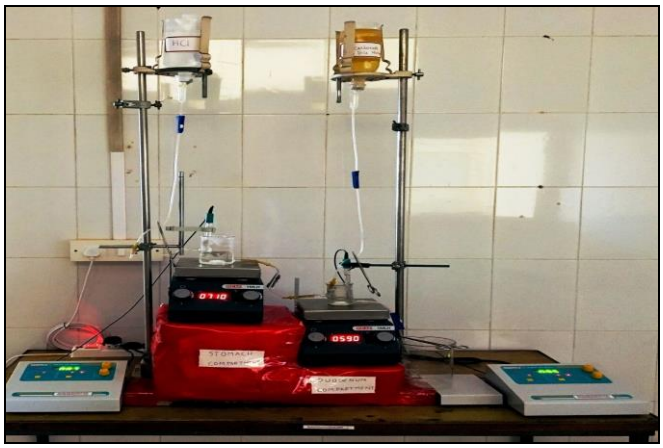

Fig 1: Artificial stomach model

Table 3: Acid neutralizing activity in gastric reservoir when antacids were added to 10 mmol of HCl

S. No.	Group	Maximum pH in stomach chamber	pH-1 recovery time (min)	mmol H^+ consumed
1	Flavoured	1.15 ± 0.04	13.67 ± 0.88	14.10 ± 0.26
2	VRC/AS/014	1.57 ± 0.058*	78.67 ± 4.41*	33.60 ± 1.32*
3	Standard - Milk of Magnesia	5.78 ± 0.14*#	96.67 ± 0.67*#	39.00 ± 0.20*#

All values are expressed as Mean ± SEM (n=3), and analysed by one way ANOVA followed by Tukey's test. Maximum pH - [Fcal (2,6) = 829.9], pH-1 recovery time -[Fcal (2,6) = 276.7] and mmol H^+ consumed - [Fcal (2,6) = 278.2]
* denotes p<0.05 when compared with flavoured syrup.
# denotes p<0.05 when compared with VRC/AS/014.

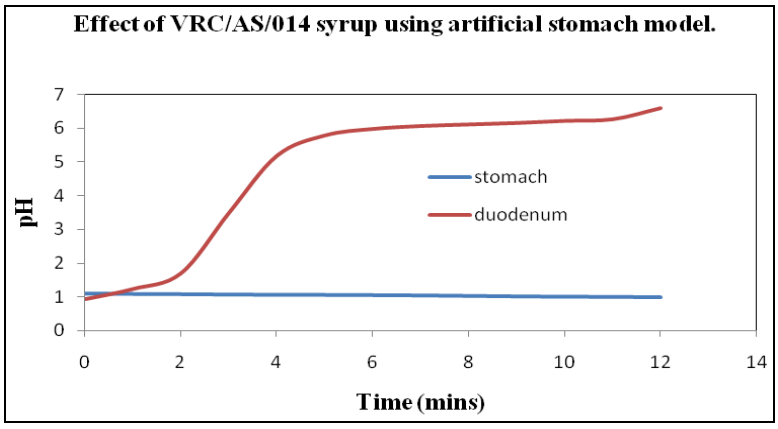

Fig 2: Change in pH of stomach-duodenum chamber (Flavoured)

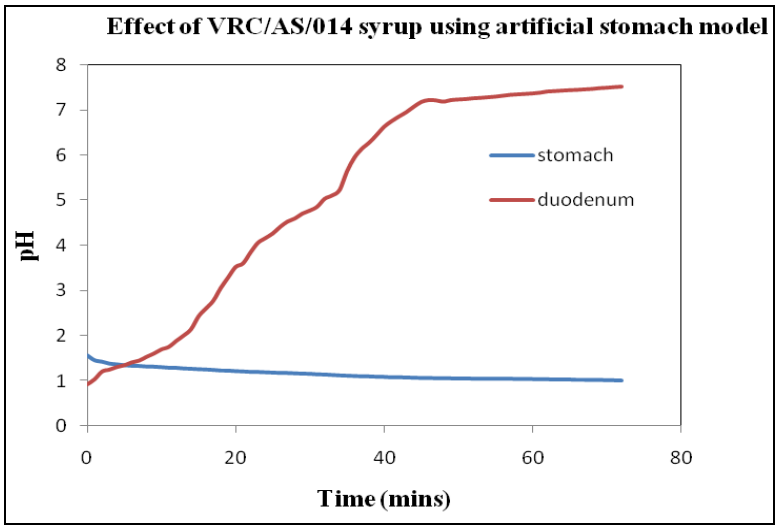

Fig 3: Change in pH of stomach-duodenum chamber (VRC/AS/014)

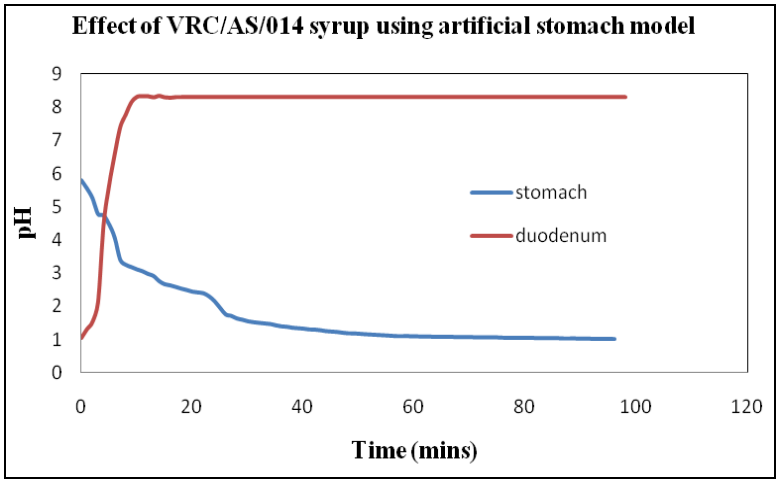

Fig 4: Change in pH of stomach-duodenum chamber (Standard- milk of magnesia)

The artificial stomach – duodenum model was established as described by Vatier *et al*.1992 [7] This is a dynamic flow model designed to mimic the conditions in the stomach. The acid neutralizing potential of test formulation in dynamic acid and digestive juice secretion conditions in stomach and duodenum was studied by this model. The maximum pH on adding flavoured syrup to 100 ml of 0.1 N HCl was not much greater than the baseline of 1.0, indicating little neutralization. Similarly, the time for recovery to baseline pH and mmol H^+ consumed was also found to be little. Milk of Magnesia (Dey's) was taken as a standard and the maximum pH (5.78 ± 0.14) was found to be significantly higher (p<0.05) than that of control i.e., flavoured syrup. This value is slightly higher than that reported by Vatier *et al*, 1992 however, they used a mixture of Aluminium and magnesium hydroxides. In line with this observation, the recovery time and mmol H^+ consumed was also significantly higher compared to flavoured syrup (p<0.05). These values were considered as an index of acid neutralizing capacity of Antacids. This also suggests that the pH of stomach on taking this antacid will be higher than 2, which might actually hamper the enzymatic activity and hence digestion. In contrast, the maximum pH of VRC/AS/014 was found to be below 2 (1.57 ± 0.05) but significantly higher than that of control. Also, the mmol H^+ consumed was significantly higher than control but less than that of Milk of Magnesia. This clearly suggests good acid neutralizing potential of test formulation, without exposing the stomach to higher pH conditions even for a short time. Hence, the test formulation might actually be better for chronic use than 'harder antacids'.

3.4 Prokinetic activity:

The effect of VRC/AS/014 syrup on intestinal motility was studied by using isolated Rat ileum. As seen in the figure below, graded doses of the syrup failed to produce any contractile effect on isolated Rat ileum. Hence, it was concluded that VRC/AS/014 does not have any prokinetic effect on the intestinal smooth muscle.

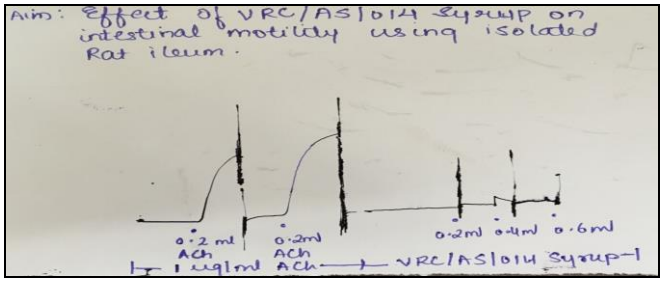

Fig 5: Effect of VRC/AS/014 syrup on intestinal motility using isolated Rat ileum

3.5 Aspirin plus pylorus ligation induced gastric ulcer model

The protective effect of test formulation against Aspirin plus PL induced gastric ulcer was also studied. On day 6, pylorus was ligated under slight ether anaesthesia, and after 6 hrs the animals were euthanized. The stomach was dissected out and the contents were collected in a test tube for determination of acid secretory parameters the stomach was opened up along the greater curvature and mounted on wax plate to determine the ulcer index. Sucralfate (300 mg/kg) was used as a standard for this model. This model was selected to enable the study of effect of formulation on acid neutralisation, effect on acid secretory parameters and overall protection against Ulcer.

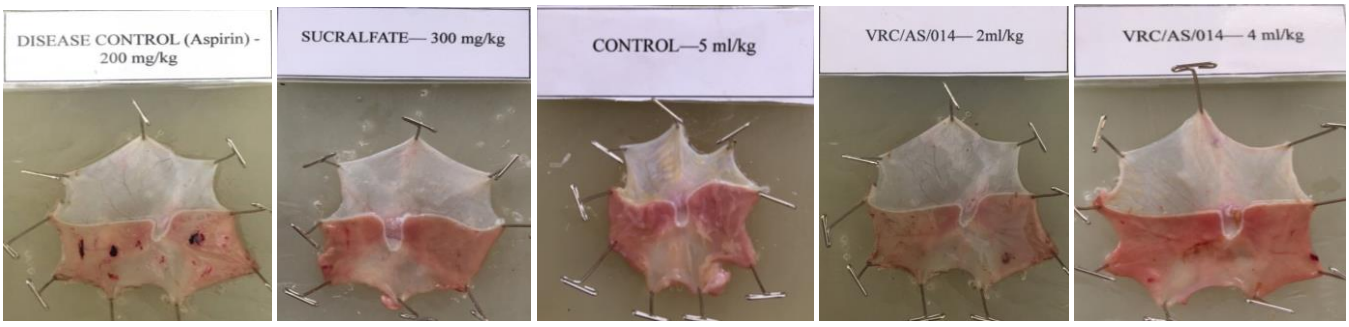

Fig 6: Effect of VRC/AS/014 syrup using aspirin plus pylorus ligation induced gastric ulcer model in Rats

3.5.1 Effect of VRC/AS/014 syrup on ulcer index using aspirin plus pylorus ligation induced gastric ulcer model in Rats

Table 4: Effect of VRC/AS/014 syrup on ulcer index using aspirin plus pylorus ligation induced gastric ulcer model in Rats

S. No.	Group	Dose (ml/kg)	No. of animal	Ulcer index
G1	Control	5	6	0.00 ± 0.00
G2	Disease control	200@	6	1.496 ± 0.092*
G3	VRC/AS/014 - lower dose	2	6	0.282 ± 0.010*#
G4	VRC/AS/014 - higher dose	4	6	0.235 ± 0.012*#
G5	Standard (sucralfate)	300@	6	0.162 ± 0.009#

@ mg/kg
All values are expressed as Mean ± SEM (n=6), and analysed by one way ANOVA followed by Tukey's test. [Fcal (4,25)= 206.6].
* denotes p<0.05 when compared with control.
# denotes p<0.05 when compared with disease control.

Aspirin plus PL, as mentioned above, produced multiple visible ulcers in the glandular portion of the stomach, as expected. The ulcer index in the disease control group was found to be 1.496 ± 0.092, which is significantly higher than the control, where no ulcers were found and hence the ulcer index was nil. In all the three treatment groups – VRC/AS/014 (2 ml/kg), VRC/AS/014 (4 ml/kg) and Sucralfate (300 mg/kg) multiple visible ulcers were observed, however, the ulcer index was significantly lower when compared with disease control (p < 0.05). Hence, it can be concluded that all the treatment groups successfully exhibited antiulcer activity.
Interestingly, though the ulcer protective effect as calculated from ulcer index was slightly higher for sucralfate (0.162 ± 0.009) compared to test formulation (high dose - 0.235 ± 0.012 and low dose - 0.282 ± 0.010), it was not found to be statistically significant. Further, the differences in acid neutralizing potential were not reflected in the protective effect against ulcer. This indicates some additional mechanism is involved in the action of VRC/AS/014. This could be due to antisecretory and/or astringent potential of test formulation which is not seen with antacids like Magnesium hydroxide.

3.5.2 Effect of VRC/AS/014 syrup on Acid secretory parameters using aspirin plus pylorus ligation induced gastric ulcer model in Rats:

Table 5: Effect of VRC/AS/014 syrup on Acid secretory parameters output using aspirin plus pylorus ligation induced gastric ulcer model in Rats.

S. No.	Group	Dose (ml/kg)	Volume of gastric secretion (ml/100 g)	Total acidity (mEq/l/100 g)	Total acid output
G1	Control	5	0.336 ± 0.083	46.53 ± 2.12	15.21 ± 0.44
G2	Disease control	200@	1.669 ± 0.099*	73.26 ± 3.48*	122.27 ± 0.47*
G3	VRC/AS/014 - lower dose	2	1.310 ± 0.105*	61.58 ± 2.74*	80.67 ± 0.33*#$
G4	VRC/AS/014 - higher dose	4	1.209 ± 0.128*#$	53.83 ± 2.09#	64.76 ± 0.16*#$
G5	Standard (sucralfate)	300@	1.657 ± 0.091*	51.61 ± 4.17#	85.44 ± 0.05*#

@ mg/kg
All values are expressed as Mean ± SEM (n=6), and analysed by one way ANOVA followed by Tukey's test. Volume of gastric secretion - [Fcal (4, 25)= 28.18], total acidity - [Fcal (4,25)= 11.81] and total acid output - [Fcal (4,25)= 13.699]
* denotes p<0.05 when compared with control.
# denotes p<0.05 when compared with disease control.
$ denotes p<0.05 when compared with Standard (sucralfate – 300 mg/kg).

3.5.2.1 Volume of gastric secretion (ml/100g)
After euthanizing the animals, on day 6, the stomach was dissected out and the total volume of gastric juice collected, measured. The volume of gastric juice was expressed in ml/100 g b. wt. As seen in the table above, the volume of gastric acid secreted in control animals was found to be 0.336 ml/100 g bw. The volume of gastric juice in the disease control group (Aspirin plus PL) was found to be significantly higher when compared with control (p<0.05). A comparable increase (p>0.05) in volume was also seen in animals treated with sucralfate (300 mg/kg). Notably, VRC/AS/014, at a dose of 4 ml/kg, attenuated this increase in gastric juice volume due to PL in a significant manner (p<0.05). This effect was also seen with doses of 2 ml/kg though it was not found to be statistically significant. We propose that this difference could be due to antisecretory effects of components like Nishoth in the formulation. However, confirmation of this requires further study with specific antisecretory models.

3.5.2.2 Total acidity and Total acid output
Total acidity was calculated by titrating against 0.01 N NaOH using phenolphthalein as indicator. The gastric acid was expressed in mEq/L/100 g. Total acid output was derived from acidity and volume of gastric secretion. The total acidity was increased in the aspirin plus PL model according to expectations. This acidity was decreased due to the treatment with Sucralfate as well as test formulation. The decrease in Total acidity and Total acid output due to Sucralfate and VRC/AS/014 was significant when compared with control Rats (p< 0.05). Again the results indicate that the decrease in acid output due to VRC/AS/014 cannot be explained on the basis of acid neutralisation alone and there is a strong possibility of antisecretory effect contributing to the beneficial effects of test formulation in the treatment of acidity.

3.5.3 Effect of VRC/AS/014 syrup on gastric wall mucus content using aspirin plus pylorus ligation induced gastric ulcer model in Rats.
The glandular area of the stomach was cut after opening the

stomach from greater curvature and was weighed. The mucus bound to Alcian blue was determined and expressed as µg/g of tissue. The mucus content was determined from the linear curve obtained for Alcian blue, in the range of 0 to 100 µg/ml and the values for various groups were extrapolated from this curve.

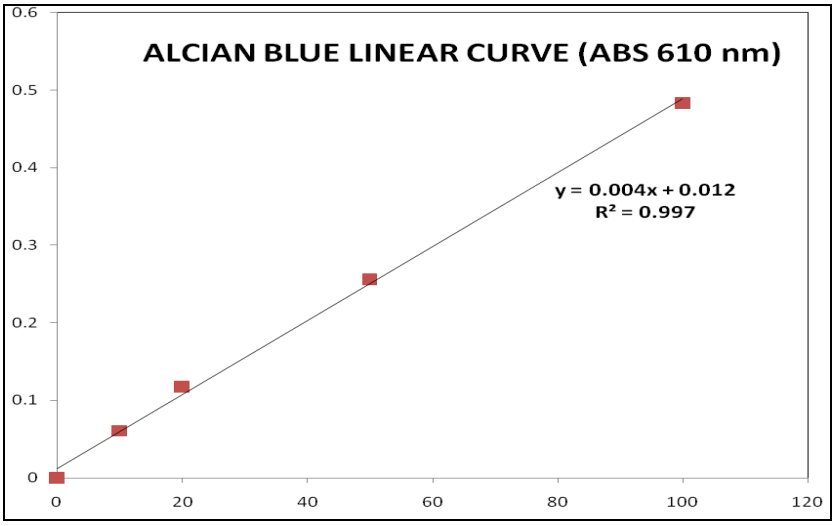

Fig 7: Linear curve of Alcian Blue.

Table 6: Effect of VRC/AS/014 syrup on gastric wall mucus content using aspirin plus pylorus ligation induced gastric ulcer model in Rats

S. No.	Group	Dose (ml/kg)	No. of animal	Gastric wall mucus content (µg/g)
G1	Control	5	6	87.83 ± 0.80
G2	Disease control	200[@]	6	29.26 ± 1.30[*]
G3	VRC/AS/014 - lower dose	2	6	44.90 ± 2.75[*#]
G4	VRC/AS/014 - higher dose	4	6	54.19 ± 2.50[*#]
G5	Standard (sucralfate)	300[@]	6	55.33 ± 3.07[*#$]

@ mg/kg

All values are expressed as Mean ± SEM (n=6), and analysed by one way ANOVA followed by Tukey's test. Gastric wall mucus content - [Fcal (4, 25)= 89.99]

* denotes $p<0.05$ when compared with control.

# denotes $p<0.05$ when compared with disease control.

$ denotes $p<0.05$ when compared with VRC/AS/014 - lower dose.

The gastric wall mucus content was significantly decreased in the Aspirin plus PL group ($p<0.05$) when compared with control animals. Sucralfate at 300 mg/k restored the loss in gastric wall mucus content produced by Aspirin plus PL in a significant manner ($p<0.05$). VRC/AS/014 at a dose of 4 ml/kg also produced a restoration of the gastric wall mucus content which was comparable to that produced by sucralfate. At a dose of 2 ml/kg (44.90 ± 2.75) also, the test formulation restored the gastric wall mucus content ($p<0.05$), however, the restoration was less than standard (55.33 ± 3.07) and higher dose (54.19 ± 2.50). The above results suggest a significant cytoprotective effect of VRC/AS/014 as indicated by mucosal content which was comparable to the protective effect of Sucralfate. Mucus content is an important defensive parameter against the formation of Ulcer and hence restoration of mucus content by the test formulation could point to another important pathway that would explain its overall antiulcer effect.

3.6 Astringent potential

The astringent potential of VRC/AS/014 was determined by slight modification in the method described by makkar *et al*, 1987[142]. The astringent potential was determined in terms of ability to precipitate proteins – albumin and expressed in terms of mg of tannin.

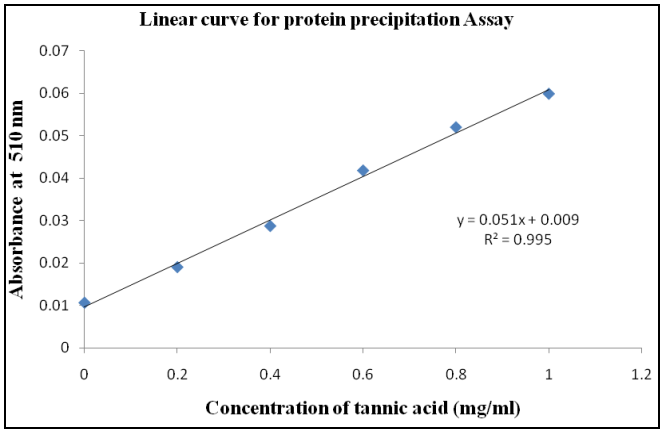

Fig 8: Linear curve for protein precipitation by tannic acid.

The absorbance at 510 nm after the procedure for protein precipitation by tannic acid as described earlier was found to follow a linear curve at concentration of tannic acid in the range 0 to 1 mg/ml. The absorbance for VRC/AS/014 syrup was measured and the astringent potential (extrapolated from graph) was found to be equivalent to that of 9.62 ± 0.57 mg/ml of tannic acid. This clearly indicates that the syrup can precipitate proteins *in vivo* and hence promote healing of peptic Ulcers. Yashtimadhu, Bibhitaki, Haritaki have been reported to have good tannin content. VRC/AS/014 contains the extracts of the above plants, which explains the astringent activity.

- **Summary**

VRC/AS/014 is a poly-herbal formulation with different components having different pharmacological properties,

which contribute to its overall acid neutralizing and antiulcer action. The safety of VRC/AS/014 was assessed using OECD 423 guideline and the formulation was found to be safe at doses of 5000 mg/kg. The acid neutralizing action of test formulation is different from conventional antacids used in the market in terms of gastric pH post treatment (test formulation is less harsh than Magnesium hydroxide), but the total acid neutralization in terms of mmol H^+ consumed is comparable. Hence this formulation may need higher dose volume but overall antacid effect would be similar. In fact, since it is having milder action, it might be more suitable for chronic use, particularly if the acidity is not very severe. Apart from its acid neutralizing action, some of the components may also have antisecretory effect which would add to its beneficial action in patients with hyperacidity. It is devoid of metals like Aluminium and Magnesium and hence the side effects on digestion and absorption of food and other drugs would also be absent. Our results also confirm the astringent action of the test formulation which would explain the protection against Ulcer produced by test formulation which was comparable to another conventional drug used for ulcer – Sucralfate.

4. Conclusion

Based on the present findings, we can conclude that VRC/AS/014 at a dose of 2 and 4 ml/kg has significant acid neutralizing action which is almost comparable to conventional antacids and has advantage over alkali antacids like Aluminium hydroxide and Magnesium hydroxide in terms of possible side effects relating to digestion and absorption. The proposed antisecretory action of test formulation needs further confirmation using specific animal or *in vitro* models. The test formulation was also found to have antiulcer activity comparable to that of Sucralfate in Rats at the administered doses, which are comparable to the human equivalent doses. The findings of the current study provide scientific preclinical data to support its clinical use for acid neutralizing and gastroprotective indication. In addition, VRC/AS/014 did not show any mortality or adverse effects during the acute toxicity study and hence the formulation was considered to be safe at oral dose of 2000 mg/kg and 5000 mg/kg in rats.

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