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Gastroprotective Activity Of Vishnukarnthi Chewable Granular Dosage Form Formulated Using *Evolvulus Alsinoides* And Its Accelerated Stability Studies

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Abstract: This study investigates the in vitro gastroprotective activity of Vishnukranthi chewable granules formulated using dry plant powder of Evolvulus alsinoides by evaluating neutralizing capacity in artificial gastric juice and the titration method using Fordtran's model. Accelerated stability studies were conducted upto three months to access the stability of granules. Each parameter was accessed at day 1, 1 month and after three months.Particle size distribution, moisture content and pH were measured as physical stability parameters. Microbial stability accessed by total viable bacteria and total viable fungi counts. Stability of the gastroprotective activity was evaluated using neutralizing capacity in artificial gastric juice and the titration method using Fordtran's model. Chewable granules has demonstrated a significant ($p<0.01$) neutralizing capacity on artificial gastric acid (mean pH 1.71±0.01) when compared with negative control. The titration conducted using Fordtran's model consumed 0.1493±0.0036 of H+ ($p<0.001$). There was a significant difference between fine percentage, percentage of weight loss, total viable count of granules at day 1 and after 1 month and 3 months ($p<0.05$). There were no significant differences between pH values, Rf values and the gastroprotective activity of granules at day 1 and after 1 month and 3 months. Granules were chemically stable but physically and microbiologically less stable. In conclusion, granules has demonstrated significant gastroprotective activity in both models. Further studies are recommended to improve the physical and microbial stability of the dosage form by adding a suitable binding agent and an appropriate preservative. Further, it is important to evaluate the efficacy of Vishnukranthi chewable granules clinically because this product has a good potential to commercialize as a herbal remedy for gastritis.

Keywords: Evolvulus alsinoides, gastroprotective activity, Fordtran's model, Accelerated stability studies, Vishnukranthi

Introduction:

Gastritis is an inflammation of gastric mucosa. It is due to excessive secretion of acid from stomach parietal cells. It may induce due to alcohol, irritant drugs e.g. non-steroidal anti-inflammatory drugs (NSAIDS), *Helicobacter pylori* infection and severe physiological stress (Waugh, A. and Grant, A. (n.d.). Ross & Wilson anatomy and physiology in health and illness.,2014). The most commonly used drugs for the treatment of peptic ulcer disease (PUD) are H2-receptor antagonists (Cimetidine), chelates

and complexes (Sucralfate), prostaglandin analogs and prostamides, proton pump inhibitors. (Omeprazole) and antacids (Sodium alginate with calcium carbonate and sodium bicarbonate) (BNF 76th edition,2018-2019).

Evolvulus alsinoides is locally known as Nil Vishnukranthi. *E. alsinoides* is an annual or perennial plant which belongs to family Convolvulaceae (Indhumol et al., 2013). The plant is used in Ayurveda and Yunani as nootropic or brain-tonic (Yadav et al., 2016).

Different formulations of *E. alsinoides* have evaluated for gastroprotective activity. Lekshmi and Reddy, 2011 has revealed *E. alsinoides* has strong dose dependent gastroprotectant activity in rats. Vishnukranthi kalka is a paste which is recommended in Ayurveda for treatment of peptic ulcers. A study has demonstrated a significant gastroprotective activity of the said powder (Hewageegana, Ariyawansa and Ratnasooriya, 2006). Our research group has formulated a chewable granular dosage form using *E. alsinoides* (Welipitiya et al., 2018).

The aim of this study was to evaluate the *in vitro* gastro protective activity of the formulation.

Furthermore, stability of Vishnukranthi chewable granules using accelerated stability testing was accessed.

Methodology:

Plant Collection and Authentication

E. alsinoides were authenticated and the voucher specimens deposited at National Herbarium, National Botanic Gardens, Peradeniya.

Formulation of Vishnukranthi chewable granular dosage form

Chewable granules were made using dried plant powder, maize starch as diluent, mannitol as the sweetening agent, starch solution as binding agent, approved chocolate flavour and chocolate colouring agents. 15 g portions of granules were packed in airtight polythene sachet and labeled properly.

***In vitro* gastroprotective activity of Vishnukranthi chewable granules**

Neutralizing effects of Vishnukranthi chewable granules on artificial gastric acid

A sachet of Vishnukranthi chewable granules (15g) was dissolved in distilled water. The solution was shaken for 2 hours. Eno (GlaxoSmithKline) and Belcid suspension (Biolab Co. Ltd) were used as positive controls whereas distilled water was taken as negative control. Initial pH of each sample (9 ml) was measured. 10 ml of freshly prepared artificial gastric juice was added to each sample and shaken for 5 minutes. pH of each solution was measured. Each solution was kept on the shaker and measure the pH at 10 minutes time intervals until reaches a constant value of pH (Thabrew and Arawwawala, 2016).

Neutralizing capacity of Vishnukranthi chewable granules using a titration method of Fordtran's model

Test sample of chewable granules, Eno, Belcid suspension and distilled water were heated and stirred to 37 ^{o}C and 30 r.p.m. respectively. Test solutions were titrated with artificial gastric juice until the pH of the solution became pH 3.00 which considered as the end point. Titrations were triplicated. The consumed volume (v) of the artificial gastric juice was measured and total consumed H+ (mmol) was calculated (Thabrew and Arawwawala, 2016).

If consumed volume for titration = V (ml),

Total Consumed H+ moles = 0.063096 (mmol/ml) x V (ml)

Accelerated stability testing of chewable granular dosage form

Accelerated stability testing was done for a period of 3 months. Specified accelerated conditions are temperature at 40 ± 2 °C and relative humidity at 75 ± 5% (USP32–NF27). Each parameter of the initial sample on the day of manufacture(day 1) and the samples kept in accelerated conditions after 1 month and after 3 months were determined..

Physical stability tests of Vishnukranthi chewable granules

Particle size distribution, moisture content and pH were measured as physical stability parameters.

The retaining percentage of weight of particles between standard pharmacopeial fine range was assessed. Moisture content was determined by loss of weight on drying. Percentage of loss on drying (LOD) was calculated (British pharmacopeia 2007).

Microbial stability tests of Vishnukranthi chewable granules

Microbial bioburdens were tested using total viable aerobic count (TVC). Number of colony forming units per gram of sample was evaluated for fungi and bacteria separately and then total viable count was calculated.

The total viable aerobic count = sum of the bacterial count (CFU) + the fungal count (CFU).

The growth mediums were Soboroud dextrose agar (SDA) for fungi and casein digest agar was for bacteria. Triplicate plates were grown for each sample (British pharmacopeia 2007).

Thin Layer Chromatography (TLC) for Vishnukranthi chewable granule samples

TLC fingerprint was used to test the chemical stability. Toluene – ethyl acetate – formic acid at a ratio of 7.5: 1.5: 1.1 (v/v/v) was used as the solvent system. The plate was visualized under UV 254 nm and UV 366 nm. Rf values were obtained (Irshad et al., 2016).

Stability of gastroprotective activity of Vishnukranthi chewable granules

Neutralizing effects of Vishnukranthi chewable granules on artificial gastric acid

Neutralizing effects of samples of Vishnukranthi chewable granules at accelerated conditions were accessed according to the methodology stated in 2.3.1.

Neutralizing capacity of Vishnukranthi chewable granules using a titration method of Fordtran's model

Neutralizing capacity of samples of Vishnukranthi chewable granules at accelerated conditions were accessed according to the methodology stated in 2.3.2.

Results and Discussion

***In vitro* gastroprotective activity of Vishnukranthi chewable granules**

Neutralizing effects of Vishnukranthi chewable granules on artificial gastric acid

Table 1: Neutralizing capacity of Vishnukranthi chewable granules on artificial gastric acid

Sample	Mean value of end pH
ENO	5.55±0.01 ***
Belcid suspension	4.22±0.02***
Distilled water	1.52±0.01
Vishnukranthi chewable granules	1.71±0.01 **

Table 6 shows the initial and end pH values of distilled water, Eno, Belcid, and vishnukranthi chewable granules. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to Control.

Mean values of end pH of Eno, Belcid, and chewable granules were statistically significant with respective negative control groups ($p<0.05$). Hence the ENO, Belcid and chewable granules are shown neutralizing capacity on artificial gastric acid.

Table 2: Fordtran's model analysis of Vishnukranthi chewable granules

Sample	Mean value of consumed H^+ (mmol)
Vishnukranthi chewable granules	0.1493±0.0036 ***
Distilled water	0.0069±0.0021
ENO	2.0695±0.0063***
Belcid suspension	3.9477±0.0036***

Table 2 shows the mean value of consumed H^+ of distilled water, Eno, Belcid, and vishnukranthi chewable granules. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to Control.

Mean values of consumed H^+ of Eno, Belcid, and chewable granules were statistically significant with respective negative control ($p<0.05$). Hence the ENO, Belcid and chewable granules are shown neutralizing capacity on titration method of Fordtran's model.

Accelerated stability testing of chewable granular dosage form

Physical stability tests of Vishnukranthi chewable granules

The fine percentage of day 1, 1 month and 3 months samples were 0.56±0.17, 1.28±0.18 and 1.47±0.02 respectively. There is a significant difference between fine percentages of day 1 and 1 month, 3 months accelerated samples ($p<0.05$). Particles has shifted towards more fine ranges within accelerated conditions.

Percentages of weight loss in drying in day 1, 1 month and 3 months were 9.3±0.30 %, 8.67±0.23 % and 7.73±0.23 %. The result shows significantly higher percentage of weight loss in drying in day 1 sample compared to 1 month and 3 months samples($p<0.05$).

pH of day 1,1 month and 3 months accelerated samples were 5.36±0.01, 5.36±0.00 and 5.35±0.06. There is no significant difference between the pH of day 1, 1 month and 3 months accelerated samples ($p>0.05$). Hence the pH of the accelerated sample at 1 month and 3 months has not changed significantly.

Microbial stability tests of Vishnukranthi chewable granules

Number CFU in Casein Soybean Digest Agar medium per gram of sample in day 1 and 3 months were 80000 CFU/g and 20500 CFU/g respectively. Results indicate there is a significant difference between total viable bacteria counts between two samples ($p<0.05$).

Number of CFU in Sobaroud Dextrose Agar Medium per gram of sample in day 1 and 3 months samples were 30 CFU/g and 250 CFU/g respectively. Results indicate there is a significant difference between total viable fungi counts between two samples($p<0.05$). Total viable count (TVC) of day 1 sample and 3 months sample were 8030 CFU/g and 20750 CFU/g respectively.

Thin Layer Chromatography (TLC) for Vishnukranthi chewable granule samples

There were three identical separate spots on day 1, 1 month and 3 months samples.

Table 3: Rf values of TLC fingerprint analysis of day 1 sample and 3 month sample

	Spot 1	Spot 2	Spot 3
Day 1 sample	0.046	0.165	0.224
1 month sample	0.047	0.165	0.224
3 months sample	0.047	0.165	0.224

Results indicate there was no significant difference between Rf values of day 1, 1 month and 3 months samples.

Stability of gastroprotective activity of Vishnukranthi chewable granules

Table 4 : Neutralizing capacity of samples of Vishnukranthi chewable granules on accelerated conditions

Sample	Mean value of end pH
Day 1	1.71±0.01
1 months after accelerated conditions	1.75±0.01
3 months after accelerated conditions	1.74±0.01

There is no significant difference of mean values of end pH of samples of Vishnukranthi chewable granules on day 1, 1 month and 3 months samples.

Neutralizing capacity of Vishnukranthi chewable granules using a titration method of Fordtran's model

Table 7 : Fordtran's model analysis of samples of Vishnukranthi chewable granules on accelerated conditions

Sample	**Mean value of consumed H^+ (mmol)**
Day 1	0.1493±0.0036
1 months after accelerated conditions	0.1598±0.0036
3 months after accelerated conditions	0.1683±0.0036

There is no significant difference of mean values of consumed H^+ of samples of Vishnukranthi chewable granules on day 1, 1 month and 3 months samples.

Conclusion:

Vishnukranthi chewable granules has demonstrated significant gastroprotective activity in both models. The granules were chemically stable under accelerated stability conditions but physical and microbiological stability need to be improved further. Granules were shifted towards to more fine range. Therefore proper binding agent should be used to reduce the degranulation during storage. Since Chewable granule dosage form was prepared using raw plant materials it was more prone to microbial contamination. Therefore, appropriate preservative should be used. Also day 1 samples were contained significantly higher amount of moisture. So granules should be dried more than recommended time.

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