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Gastroprotective activity of the paste of *Evolvulus alsinoides* L. (vishnukranti kalka) in rats

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Abstract

This study examined the gastroprotective activity of vishnukranti kalka (paste) which is recommended in Ayurveda for the treatment of peptic ulcers. The kalka was made by mixing the dry powder of the whole plant *of Evolvulus alsinoides* L with cow ghee, bee honey and common sugar (1:2:2:1 w/w). The gastroprotective activity was tested using the rat alcohol induced gastric lesion model following oral administration of either the kalka (1250 mg/kg, 2500 mg/kg, 5000 mg/kg), placebo-1 (5000 mg/kg of wheat flour, cow ghee, bee honey and common sugar), placebo-2 (5000 mg/kg of cow ghee, bee honey and common sugar), cimetidine (100 mg/kg) and distilled water. The results showed a strong and dose depended gastroprotective activity of vishnukranti kalka (in terms of length and number of macroscopical mucosal haemorrhagic lesions). Placebo-1 and placebo-2 also had gastroprotective activity but was less potent to kalka. Vishnukranti kalka significantly increased the pH of gastric juice and its mucus and carbohydrate contents. The kalka was well tolerated even with chronic administration with no renaltoxicity (in terms of urea and cratinine), hepatotoxicity (in terms of SGOT and SGPT) and haematotoxiciry (in terms of packed cell volume, red blood cell count, white blood cell count, and haemoglobin concentrations). It is concluded that vishnukranti kalka has strong and safe oral gastroprotective activity and the study also provides scientific evidence for the justification of its use as a gastroprotective agent in Ayurvedic medicine.

Key words: *Evolvulus alsinoides* L., kalka, paste, gastroprotective activity, peptic ulcer.

1. Introduction

Peptic ulcer disease is a common gastrointestinal disorder in many western countries and it still affects approximately 10% of all adults at some time in their

lives (Haslett *et al.,* 1999).In Sri Lanka, prevalence of peptic ulcer disease is 1.9% and its incidence is 1.4% (http://wrong diagnosis.com, 2003). Several drugs are recommended for the treatment of gastric ulcers in allopathic medicine (Haslett *et al.,* 1999). Although these drugs are effective, there are undesirerable side effects such as arrhythmias, impotency, gynaecomastia, (Bafna *et al.,* 2004), constipation, diarrhoea, nausea, vomiting, indigestion, gastric discomfort (Mehta et al., 2002). Further, most of these drugs are expensive. Therefore, there is need to develop safe and effective gastroprotective drugs which are affordable to people in developing countries including Sri Lanka. In this connection, we have scientifically tested the gastroprotective activity of *Evolvulus alsinoides* L. (vishnukranti) powder (Ratnasooriya *et al.,* 2005) which is the main ingredient of the paste of *E. alsinoides* (vishnukranti kalka) that is recommended in Ayurveda for peptic ulcer disease (Vidyasagara, 1920). Vishnukranti (VK) is formulated with cow ghee, bee honey and common sugar (sucrose). The results showed that *E. alsinoides* powder had safe and strong oral gastroprotective activity. But in clinical practice most of the patients do not prefer to swallow the *E. alsinoides* powder and indicate that there is rapid relief of burning sensation in epigastric region with the treatment of VK. However, the gastroprotective activity of VK is not scientifically investigated and proven. Therefore the aim of this study was to investigate the gastroprotective activity of VK and also its toxicity using rats. Interestingly vishnukranti also recommended for bowel complaints, dysentery, nervous debility, chronic bronchitis and asthma (Jayaweera, 1980).

E. alsinoides L. (Family: Convolvulaceae) is a common weed that grows in open and grassy places in tropical and subtropical countries including India and Sri Lanka. In Sri Lanka, this plant is widely distributed in dry and sandy grounds in low country. It is a perennial herb with small woody-branched roots with simple and alternate leaves. It's stem usually wiry with long spreading hair. Flowers are regular, bisexual, bright blue in colour, solitary or paired with short filiform peduncle. Petals 5, fused into a nearly rotate corolla about 9mm in diameter. Flowers occur throughout the year (Jayaweera, 1980).

2. Materials and methods

Collection of the plant and other materials

Fresh *E. alsinoides* plants were collected from a government plot at Ussangoda in Hambantota district in August 2002. The fresh plants were immediately brought to the department of Zoology, University of Colombo, washed in tap water and shade dried for one week. The plant was authenticated by Mrs S. Sugatadasa, Botanist, Bandaranayake Memorial Ayurvedic research institute, Navinna where the voucher specimen (No: Acc 1006 a) was deposited.

Bee honey (J.A.Wilsons, Rajagiriya, Sri Lanka), cow ghee (Highland products, Ambewela, Sri Lanka), common sugar ("Satosa" products, Sri Lanka) and wheat flour (Ceylon Agro-industries, Seeduwa, Sri Lanka) were purchased during the same time period.

Preparation of VK, placebo-1, placebo-2 and control

The dried plants were finely powdered using a grinding machine (No 0509, model FFC -23 A, Shanhi, China) at 5800 rpm for 5 - 10 minutes (yield 94% w/w). Fixed weight of fine brownish powder was mixed with double the quantity of bee honey, cow ghee and equal quantity of common sugar and grounded into a homogenous paste of VK using motor and pestle. Equal weight of wheat flour was mixed with double the quantity of cow ghee, bee honey and equal quantity of common sugar was grounded into a homogenous paste using motor and pestle (Placebo-1). Similarly equal quantity of cow ghee, bee honey and common sugar was mixed in same proportions and grounded into a homogenous paste using motor and pestle (Placebo-2). Two milliliters of distilled water (DW) was used as the control.

Animals

Healthy adult Wistar rats (weight 200±50g) purchased from Medical Research Institute, Colombo were used. Animals were acclimatized for one week under standardized animal house conditions (temperature 28 - 30 ° C, photo period: approximately 12 h day light and 12 h dark, humidity: 50 - 55%). with free access to pelleted food (Vet house Ltd, Colombo, Sri Lanka) and tap water.

Administration

Three doses of VK (5000 mg/kg, 2500 mg/kg, 1250 mg/kg) were made in 2 ml of DW, 1 dose of placebo-1 (5000 mg/kg) in 2 ml of DW, 1 dose of placebo-2 (5000 mg/kg) in 2 ml of DW and 1 dose of cimetidine (100 mg/kg) in 2 ml of DW and 2ml of DW were given orally for seven groups of rats. The highest dose selected was ten times of the usual dose recommended for herbal paste (kalka) by Ayurvedic physicians (Vidyasagara 1920).

Evaluation of the effect of VK on gastroprotective activity

Food was withheld for 36 h and water for 24 h in 68 rats before the commencement of the experiment. The rats were divided into seven groups and treated orally in the following manner. Rats in group 1 (n= 14/group) with 2ml of DW, 2 with 5000 mg/kg of placebo-1 (n=8/group), group 3 with 5000 mg/kg of placebo-2 (n= 8/group), group 4 with 5000 mg/kg of VK (n= 14/group), group 5 with 2500 mg/kg of VK (n=8/group), group 6 with 1250 mg/kg of VK (n=8/group) and group 7 with

100 mg/kg of cimetidene, the reference drug (n=8/group). Thirty minutes later, gastric lesions were induced using absolute ethanol (Fluka chemical Co, Buchs, Switzerland) as described by Robert (1979). These animals were killed Ih after administrating with an over dose of ether. There stomachs were excised and slit open along the grater curvature and pined on to a ridgeform sheet (Richard Peris Company, Maharagama, Sri Lanka). The number and length of macroscopic mucosal hemorrhagic lesions in the glandular portion were determined and summed up per stomach.

Evaluation of the histopathology of stomach mucosa

After macroscopic examination, parts from the glandular portion of the stomachs (from control, placebo - 1 and VK 5000 mg/kg treated animal groups) were fixed in Bouin's solution for three days. Tissues were thoroughly washed in 70% alcohol and dehydrated 1-2 h in 95% ethanol and 1-2 h in absolute ethanol, again for 1-2 h in absolute ethanol. These tissues were cleared in xylene (1-2 h). These were embedded in paraffin wax 1 1/2 - 2 h and sections (5 μm) were cut using rotary microtome (LR85, Yamato kohki industrial Co Ltd, Tokyo, Japan). Tissues were stained using heamotoxalin (Fluka chemika, Buchs, Switzerland) and alcohol soluble eosin (BDH chemical Ltd, Poole, England) as described by Humanson (1962). The gastric mucosae were observed microscopically for pathological changes at 400 magnifications using a photomicrographic microscope (Microflex AFX-DX, Nikon Cooperation, Tokyo, Japan).

Assessment of quantity of mucus adhered to gastric mucosa.

The quantity of mucus adhered to gastric mucosa was assessed in orally treated rats (n=8/group) either with 2 ml of DW or 5000 mg/kg VK using alcian blue (SGX, Sigma, St.Louis, USA) technique as described by Corn (1974).

Determination of gastric juice volume, pH, and total acidity

Sixteen rats were divided into two equal groups (n=8/group) and one group was orally treated with 2 ml of DW and other with 5000 mg/kg VK. Thirty minutes later, animals were anesthetized with ether and mid ventral incision in the upper abdominal region was made with aseptic precautions. The pylorus was legated without causing any damage to the blood supply. Animals were then sutured and kept 4 h without access to water. The animals were killed with ether and the gastric content was collected (Ratnasooriya *et al.*, 1995). This was centrifuged at 4000 rpm 10 minutes and the supernatant was pipetted out and its volume was measured. The pH was determined by using a pH meter (TOA electronics, Tokyo, Japan). Total acidity of the supernatant of the gastric juice was measured tritrometrically using methyl orange and phenol red as indicators as directed by Varley (1962).

Estimation of the amount of carbohydrate in gastric juice

Supernatant of the gastric juice collected from 2.8 was used to evaluate the carbohydrate quantity as described by Nair (1976).

Evaluation of chronic toxicity

Eighteen rats were divided into two equal groups (n=9/group) and one group was orally administered 2ml of DW and the other group was 5000 mg/kg of VK for 42 consecutive days between 12.00-13.00 h. These rats were observed for overt signs of toxicity (diarrhoea, restlessness, salivation, rhinorrhoea, lacrimation, ptosis, tremor, yellowing of fur, loss of hair, and pallor of lips), aversive behaviors (biting, scratching of body, licking of tail and paws) 30 minutes after each administration. On day one post treatment, blood was collected from the tail under light ether anesthesia using aseptic precautions and was allowed to clot at room temperature (28°-30 °C). The serum was separated and some serum parameters [serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), urea, creatinine, triglycerides, cholesterol and fasting blood glucose] were determined using respective Randox assay kits (Randox laboratories, Antriam, UK) and a spectrophotometer (V 500, Jasco Co operation, Tokyo, Japan).

On day two post treatment, blood was collected from the tail and the anticoagulant EDTA (1 mg/ml) was added (Marcus 1966). Some serum parameters [packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, and haemoglobin levels] were determined using standard techniques as described by Ghai (1993).

Immediately after the collecting of blood, rats were weighed (MP Chyo Cooperation, Tokyo, Japan) and then killed with an overdose of ether. Heart, liver, kidney, spleen and adrenal glands were removed, blotted free of blood and weighed using an electronic balance (Shimadzu Cooperation, Tokyo, Japan).

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was made using Mann Whitney- U Test. Significance was set at $P<0.05$.

3. Results

Evaluation of the gastroprotective activity

This study clearly demonstrates that VK can protect the gastric mucosa against 100% absolute ethanol. As shown in Table 1, VK had a dose depended ($r^2=0.99$, $P<0.05$) gastro protective activity. The inhibitions of the ethanol induced gastric lesions in the mid and high doses were by 91% and 100% respectively. The reference drug, placebo- 1 and placebo-2 also showed gastroprotection, but to a

lesser degree (in length by 84% and number by 56% in reference drug, in length by 84% and number by 53 % in placebo- 1 and in length by 89% and number by 70 % in placebo -2).

Evaluation of histopathological changes of gastric mucosa

Appearance of the gastric mucosa or in the gastric glands of the VK treated rats was essentially normal with no gastric damage in the mucosal epithelium or in the gastric gland. On the other hand, gastric mucosal cells in control rats were severely damaged and some cells were moderately exfoliated. In rats of placebo- 1, a similar patterns of damage was visible in the gastric mucosa but was to a lesser degree.

Assessment of quantity of mucus adhere to gastric mucosa

The highest dose of VK significantly ($p<0.05$) increased (by 27.7%) the gastric mucus content (control vs. treatment 274.9 ±13.3 vs. 380 ± 19.2 µg alcian blue /stomach).

Determination of gastric juice volume, pH and total acidity

Compared to control, the highest dose VK did not significantly ($p>0.05$) changed the gastric juice volume (control vs. treatment 2.9 ± 0.3 vs. 3.1 ± 0.1 ml) and total acidity (control vs treatment 0.09 ± 0.007 vs. 0.07 ± 0.003 mol $^{-}$L). In contrast, the highest dose of VK significantly ($p<0.05$) increased the pH of gastric juice (control vs. treatment 1.6 ±0.8 vs. 2.9 ±0.1).

Estimation of the amount of carbohydrate in gastric juice

The highest dose of VK significantly ($p<0.05$) increased (by 80%) the carbohydrate content of gastric juice (control vs. treatment 0.01 ±0.1 vs. 4.6 ± 0.4mg/dl).

Evaluation of chronic toxicity

Administration of the highest dose of VK did not produce overt signs of toxicity in chronically treated rats through out the study. Further, the treatment neither suppressed the body weight nor organ weights nor altered any of the haematological parameters investigated significantly (data not showed). VK treatment slightly but not significantly ($p>0.05$) reduced the SGOT (by 10.5%), SGPT (by 14.2%), urea (by 2.9%), cholesterol (by 6.1%) and fasting blood glucose (by 4.9%). On the other hand, serum triglyceride level was significantly ($p<0.05$) reduced (by 10.5%) by VK (control vs. treatment 100.7 ±1.9 vs. 90.1 ± 2.9mg/dl).

4. Discussion

The results of present study demonstrate that VK possesses strong oral gastroprotective activity (in terms of length and number of macroscopic mucosal heamorrhagic lesions). This effect was dose depended. The materials used in the

formulation of kalka (cow ghee, bee honey and common sugar) by itself and the reference drug cimetidine, H_2 receptor blocker (Yamamoto *et al.,* 1994) also showed marked gastroprotective activity but it was less potent compared to VK. The results of the study also indicated that gastroprotective activity of VK was mildly superior (by 12.3 %) to the vishnukranti powder when orally administered to rats (Ratnasooriya *et al.,* 2005).

VK did not alter the volume of the gastric juice and its total acidity. However, VK significantly increased the mucus adhered to the gastric mucosa, gastric pH and the carbohydrate content of the gastric juice. It is well recognized that over production of gastric acid and decrease in gastric protective mechanisms contribute the formation of gastric ulcers (Dharmani *et al.,* 2004). Thus it is possible that increase in mucus and carbohydrate content and gastric pH induced by VK may have contributed to it's gastroprotective activity.

Helicobacter pylori are implicated with the pathogenesis of gastric lesions (O' corner *et al.,* 1994). Interestingly, recent *in vitro* studies conducted by us have revealed that vishnukranti powder has promising bactericidal activity against human *H. pylori* (when evaluated by dise diffusion method and kill curve) (Fernando *et al.,* 2006). It is also likely that this mode of action of vishnukranti powder may also have played pivotal role in inducing gastroprotective activity by VK.

Other than these, oxygen derived free radicals play an important role in the pathogenesis of gastric lesions (Vanisree *et al.,* 1996). Vishnukranti powder had marked antioxidant activity when evaluated with Theobarbituric acid relative substances (TBARS) assay for antioxidant properties (Ratnasooriya *et al.,* 2005). In addition, bee honey also possesses marked antioxidant activity (http://www.nutraingredients.com, 2001) which could have increase the antioxidant potential of VK. So it is possible that this combine antioxidant activity may also have contributed to the gastroprotective activity of VK.

VK was well tolerated with no overt signs of toxicity, liver toxicity (by SGOT and SGPT levels), renal toxicity (by serum creatinine and urea levels), haemotoxicity (by RBC and WBC counts, haemoglobin levels and PCV) in chronically treated rats. Other than this VK also induced moderate reduction in serum tryglycerides and did not increase the serum glucose level though it contains cow ghee, bee honey and common sugar. Collectively, these observations suggest that VK is a safe and cheap gastroprotective agent which may be beneficial even to diabetic and hyperlipidemic patients.

In conclusion, this study for the first time, provide experimental evidence for the presence of safe and marked oral gastroprotective activity of VK and indicate its potential to be used in the treatment of peptic ulcers by Ayurvedic practitioners. The sweet taste of VK may increase the patient compliance.

Table 1: Effect of orally administered paste of *Evolvulus alsinoides* (VK) on ethanol induced gastric lesions in rats (means ± SEM)

Treatment	Number (N)	Length of lesions (mm)	% Inhibition (length)	Number of lesions	% Inhibition (number)
Control (2 ml DW)	14	87.0±4.9	-	7.7±0.4	-
Placebo- 1 (5000 mg/kg)	8	13.7±1.9	84.3	3.6±0.6	53.2
Placebo-2 (5000mg/kg)	8	9.8±1.6	88.7	2.3±0.3	70.1
VK(1250 mg/kg)	8	72.3±5.2	16.8	7.3+0.4	5.1
VK (2500 mg/kg)	8	8.2±1.5*	90.5	3.0±0.7 *	61.0
VK (5000 mg/kg)	14	Q*[ab]	100.00	0*[ab]	100.00
Cimetidine (100 mg/kg)	8	13.6±0.8	84.3	3.4±0.3	55.8

Statistical significant determined at * $p<0.05$. DW - Distilled water
(*) as compared with control. ([a]) as compared with placebo-2. ([b]) As compared with cimetidine. Inhibition % is expressed against the control, placebo-1 = wheat flour, cow ghee, bee honey, common sugar, placebo-2 =cow ghee, bee honey, sugar, control = DW.

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