Protective effect of vilva juice on glycoconjugate levels in experimentally induced constipation in rats

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Received 9 February 2004; revised 29 June 2004

Efficacy of vilva, a polyherbal formulation was evaluated in morphine induced constipated rats. Vilva juice, at a dose of 1.5 ml/100 g body wt was given orally for a period of 7 days. Morphine sulfate was injected to induce constipation on 8th day, 45 min before the experiments. Protein bound glycoconjugates were estimated in intestinal tissue. Altered levels of glycoconjugates were maintained at near normalcy when pretreated with vilva juice in morphine induced rats. Histological changes were observed in the colon tissue. The damage to crypts of Lieberkunn in constipated rats were found to be reduced in vilva pretreated rats. Vilva, thus, offered significant protection against morphine induced constipation by way of augmenting mucus secretion.

Keywords: Constipation, Glycoconjugates, Morphine sulfate, Vilva juice

IPC Code: Int Cl.7 A61P

Constipation is a sluggish action of bowels usually with difficulty in evacuation. The symptom of incomplete evacuation is unreliable, since rectal sensation is extremely variable and the rectum can accommodate itself to a wide range of stool volumes before a desire to defecate is appreciated. Constipation is actually a symptom and not a disease. Certain obstructive bowel diseases such as carcinoma, hernia, rectal prolapse, solitary rectal ulcer, volvulus may be responsible for chronic constipation. In this study, constipation was induced by morphine sulfate, a narcotic analgesic, which exerts its constipating effect by inhibiting intestinal transit. Morphine increases tone and reduces motility in many parts of gastrointestinal system.

Through the ages, India's myriad herbs, spices and plants have played a role in the accumulation of ancient medical knowledge. Vilva (trade name) is a polyherbal formulation consisting of Aegle marmelos, Glycyrrhiza glabra, citrus aurantifolia, Elettaria cardamomum, Saccharum officinarum and Rosa damascena. These ingredients have digestive and laxative actions as reported in Ayurvedic literature. Aegle marmelos (Bael) is used, especially in Indian Medicine for constipation. Elettaria cardamomum promotes digestion and is administered with purgatives. Saccharum officinarum is useful in healing ulcers of the mucous membrane. It has digestive and laxative properties as well. Glycyrrhiza glabra (Liquorice), the various preparations of which is used in cough, gastric and duodenal ulcers and dermatitis. Hence, an attempt has been made to reveal the protective role of vilva in ameliorating constipation.

Drugs and chemicals—Vilva, vilvam herbal juice was obtained from Rumi Herbals Pvt. Ltd., Chennai, India. Morphine sulfate was purchased commercially. All other chemicals used in this study were of analytical grade.

Animals—Female Wistar rats weighing 100-150 g obtained from Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai, India and were fed with standard pelleted diet (M/s. Hindustan Lever Limited, Bangalore, India) and water ad libitum. The animals were housed under standard environmental conditions. All animal experiments were carried out as per the guidelines provided by Institutional Animals Ethics Committee (IAEC). Animals were divided into four groups of six animals each. Group I served as normal control; Group II received vilva juice (1.5 ml/100g body weight) for 7 days orally; Group III (constipated) rats received morphine sulfate (3.2 mg/kg body weight; SC) 45 min prior to sacrifice; and Group IV rats were pretreated with vilva juice (1.5 ml/100 g body weight) for 7 days orally and constipated by Morphine sulfate (as in group III).

The animals were sacrificed on day 8 and the intestinal tissue was dissected out immediately and homogenized in Tris HCl buffer (pH 7.4) and used for estimation of glycoconjugates namely hexose, hexosamine, fucose, sialic acid. Intestinal mucosal studies were carried out using alcian blue as described by Corney et al.
Histology—Pieces of colon were collected and washed with physiological saline, then fixed in formalin (10%) for 24 hr and processed by paraffin techniques. Sections of 5 μm thickness were cut and stained with hematoxylin - eosin. Statistical analysis—Data was subjected to Student's unpaired t-test. Values were considered significant at P<0.05.

Table 1 shows the levels of glycoconjugates and mucus content in the intestine of control and experimental group of rats. A significant decrease in the level of hexose, hexosamine and sialic acid (P<0.001) and fucose (P<0.05) was observed in group III morphine administered rats as compared to normal control. No significant changes were observed in group II rats as compared to group I rats. In case of vilva pretreated (group IV) rats, the levels of these glycoconjugates were maintained at near normal levels as compared to group I. A significant increase in the level of hexose, hexosamine, fucose and sialic acid was noted in group IV rats when compared with group III rats (P<0.001). The mucus content in group III rats were decreased as compared to group I rats (P<0.001). But in case of vilva pretreated rats, group IV showed significant increase in mucus content in comparison with Group III.

Membrane glycoprotein are the molecules composed of covalently linked protein with carbohydrate which apparently have an important role in membrane structure and transport process. Glycoprotein has been shown to be the preferential target for free radicals. Due to oxidative assault, there was an increase in peroxidation byproducts during morphine administration which might have prevented the membrane damage by scavenging the free radicals. Antioxidant activities are present in herbal preparation which might have prevented the membrane damage by scavenging the free radicals. Antioxidant action of *Aegle marmelos* is well reported. Glycyrrhiza glabra stimulates and/or accelerates the differentiation to glandular cell formation and secretion in stomach leading to enhanced mucous secretion.

The results obtained from the present study indicated that vilva pretreatment offered significant protection to intestinal mucosa against morphine induced damage.
Fig. 1—(A-D) shows the histological changes in the colon of experimental group of rats. (H & E 100x). (A) Colon showing the normal architecture, with normal crypts of Liberkunn and goblet cell population in Group I rats; (B) Colon showing normal architecture of the intestine as compared to control in Group II rats; (C) Constipated: Colon showing shortening of crypts of Liberkunn with marked ulceration and polymorphonuclear cell infiltration. Stasis of the undigested food material (UFM) in the lumen was also observed; and (D) Colon showing the section of drug pretreated rat with restricted damage to crypts and less number of goblet cells. No stasis of the food particle was seen and absence of inflammatory cell infiltration.
peroxidative damage. The exact mechanism by which vilva reduced the goblet cell population, restricted histological damage is undergoing further research.

The author KES, acknowledges the financial support rendered by CSIR, New Delhi.

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