Histological changes in intestine in semichronic diarrhoea induced by lactose enriched diet in rats: Effect of Diarex-vet

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Diarrhoea is regarded as the characteristic symptom of intestinal disturbances. Lactose, a disaccharide present in mammalian milk, is hydrolysed by β-galactosidase (lactase) localised on the brush border of small intestine. Lactase hydrolyses lactose to monosaccharides namely, glucose and galactose which are then actively absorbed into the circulation. Intestinal lactase activity in mammals is high at birth but begins to decline around weaning and reaches very low levels in adult life. The decrease in activity of lactase is genetically programmed and is referred to as “primary lactase deficiency”. The deficiency of lactase leads to symptoms of lactose malabsorption such as diarrhoea, bloating and flatulence. These symptoms originate from fermentation of undigested lactose entering colon resulting in osmotic diarrhoea. Osmotic diarrhoea occurs when inadequate nutrient absorption or malabsorption results in a collection of solutes in the gut lumen, which cause water to be retained by their osmotic activity. This physiological phenomenon of lactase deficiency and lactose intolerance is ideally exploited to serve as a model of diarrhoea to evaluate antidiarroheal drugs, though the histological evaluation of such models remain inadequate. In the present study histological response in ileum, caecum and colon in lactose induced semichronic diarrhoea was evaluated. The responses of the same in effect to a herbal antidiarroheal Diarex-Vet are highlighted.

Materials and Methods
Lactose monohydrate (C_{12}H_{22}O_{11}·H_2O) from Acros Organics and Diarex-Vet from The Himalaya Drug Company were used. Each 10 g powder of Diarex-Vet contained extracts of Holarrhena antidysenterica (1.59 g); Tinospora cordifolia (0.103 g); and powders of Aegle marmelos (1.59 g); Punic granatum (0.352 g); Cyperus rotundus (0.331 g); Bombay celiba (0.454 g); and Aconitum heterophyllum (0.454 g).

Animals—Inbred Wistar male rats weighing between 250-275 g were used. The animals were maintained as per the guidelines of National Research Council. Care was taken in accordance to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institute of Health. The Animal Ethics Committee of the Institute approved the experimental protocol. Synthetic pelleted feed (Lipton India Limited, Mumbai) and water were provided ad libitum throughout the study period.

Experimental design and methods—A total of 60 male rats were randomized into 5 groups of 12 animals each and the treatment schedule followed was as hereunder:
Group I: Received standard commercial pellet feed and served as control.

Group II: Received lactose mixed diet for 7 days and served as the model for semichronic diarrhoea.

Group III, IV and V: Served as treatment groups and were given the same diet as in Group II in addition to Diarex-Vet at a dose of 250, 500 and 750 mg/kg body weight respectively, as an oral aqueous suspension.

The rats were fasted overnight and were then fed with a combination diet of commercially available feed and lactose to induce semichronic diarrhoea. The combination diet was fed to the treated groups for 7 days. The treatment with Diarex-Vet in groups III, IV and V was started from the 3rd day onwards. The animals were euthanized at the end of 5 days of treatment and organs were collected for histological evaluation. Faecal examination of the rats was done routinely to rule out any infectious causes of diarrhoea.

**Histology**—Pieces of ileum, caecum and colon were collected after flushing with normal saline and then fixed in 10% neutral buffered formalin and processed by paraffin technique. Sections of 5µm thickness were cut and stained with Haematoxylin-Eosin method.

**Semi-quantitative analysis**—Semi-quantitative analysis of the goblet cell activity was done by counting the number of goblet cells per high power field.

**Statistical analysis**—The data of the semi-quantitative analysis was expressed as mean ± SE and the statistical significance was ascertained using Student’s t test. The minimum level of significance was fixed at $P \leq 0.05$.

**Results**

The induction of diarrhoea as observed by the examination of faecal mass was complete by 36-48 hr. Faecal examination ruled out any infectious causes of diarrhoea. Cage side observations did not reveal any evidence for abnormal clinical signs except for the matting of the hair around the hind quarters due to watery diarrhoea. The rats were alert and active throughout the study period.

**Light microscopy**—In group I, portions of the small and large intestine showed normal structural and architectural intactness. In group II ileum appeared disrupted with goblet cell hyperplasia and polymorphonuclear cell infiltration in the lamina propria (Fig. 1). Mucosa of caecum and colon showed severe congestion and presence of inflammatory cells (Figs 2 and 3). The mucosa and muscularis mucosal layers of the ileum, caecum and colon appeared disrupted resulting in loss of structural alignments. In groups III, IV and V the above described histological changes were restricted and showed a dose dependent improvement with maximal efficiency by 750 mg/kg body wt dose of Diarex-Vet (Figs 4, 5 and 6).

**Semi-quantitative analysis of goblet cell**—The goblet cell hyperplasia in ileum, caecum and colon were significantly high in groups II, III and IV as compared to group I (Table 1). No statistical differences were observed between group V and group I though the values in group V remained apparently high.

**Discussion**

Lactose malabsorption is characterized by a deficiency of mucosal lactase. As a consequence undegraded lactose reaches the colon where it is broken down by bacteria to short chain fatty acids, carbon dioxide and hydrogen which results in bloating, osmotic diarrhoea and other symptoms similar to irritable bowel syndrome. However, these reports lacked the anticipated histological changes in the intestines.

The histological changes in ileum in the present study were similar to the observations recorded by Amend et al. who reported hyperplastic mucosa with submucosal accumulation of inflammatory cells. The short term exposure of lactose in the present

| Table 1 — Semi-quantitative analysis of goblet cell (number per high power field) activity [Values are mean ± SE of 12 observations] |
|---|---|---|---|---|---|
| Organs | Groups | I | II | III | IV | V |
| Ileum | 13.92±0.78a | 18.17±1.34b | 18.67±1.34b | 17.83±1.08b | 14.83±0.95b |
| Caeceum | 11.83±1.13b | 41.25±1.56b | 38.75±2.51b | 23.92±1.82b | 15.08±1.42b |
| Colon | 12.92±0.98b | 18.50±1.48b | 17.58±1.47b | 16.17±1.06b | 15.50±1.08b |

Means bearing the same superscript between rows do not vary significantly and those bearing different superscripts (a, b and c) vary significantly ($P \leq 0.05$).
Figs 1-6—(1)—Ileum showing goblet cell hyperplasia and polymorphonuclear cell infiltration in Group II (H & E, 1000×); (2)—Caecum showing congestion, infiltration of polymorphonuclear cells and increased goblet cell hyperplasia in Group II (H & E, 1000×); (3)—Colon showing increased goblet cell activity in Group II (H & E, 250×); (4)—Ileum showing restricted goblet cell activity and absence of inflammatory cell infiltration in Group V (H & E, 1000×); (5)—Caecum showing intactness of villi and reduced goblet cell activity in Group V (H & E, 250×); and (6)—Colon section showing restricted damage to villi and less number of goblet cells in Group V (H & E, 250×)
The effectiveness of Diarex-Vet in the present study may be attributed to the activities analogous to enzyme lactase or hindrance of the osmotic process, which draws water into the intestinal lumen contributing to diarrhoea. It is anticipated that use of Diarex-Vet in osmotic diarrhoea can be extended in the treatment of milk intolerance and as an adjuvant therapy in infectious causes of diarrhoea caused by Coronavirus, Rotavirus or Cryptosporidia where most of the clinicopathological changes were observed because of malabsorptive osmotic diarrhoea\textsuperscript{21}.

The exact mechanism by which Diarex-Vet reduced the goblet cell activity, restricting the histological damage is undergoing further research. Further investigations in this regard are done keeping in view the pharmacological activities of the individual ingredients of the polyherbal anti diarrhoeal Diarex-Vet. The active ingredients of the herbal formulation Diarex-Vet have been effectively used in treatment of indigestion, flatulence and diarrhoeas of varying etiologies including bacterial, fungal and protozoal. Some of the published reports on the ingredients of Diarex-Vet as reported by Khory and Katrak\textsuperscript{22} include antidiarrhoeal and antisyndromic properties associated with Halarrhena antidysenterica. Chopra et al.\textsuperscript{23} reported the usefulness of Tinospora cordifolia in chronic diarrhoea and dysentery. Das et al.\textsuperscript{24} reported the astringent activity of Punica granatum in the cases of diarrhoea and dysentery. Antidiarrhoeal property of Aegle marmelos in castor oil induced diarrhoea in rats was reported by Pandey\textsuperscript{25}. However, published research on histomorphological effects of herbal antidiarrhoeals is inadequate. Further work needs to be done to histologically standardise antidiarrhoeal models using herbal compounds, which are fast regaining their importance.

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