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

Mitra, S.K., Ashish Sachan, Venkatesha Udupa and Seshadri, S.J.
R&D Center, The Himalaya Drug Company, Makali, Bangalore, India
and Jayakumar, K.
Department of Pharmacology and Toxicology, Veterinary College, Bangalore, India.


SUMMARY
Efficacy of Diarex Vet (The Himalaya Drug Company, Makali, Bangalore, India) was evaluated histologically in semichronic diarrhoea induced by lactose-enriched diet in rats. The rats in different groups were given lactose enriched diet alone for 2 days before starting the treatment with Diarex Vet at a dose of 250, 500 and 750 mg/kg body weight along with lactose enriched diet for 5 days. Animals were euthanised at the end of 5 days of treatment and histological changes were observed in the ileum, caecum and colon. Semiquantitative analysis of goblet cells in intestines showed dose dependent response among the treated groups. The morphological changes were comparable to normal in the group given 750 mg/kg body weight Diarex Vet. The effects observed were attributed to the lactase like analogous activity of Diarex Vet or the inhibition of the intestinal lumen thereby reducing the morphological changes in the intestines.

Diarrhoea is regarded as the characteristic symptom of intestinal disturbances. Lactose, a disaccharide present in mammalian milk, is hydrolysed by b-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 maldigestion 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 antidiarrhoeal drugs, though the histological evaluation of such models remains 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 antidiarrhoeal 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 Holrrhena antidysenterica (1.59 g); Tinospora cordifolia (0.103 g); and powders of Aegle...
marmelos (1.59g); Punic granatum (0.532 g); Cyperus rotundus (0.331 g); Bombax ceiba (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 euthanised 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 5 µm thickness were cut and stained with Hematoxylin-eosin method.

Semiquantitative analysis: Semiquantitative 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 semiquantitative 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 ≤0.05.

RESULTS
The induction of diarrhoea as observed by the examination of fecal mass was complete by 36-48 hr. Faecal examination ruled out any infectious causes of diarrhea. 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 (Figure 1). Mucosa of caecum and colon showed severe congestion and presence of inflammatory cells (Figures 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 efficacy by 750 mg/kg body weight dose of Diarex Vet (Figures 4, 5 and 6).

**Semiquantitative 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.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
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<tbody>
<tr>
<td></td>
<td>(Values are mean ± SE of 12 observations)</td>
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<tr>
<td>Ileum</td>
<td>13.92 ± 0.78a</td>
<td>18.17 ± 1.34b</td>
<td>18.67 ± 1.34b</td>
<td>17.83 ± 1.08b</td>
<td>14.83 ± 0.95a</td>
</tr>
<tr>
<td>Caecum</td>
<td>11.83 ± 1.13a</td>
<td>41.25 ± 1.56b</td>
<td>38.75 ± 2.51b</td>
<td>23.92 ± 1.82c</td>
<td>15.08 ± 1.42a</td>
</tr>
<tr>
<td>Colon</td>
<td>12.92 ± 0.98a</td>
<td>18.50 ± 1.48b</td>
<td>17.58 ± 1.47b</td>
<td>16.17 ± 1.06b</td>
<td>15.50 ± 1.08a</td>
</tr>
</tbody>
</table>

Means bearing the same superscript between rows do not vary significantly and those bearing different superscripts (a, b and c) vary significantly (p 0.05)

**Figures 1-6:** (1) Ileum showing goblet cell hyperplasia and polymorphonuclear cell infiltration in group II (H&E, 1000x); (2) Caecum showing congestion, infiltration of polymorphonuclear cells and increased goblet cell hyperplasia in Group II (H&E, 1000x); (3) Colon showing increased goblet cell activity in Group II (H&E, 250x); (4) Ileum showing restricted goblet cell activity and absence of inflammatory cell infiltration in Group V (H&E, 1000x); (5) Caecum showing intactness of villi and reduced goblet cell activity in Group V (H&E, 250x); and (6) Colon section showing restricted damage to villi and less number of goblet cells in Group V (H&E, 250x).
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 bacterial to short chain fatty acids, carbon dioxide and hydrogen, which results in bloating, osmotic diarrhoea and other symptoms similar to irritable bowel syndrome\textsuperscript{13-16}. 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 \textit{et al.}\textsuperscript{17} who reported hyperplastic mucosa with submucosal accumulation of inflammatory cells. The short-term exposure of lactose in the present study resulted in histological changes similar to 14 days lactose administration to rats in a study conducted by Galvez \textit{et al.}\textsuperscript{18} who reported colonic mucosal hyperplasia and reduced average cell size in lactose fed rats. However, the cytopathological alterations reported by these authors could not be anticipated in the present study, which could be due to the differences in the duration of exposure of lactose.

Feed additives, drugs and other chemicals are known to influence the lower gastrointestinal tract under some defined conditions resulting in morphological alterations in the mucosa. The changes in caecum and colon observed in the present study were similar to the observations of Newberne \textit{et al.}\textsuperscript{19} according to whom the caecal enlargement and variable hyperplasia of the colon mucosa in animals exposed to lactose is due to incomplete absorption of lactose in the small intestine and subsequent microbial metabolism in the caecum and colon.

Histological changes in the caecum in the present study were in concurrence with the findings of Tellez \textit{et al.}\textsuperscript{20} who reported a marked reduction in lamina propria thickness and subjective epithelial cell proliferation in chicks following either 14 or 19 days of lactose administration. In addition, severe inflammatory cell infiltration, goblet cell hyperplasia and yperemia were observed. Possibility of an infective aetiology had also been excluded by carrying out aerobic and anaerobic cultures for micro-organisms. The supporting references for these changes were found to be not available.

The semiquantitative analysis of the goblet cells revealed that morphologically the ileum, caecum and colon in group V were similar to group I. Also in the caecum significant reduction was observed in groups IV when compared to groups II and III. The effective dose at which the goblet cell activity was found to be statistically similar was 750 mg/kg body weight of Diarex Vet.

Overall, the morphological changes int eh ileum and caecum showed a dose dependent decrease in the intensity of morphological alterations as also supported by semiquantitative analysis. The choice of the portions of the intestines for histological evaluation was based on the gross morphology as in caecum extensive distension due to the accumulation of gas was observed. Ileum and caecum being in the immediate proximity were hence also considered for histological evaluation. The morphological alterations in the other portions of the intestines such as duodenum, jejunum and rectum are also anticipated and further studies in this regard are in progress.

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, Rotaviurs or Cryptosporidia where
most of the clinicopathological changes were observed because of maldigestive osmotic diarrhoea21.

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 antidiarrhoeal 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 Katrak22 include antidiarrhoealand ant dysenterica properties associated with Holarrhena antidysenterica. Chopra et al.23 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 Pandey25. However, published research on histomorphological effects of herbal antidiarrhoeals is inadequate. Further work needs to be done to histologically standardize antidiarrhoeal models using herbal compounds, which are fast regaining their importance.

Acknowledgement
The authors are thankful to Ms. T.J. Vidya for formulation of Diarex Vet.

References


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