Experimental Study of Disciplined Regular Diet on Diseases of Purushvaha Srotas in Mice

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ABSTRACT
By considering the beneficial references concerning with disciplined diet which are mentioned in Ayurveda viz, Charak Samhita, Sushruta Samhita, yogaratnakara and Sharandrha Samhita and also about the laboratory approved diet for animals, three diets were formulated which are convenient for animals, which are as follows, Diet-1: Lab approved diet, content of 10 kg mixture which includes, Wheat flour: 6 kg, maize flour: 3 kg, fibre husk: 500 g, Sugar: 500 g, Salt: 100 g, mineral powder (Agrimine forte, Galaxo, INDIA): 1000 g. Diet-2: Sarvarasahara (Disciplined regular diet mentioned in Ayurveda), which contains, Vilepi: It is a form of paste or homogeneous mixture of nutrient flours of Yava (malt), Saindhava (rock salt), Shalishashtika (old rice), Tuvar dal (pea), Msoora (Lentil) spiced or zest with Ghrita, Saindhava (Rock salt), Jira (Cuminum cyminum), Ardakra (ginger) and garnished with fresh leaves of Dhanyak (coriander), Rasayana: It is a sort of best tonic mentioned in Ayurveda i.e. the mixture of Ghrita and Godugdha (cow's milk) and Diet-3: Ekarasahara (continuous use of same diet). Histological changes were observed after 15 days of stoppage of medicine which are taken as causative factors for inducing the symptoms coated into Purushvaha Srotodushi lakshana, mainly constipation and diarrhoea. It was observed that Group- II and Group- III showed large ulcers some superficial as well deep nidus of inflammation, infiltration of neutrophils was more in comparison to Group- IV and V, blunting of the villi as well as loss of villi compared than Group- IV and Group- V respectively. Thus, it can be suggested that maintenance of the disciplined diet as mentioned in “Charak Samhita” facilitates better healing to the intestinal mucosa injury induced both in constipation as well as in diarrhoea.

Keywords: Ayurveda, Sarvarasahara, Vilepi, Rasayana, Ekarasahara Histology, Neutrophils.

INTRODUCTION
There is an ancient belief that, “What we eat, that we produce.” All prime scriptures of Hindu mythology quotes that existence of every living being is only because of food which is in turn a dependent product of highly specific ladder of Panchmahabhuta. Ayurveda also proves the sayings of Vedic and Ayupanaishdhika literature in very scientific and logical way. Aaycharya Charaka had stated the importance of daily routine diet i.e. Pathya Aahara. In Agrya Sangrah he suggested that the complete diet Sarvarasahar is the only prime thing which can relieve the illness and rejuvenate the health, while incomplete or similar kind of diet Ekarasahar is the prime cause of illness. Further he states that, diet is the only factor, which is responsible for life of every living being, not only life but everything they have i.e. their complexion, voice, brilliance, instinct, intellect etc are only dependent on that diet which they ingest. Hence he might have included the diet in three Upsthambha of the life. Diet can influence the structural characteristics of large intestine. In this study, it was investigated that colon of mice fed on Sarvarasahara mentioned in Ayurveda (for 2.5 months) by focusing our attention on the histological and ultra structural characteristics of the epithelium, the histological pattern of goblet cell, micro villi pattern and folds of the mucus membrane. Diet can influence the characteristics of the gastrointestinal tract since the intestinal mucous membrane is directly in contact with food and absorbs the substances produced by digestion. In particular, it has been reported that the diet may affect both small and large intestine in terms of mucosal architecture, villous height and crypt depth, epithelial cell proliferation and other features. Moreover, it is known that diet and the histological characteristics of goblet cell and mucous membrane are strictly correlated. While extensive literature exists on the changes induced on gut histology by different dietary factors such as fibres, pectin, cellulose, nucleotides, etc. In particular, the high sensitivity of goblet cell mucins to different physiological and pathological conditions makes them suitable markers for monitoring diet effects on the intestine.

MATERIALS AND METHODS
Twenty mature Swiss Albino mice, weighing 22-32 g of an average age of about 50 days were used. (Ethical clearance number = Dean/2012-13/EC 541) Then animals were divided in five distinct groups i.e. Group I, Group II, Group III, Group IV and Group V, containing four mice in each. The animals were classified in three distinct categories those are, Category- 1: Classification based on management used to induce signs of vitiation of srotas, Category- 2: Classification based on signs, Category-3: Classification based on provided diet. These categories are well denoted in under mentioned Tables i.e. in Table 1-3. Out of which group were considered as controls (Group I) and were fed on Diet-1 i.e. Lab
approved diet, contents of 10 kg mixture includes, Wheat flour: 6 kg, Maize flour: 3 kg, Fibre husk: 500 g, Sugar: 500 g, Salt: 100 g, Mineral powder (Agrimin forte, Galaxo, India.): 1000 g. Group II and Group III mice were fed on Ekarasahara containing only chick pea (Chana). Charaka Aacharya had clearly stated that continuous usage of similar kind of Rasas is a prime reason for illness of a being. Group VI and Group V mice were fed on Sarvarasahara, containing Rasayana and Vilepi (5 g/ mice/day), Rasayana contain a mixture of milk and ghee, where as Vilepi contains a homogeneous mixture of barley (Yava), old rice (Shalishastika) region pea (Tuvara dal), lentils (Masoor Dal) rock salt, ginger root, cumin seeds. These contents are described in Charaka Samhita as daily routine diet (Pathya Aahara). Then study was focused on “How food influences and cures the wounds produced by diseases of Purushapak Shalishastika”. These contents are described in Charaka Samhita as daily routine diet (Pathya Aahara). Then study was focused on “How food influences and cures the wounds produced by diseases of Purushapak Shalishastika”. Then study was focused on “How food influences and cures the wounds produced by diseases of Purushapak Shalishastika”.

RESULTS

Micro structural changes

At first Category -I was taken on focus (Table 1), it was found that histological slides of all symptom induced groups had pathological changes in their intestinal lining and mucosal layer as they were compared with histological slides of control group, which were snapped on Motic System Microscope, Control group (Group -I):

The mucosa of the colon is lined by a simple columnar epithelium with a thin brush border and numerous goblet cells. Note that there are no plicae or villi. The crypts of Lieberkuhn are straight and un-branched and lined largely with goblet cells. In many regions the mucus is partially preserved and stains with Hematoxylin. At the base of the crypts, undifferentiated cells and endocrine cells are present; however, Paneth cells are not usually present. The appearance of the lamina propria is essentially the same as in the small intestine; Leukocytes are abundant and the isolated lymphoid nodules present in this tissue extend into the submucosal layer. The muscularis mucosae are a bit more prominent compared to the small intestine, and consists of distinct inner circular and outer longitudinal layers. The submucosa of this specimen is particularly well fixed such that you may better appreciate the mixture of irregular connective and adipose tissue, numerous blood vessels, and several excellent examples of ganglionic nerves and nerves of the submucosal plexus. The muscularis externa of the large intestine is different from that of the small intestine in that the outer longitudinal layer of smooth muscle varies in thickness and forms three thick longitudinal bands, the taeniae coli. Then in Category II- (Table 2) it was found that, there were similar kinds of wounds, like ulceration, breach in epithelium etc. are abundant and the isolated lymphoid nodules present in this tissue extend into the submucosal layer. The muscularis mucosae are a bit more prominent compared to the small intestine, and consists of distinct inner circular and outer longitudinal layers. The submucosa of this specimen is particularly well fixed such that you may better appreciate the mixture of irregular connective and adipose tissue, numerous blood vessels, and several excellent examples of ganglionic nerves and nerves of the submucosal plexus. The muscularis externa of the large intestine is different from that of the small intestine in that the outer longitudinal layer of smooth muscle varies in thickness and forms three thick longitudinal bands, the taeniae coli.

Table 1: Classification 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Castor Oil (5 ml/mice/day)</th>
<th>Codeine (5 ml/mice/day)</th>
<th>Distilled water (5 ml/mice/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-III and Group-V</td>
<td>Group-II and Group-IV</td>
<td>Group-I</td>
</tr>
</tbody>
</table>

Table 1 showing category-1 i.e. classification based on management used to induce signs of vitiation of srotas

Table 2: Classification 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Constipated animals</th>
<th>Diarrheal animals</th>
<th>Healthy animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-II and Group-IV</td>
<td>Group-III and Group-V</td>
<td>Group-I</td>
</tr>
</tbody>
</table>

Table 2 showing category-2, Classification based on signs

Table 3: Classification 3

<table>
<thead>
<tr>
<th>No.</th>
<th>Sarvarasa ahaara</th>
<th>Ekarasa ahaara</th>
<th>Lab approved diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-IV and Group-V</td>
<td>Group-II and Group-III</td>
<td>Group-I</td>
</tr>
</tbody>
</table>
Figure 1: Microphotograph of Colon of control animals i.e. Group - I handled with distilled water induction per orally and fed with Lab approved diet. Note that, Normal Colon Mucosa, systematically arranged crypts embedded with goblet cells 40 X

Figure 2: Microphotograph of Colon of constipation induced animals i.e. Group - II with help of codeine syrup treatment and fed with Ekarasahara. Note epithelial disruption and deep large ulcer reaching the muscularis layer (Red arrow) as compared to Group – IV (Figure-4) 40 X

Figure 3: Microphotograph of Colon of diarrhea induced animals i.e. Group - III with help of codeine syrup treatment and fed with Ekarasahara. Note the disruption of the epithelium causing large ulcer, loss of mucosal folds and disruption of the muscular layer 40 X

Figure 4: Microphotograph of Colon of constipated induced animals i.e. Group - IV like Group-II with help of castor oil treatment but fed with Sarvarasahara; Note the shallowing of ulcer (Green Arrow) as compared to Group – II (Figure 2) 40 X

Figure 5: Microphotograph of Colon of diarrhea induced animals i.e. Group V like Group - III with help of castor oil treatment but fed with Sarvarasahara. Note that rapid regeneration of crypt patterns which are though short and vacuolated but showing the healing
Throughout the experimental study, the relation between complications present in barley helps prevent constipation and it prevent certain intestinal disorders (dyspepsia), Gastro duodenal ulcer, Colitis, Barley (Yava) cancerous anti-concentration of butyric acid, a fatty acid that contains se
body. Ghee is rich of fatty acids is also metabolized very readily by the dangerous free radicals when cooking. Ghee's short chain doesn't burn easily during cooking. Ghee has stable
Diet
These above said contents of diet 1, are universally accepted diet for healthy growth of animals. It is also known as animal feed or animal diet.

Diet 2
Ghee (Ghrita) - It has a very high smoke point and doesn't burn easily during cooking. Ghee has stable saturated bonds and so it is less likely to form the dangerous free radicals when cooking. Ghee's short chain of fatty acids is also metabolized very readily by the body. Ghee is rich in antioxidants and acts as an aid in the absorption of vitamins and minerals from other foods, serving to strengthen the immune system. A high concentration of butyric acid, a fatty acid that contains anti-viral properties, is believed to inhibit the growth of cancerous tumours.

Barley (Yava) - In the form of flour, malt and pearl, are highly recommended in cases of Indigestion (Gastritis dyspepsia), Gastro duodenal ulcer, Colitis, Gastroenteritis, and Intestinal disorders – Barley helps prevent certain intestinal disorders. For example, the fibre present in barley helps prevent constipation and its complications.

Coriander (Dhanyaka) - Like many spices, contains antioxidants, which can delay or prevent the spoilage of food seasoned with this spice. A study found both the leaves and seed to contain antioxidants, but the leaves were found to have a stronger effect. Chemicals derived from coriander leaves were found to have antibacterial activity.

The Cumin seeds (Jiraka) - These seeds are most effective carminatives. They are aromatic, stomachic (i.e. good for the stomach), stimulant and astringent.

Pigeon pea (Tuvara dala) - In recent years it has also been explored that daily use of Pigeon pea (Tuvara dal) for the treatment of ischemic necrosis of the caput femoris, aphtha, bedsores and wound healing dysentery and many other illnesses.

Rice (Salishashtika) - Anti diarrhoeal, the broth of boiled rice has been used from old times to stop the diarrhoea. The water of rice’ seats the belly and stops the diarrhoea. Antigastritic and demulcent: Rice is very rich in starch. The starch mixed with water, has demulcent, properties. Demulcent components are the ones that protect the internal mucus or the external skin.

Lentils (Masoora dala) - It has been found to clear the digestive system as they are extremely rich in soluble fibre, which forms a gel like substance in the digestive tract, it has also antioxidant properties, hence positively affect colon function.

Ginger Root (Aadraka) - A study carried out at the University of Michigan Medical School found that Ginger Root Supplement administered to volunteer participants reduced inflammation markers in the colon within a month.

Rock salt (Saindhava lavana) - It contains 84 out of the 92 trace elements required by body including calcium, zinc, magnesium, etc.

These above said content of diet 1, were planned to give according to Charaka Samhita as Pathya Ahara, which is also correlated with medicinal properties according to modern science.

Diet 3
Chick pea. has high protein content, long term use of high protein diet without other nutrient supply may causes nutritional deficiencies and insufficient fibre which can cause health problems such as constipation. The content of Diet 3 consists of only high protein diet, which can be correlated with Ekrasahaara mentioned in Ayurveda. The histological finding mentioned in observations, can be correlated as follows wound healing has phases as haemostatic, inflammatory, proliferative and remodeling phase; every phase has its own characteristics. Neutrophills are the predominant cells in inflammatory phase of wound healing process. As inflammation plays roles in fighting infection, clearing debris and inducing the proliferation phase, it is a necessary part of healing.
However inflammation can leads to tissue damage if it lasts too long and delays proliferative phase. This reference suggests that wound healing is delayed in group II and group III than group IV and V. Shallowing of wound in group IV and V and blunting and loss of some micro villi in group II and III also justify the above statement. Thus, it is justified that maintenance of the Pathya Ahara, i.e. Diet-2 considered here in this study as Sarvarasahar/Disciplined regular diet, as mentioned in “Ayurveda” facilitates better healing to the intestinal mucosa injury induced both in constipation as well as in diarrhoea.

CONCLUSION
Thus, it can be concluded that maintenance of the Pathya Ahara as mentioned in “Charak Samhita” facilitates better healing to the intestinal mucosa injury induced both in constipation as well as in diarrhoea.

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