Research Article

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EXPERIMENTAL STUDY OF DISCIPLINED REGULAR DIET ON DISEASES OF PURISHVAHA SROTAS IN MICE

Jadhav Amol B^{1*}, Singh K N², Singh Royana³

¹Junior Resident, Department of Rachana Sharir, Faculty of Ayurveda, IMS, Banaras Hindu University, Varanasi, U P, India

²Assistant Professor, Department of Rachana Sharir, Faculty of Ayurveda, IMS, Banaras Hindu University, Varanasi, U P, India

³Associate Professor, Department of Anatomy, Faculty of Medicine, IMS, Banaras Hindu University, Varanasi, U P, India

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*Corresponding author

Dr. Jadhav Amol B, Junior Resident, Department of Rachana Sharir, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, U P, India E-mail: amoljadhav.bhu@gmail.com

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ABSTRACT

By considering the beneficial references concerning with disciplined diet which are mentioned in Ayurveda viz, in Charak Samhita, Sushruta Samhita, Yogaratnakara and Sharandhara 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 (Agrimin 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), Shalishashtika (old rice), Tuvardal (pigeon pea), Msoora (Lentil) spiced or zesty with Ghrita, Saindhava (Rock salt), Jira (*Cuminum cyminum*), Ardraka (ginger) and garnished with fresh leaves of Dhanyaka (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 Pureeshvaha Srotodushti lakshana, mainly constipation and diarrhoea. It was observed that Group- II and Group- III showed large ulcers some superficial as well deep reaching the muscular layer, infiltration of neutrophil 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 Panchmahabhuta¹. Ayurveda also proves the sayings of Vedic and Aupanaishadhika literature in very scientific and logical way. Acharya Charaka had stated the importance of daily routine diet i.e. Pathya Aahara.² In Agrya Sangraha he suggested that the complete diet Sarvarasahara is the only prime thing which can relieve the illness and rejuvenate the health,³ while incomplete or similar kind of diet Ekarasahara 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 etc⁴ are only dependent on that diet which they ingest. Hence he might have included the diet in three Upstambha of the life.⁵ 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.^{6,7} Moreover, it is known that diet and the histological characteristics of goblet cell and mucous membrane are strictly correlated.⁸ While extensive literature exists on the changes induced on gut histology by different dietary factors such as fibres, pectin, cellulose, nucleotides, etc.⁹ In particular, the high sensitivity of goblet cell mucins to different physiological and pathological conditions⁸ 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 is = 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 Acharya had clearly stated that continuous usage of similar kind of Rasa is a prime reason for illness of a being.³ 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 ghee³, where as Vilepi contains a homogeneous mixture of barley (Yava), old rice (Shalishastika) pigeon 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).² Then study was focused on "How food influences and cures the wounds produced by diseases of Pureeshavaha srotas in one of its Moolasthanas i.e. Pakvashaya. Then Group- II and Group- IV were exposed to drug (linctus codeine syrup purchased from the market), which is capable of inducing constipation and Group- III and Group- V were exposed to drug (castor oil, obtained from Ayurveda pharmacy of faculty of Ayurveda, IMS BHU, Varanasi, India), which is capable of inducing diarrhoea, and control group was introduced with distilled water (obtained from distillation plant, Histology lab, IMS BHU) to maintain the same stress factor (which occur while ingestion of the drug by oral route to the mice) among all grouped mice. The mice were sacrificed by overdose of chloroform anaesthesia, 15 days after the stoppage of continuous induction of symptoms by drugs. Samples of the colon were quickly removed and immediately processed for light microscopy. For histological study in light microscope, samples were fixed with 10 % neutral buffered formalin and embedded in paraffin wax. Eight μ m-thick cross sections of colon were stained with haematoxylin-eosin according to the routine methods. For this method, twenty sections from each part of the colon viz; caecum, proximal colon, distal colon and anorectal region were stained. Observations were performed by optical microscope (Motic system microscope DMW B1 223 ASC) light microscope. Morphometrical analyses were performed on micrographs (10X, 40X and 100X) for each sample; intact and well-oriented villi and/or crypt patterns were evaluated by observing control group slides and compared with treated groups. There after treated groups were compared with

each other. Various phases of wound healing were traced carefully viz; ulceration, breach in epithelium, infiltration of neutrophils etc.

RESULTS

Micro structural changes

At first Category -1 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 ganglion cells 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 2-(Table 2) it was found that, there were similar kinds of wounds, like ulceration, breach in epithelium etc. are formed in both of the treated groups i.e. Group- II-III and Group- IV-V. Then Category-3, It was observed that when constipation and diarrhea were induced in Group- II and Group-III respectively, showed large ulcers, some superficial as well as deep, reaching the muscular layer of large intestine, infiltration of neutrophil was also more in comparison to Group- IV and V, blunting of the micro villi as well as loss of micro villi was also more as compared with Group- IV and Group- V respectively. (Figure 1-5)

Table 1: Classification 1

No.	Castor Oil (5 ml/mice/day)	Codeine (5 ml/mice/day)	Distilled water (5 ml/mice/day)
1	Group- III and Group- V	Group- II and Group- IV	Group- I

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

Table 2: Classification 2

No.	Constipated animals	Diarrheal animals	Healthy animals
1	Group- II and Group- IV	Group- III and Group- V	Group- I

Table 2 showing category- 2, Classification based on signs

Table 3: Classification 3

No.	Sarvarasa ahaara	Ekarasa ahaara	Lab approved diet
1	Group- IV and Group- V	Group- II and Group- III	Group- I

Table 3 showing category-3, Classification based on provided diet

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 castor oil 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

DISCUSSION

Throughout the experimental study, the relation between diet and Pureeshvaha Srotas was taken under consideration. Then diet plan was decided, which was group specific. Each ingredient of all diets i.e. Diet-1, Diet-2 and Diet-3 was specified, evaluated and correlated nutritionally with modern medical science which is as follows,

Diet 1

Wheat- It is considered good source of protein, minerals, B-group vitamins and dietary fibre i.e. an excellent health-building food. Thus, it has become the principal cereal, being more widely used for the making of bread than any other cereal because of the quality and quantity of its characteristic protein called gluten. Gluten makes bread dough stick together and gives it the ability to retain gas. Wheat has several medicinal virtues; starch and gluten in wheat provide heat and energy; the inner bran coats, phosphates and other mineral salts; the outer bran, the much-needed rough age the indigestible portion that helps easy movement of bowels; the germ, vitamins B and E; and protein of wheat helps build and repair muscular tissue.¹⁰

The maize flour - It is naturally rich in dietary fibre, antioxidant, Vitamin B, omega-6 unsaturated fat and also rich in magnesium.¹¹

Salt and sugar- Adequate intake of Salt and sugar maintain the electrolyte level hence in turn maintain normal functions of the body.¹²

Agrimin forte powder, Galaxo, India- This contains mineral powder, which helps to improve growth rate and keeps animal healthy.¹³

These above said contents of diet I, 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.¹⁴

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.¹⁵

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.¹⁶

The Cumin seeds (Jiraka) - These seeds are most effective carminatives. They are aromatic, stomachic (i.e. good for the stomach), stimulant and astringent.¹⁷

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, bed sore and wound healing dysentery and many other illnesses.¹⁸

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. Antigastric 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.¹⁹

Lentils (Masoor 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.²⁰

Ginger Root (Aardraka) - 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.²¹

Rock salt (Saindhava lavana) - It contains 84 out of the 92 trace elements required by body including calcium, zinc, magnesium, etc.²²

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 cause nutritional deficiencies and insufficient fibre which can cause health problems such as constipation.²³ The content of Diet 3 consists of only high protein diet, which can be correlated with Ekrasaahaara 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. Neutrophils 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,² 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 diarrhea.

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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