ORIGINAL

Effect of Kampo medicine "Dai-kenchu-to" on microbiome in the intestine of the rats with fast stress

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Abstract : [Purpose] Diversity of gut microbiome has been recently reported to be lost in inflammatory bowel disease. We have previously reported that the Dai-kenchu-to (DKT) prevented the bacterial translocation through suppression of cytokine and apoptosis in rat's fast stress model. The aim of this study was to evaluate the effect of DKT on maintenance of microbial diversity in rat's intestine with inflammation. [Method] Wister rats were received the fast stress for 5 days. In DKT group, rats were administered with DKT (300 mg/kg/day) during the fast stress (DKT-group). The gut microbiomes were analyzed at before- and after- fast stress, and the effect of DKT for on microbial diversities of the gut were evaluated by the PCR-clone library method targeting the 16 S ribosomal RNA gene. [Result] In Control-group, Erysipelotrichaceae increased to 86% in after fast stress, OTU of before-fast stress was 111 and after fast stress was only 9 (changing rate : 58%). The diversity of microbiome was severely decreased. On the other hand, in DKT-group, diversity of microbiome was kept after fast stress (*Lachnospiraceae : Ruminococcaceae : Coriobacteriales* 54%, 22%, 5%), Operational taxonomic units of before fast stress was 52 and after fast stress was 55 (changing rate : 6%). Family *Lachnospiraceae* which includes butyrate-producing Clostridia (Clostridium IV and XIVa). [Conclusion] DKT prevented the reduction of diversity of microbiome in rat's fast stress model. Our data suggested the new anti-inflammatory mechanism of DKT through gut microbiome. J. Med. Invest. 60 : 221-227, August, 2013

Keywords **:** *Daikenchuto, microbiome, fast stress*

INTRODUCTION

The gut microbiome plays a key role in a wide range of host-related processes and has a profound effect on human health. Comparative analyses of the human gut microbiome have revealed its substantial

Abbreviations : DKT : Dai-kenchu-to

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variation in species and gene composition within a variety of disease states and even among healthy individuals but may fall short of providing a comprehensive understanding of the impact of this variation on the microbial community and on the host (1). It has been argued that an imbalance in microbial population in the intestinal tract is a trigger factor for gut inflammation. Inflammatory bowel disease arises from disruption of immune tolerance to the gut commensal microbes, leading to chronic intestinal inflammation and mucosal damage in genetically predisposed hosts (2). Many reports have shown that the integrity of gut microbiome is important to prevent the inflammatory bowel disease (2). In addition, the relationship between gut microbiome and development of colorectal cancer was suggested in some reports (3).

Herbal medicines can affect health *via* the gut microbiome in two ways. Like ginseng, a variety of herbal medicines are known to provide pharmacological effect only after being processed by bacteria in the gut. The list includes the dried fruits of *Gardenia jasminoides*, containing the compound geniposide, which is converted by gut microbes into its active form, genipin, another anti-inflammatory and anticancer compound. Similarly, the root of the liquorice plant, *Glycyrrhiza glabra*, contains glycyrrhizin, which can be processed by gut microbes into 18β-glycyrrhetic acid, which is effective in the treatment of peptic ulcers but also has antiviral and antifungal activities. In other way, certain ingredients of herbal medicines influence the microbial composition in the gut. For example, extracts from the *Ginkgo* leaf have been shown to increase the abundance of symbiotic bacteria such as *Lactobacillus* and *Bifidobacterium* in the gut. These microbes have been linked with a number of health benefits in the human host ; in particular, they can modulate the immune system as to reduce the risk of autoimmune diseases like type-I diabetes mellitus (2).

Dai-kenchu-to (DKT) is used for the treatment of adhesive bowel obstruction and a feeling of coldness in the abdomen (4-8). The pharmacological action of DKT is gradually understood, and several studies have shown that DKT accelerates the transit of gastrointestinal contents (9-13). Since the delayed transit of luminal contents could be responsible for the bacterial overgrowth in the gut (14-17), DKT may prohibit the harmful microbial activities. It has also been reported that DKT increases intestinal and portal blood flow (7, 18-21), resulting in the reduction of blood ammonia level after hepatectomy (9) and an anti-inflammation (11). We have previously reported that DKT prevents the inflammatory cytokine production in the gut and bacterial translocation in the rat model with fast stress (4). In addition, we have shown that DKT suppresses the inflammatory response after surgical operation in human (6).

The aim of this study was to evaluate the effect of DKT on the microbial diversity in inflamed gut using the rat model with fast stress.

MATERIALS AND METHODS

Dai-kenchu-to (DKT)

A commercially available kampo medicine, DKT, was purchased from Tumura Co. Ltd (Tokyo, Japan). It was prepared as a dried extract powder of Ginseng radix, Zanthoxyli fructus and Zingiberis siccaatum rhizome in the ratio of 3 : 2 : 5 respectively. DKT (15 g) has been used for the patients with adhesive ileus. The dose of DKT for the rats was calculated to be 300 mg/kg from the amount of DKT (15 g) administered to a patient with 50 kg body weight.

Animals

Male Wister rats (200 to 220 g of body weight, 6-week-old) were purchased from Charles River Inc. (Yokohama, Japan). The experimental protocol use in this study was reviewed and approved by the animal committee of the Institute of Health Biosciences, the University of Tokushima Graduate School. Animals were checked to be specific-pathogen-free and housed under standard conditions (Room temperature 22°C, humidity 50±5%, 12 h/12 h light-dark cycle).

Experimental design

Four 6-week-old male were received the fast stress for 5 days as described previously (4). The three rats were administered daily with DKT (300 mg/kg/day) by oral gavage during the fast stress (DKT-group), and the remaining rat was kept without treatment for comparison (control group). The alteration of gut microbiome was evaluated by PCR-clone library method targeting the 16S ribosomal RNA gene (16S rDNA) of the stools collected at before and after fast stress.

Analysis of gut microbiome by sequencing the 16S rRNA gene clone libraries

To evaluate the effect of DKT on gut micorbiome

of the fasted rats, we employed the sequencing analysis of 16S rRNA gene (16S rDNA) clone libraries method. DNAs were extracted from the rat feces according to the method described by Morita *et al.* (22), in which lysozyme and achromopeptidase were used as lytic enzymes. Bacterial 16S rDNA were amplified with the universal primers, 27F and 1492R (22). The amplicons were cloned into pGEM-T Easy Vector (Promega), and the libraries were constructed with *Escherichia coli* DH5α. From the each library, 96 colonies were randomly selected and the insert DNA fragment was amplified with primer SP6 and T7, whose annealing sites locate just outside the cloning site of the pGEM-T Easy Vector. The amplicons were purified, and their nucleotide sequences using the 27F as a sequencing primer were determined by Takara Bio Inc (Otsu, Shiga, Japan). Of the obtained sequences, low quality sequences were removed. The uncertain nucleotides at both ends were also trimmed. The assignment of each 16S rDNA sequence was done by Classifier program from the Ribosomal Database Project (22) with confidence level of over 80%. Sequences were aligned with Clustal W2 program and assembled into operational taxonomic units (OTUs) with the parameters of > 97% identity and > 90% alignment length, and the number of OUT was used to evaluate the diversity of gut microbiome. The phylogenetic tree was drawn by MEGA5 program.

RESULTS

< Control group >

Figure 1 and Figure 2 showed the influence of fast stress on microbiome in the intestine, Figure 1

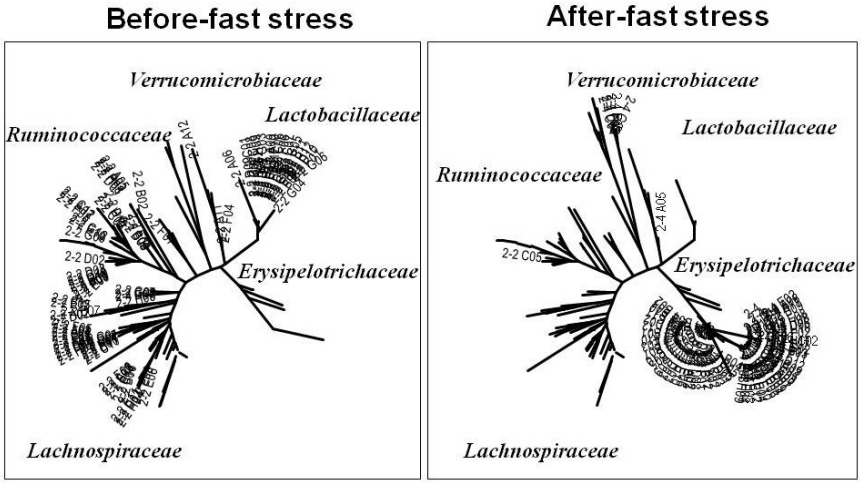

Figure 1 The phylogenetic tree of the microbiome component in control-group.

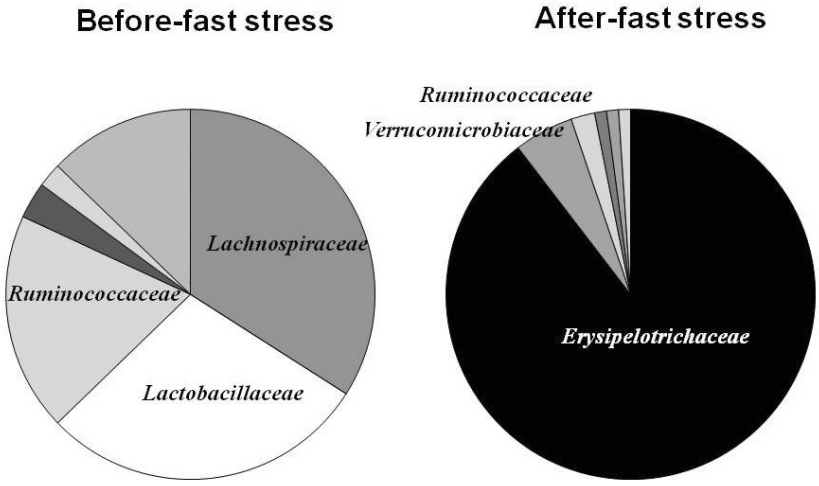

Figure 2 The composition of microbiome as circle figure in control-group.

showed the tree of the microbiome component, Figure 2 showed the composition of microbiome as circle figure. In Control-group, Erysipelotrichaceae increased to 86% in after-fast stress, OTU of before-fast stress was 111 and after-fast stress was 46 (changing rate : 58%). The diversity of microbiome was severely decreased.

< DKT group >

Figure 3 and Figure 4 showed the effect of DKT on microbiome in the intestine after fast stress. Figure 3 showed the tree of the microbiome component, Figure 4 showed the composition of microbiome as circle figure. In DKT-group, diversity of microbiome was kept after fast stress (*Lachnospiraceae : Ruminococcaceae : Coriobacteriales* 54%, 22%, 5%). OTU of before-fast stress was 52 and after-fast stress with DKT was 55 (changing rate : 6%). DKT maintained the diversity of microbiome in fast stress model.

< Composition of microbiome change between the control and DKT group >

Figure 5 showed the component of microbiome. Compared with the microbiome of control-group, the variation of microbiome after-fast stress with DKT was not changed significantly. DKT maintained the diversity of microbiome almost normal condition.

Figure 6 showed the OTU changing rate. The OTU in control-group decreased to 42%, while the OTU in DKT-group maintained at 105% compared with before fast stress respectively.

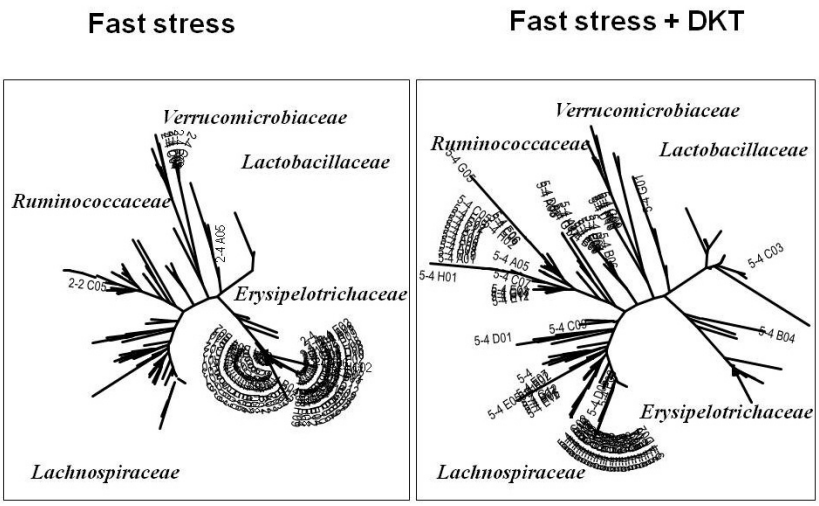

Figure 3 The phylogenetic tree of the microbiome component in DKT-group.

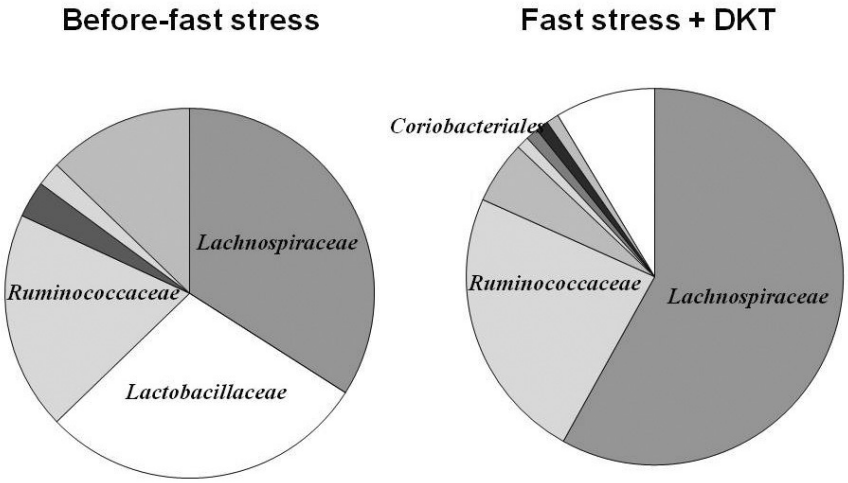

Figure 4 The composition of microbiome as circle figure in DKT-group.

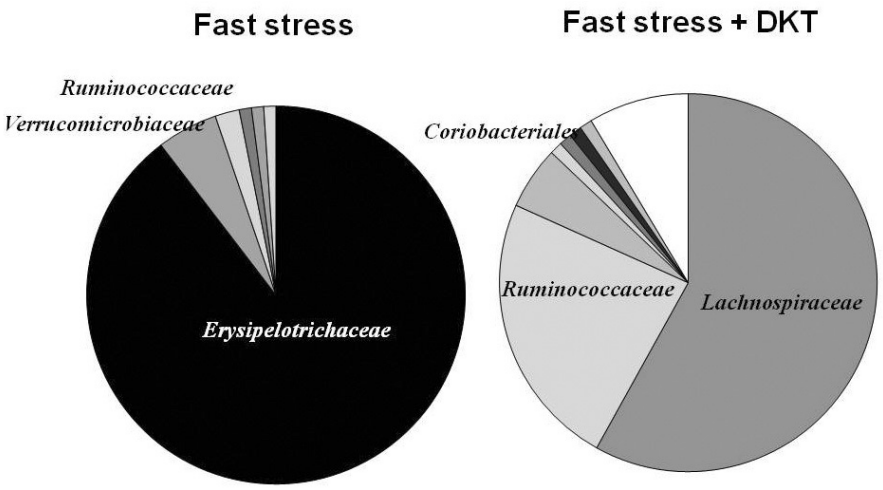

Figure 5 Composition of microbiome change between the control and DKT group.

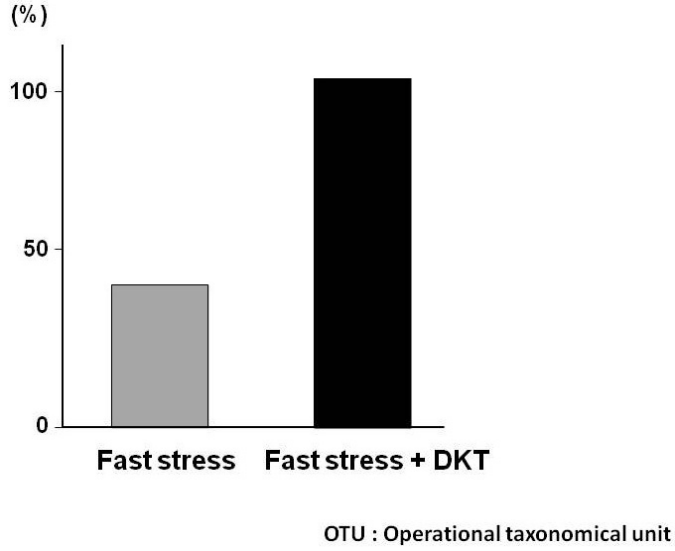

Figure 6 OTU changing rate between control and DKT group.

DISCUSSION

The global composition of the intestinal bacterial microbiome rather than the presence of single pathogens appear to be relevant for inflammatory bowel disease (IBD) pathogenesis and etiology. Analysis of bacterial 16S rRNA genes, amplified directly from complex communities, provides an efficient strategy to explore the bacterial diversity of complex microbiome. Alteration of the bacterial microbiome in mucosal inflammation reflects a metabolic imbalance of the complex microbial ecosystem with severe consequences for the mucosal barrier rather than disrupted defence to single microorganisms (1, 2).

The malnutrition is associated with cometabolism of microbiome and abnormal increase of microbiome is also associated with malnutrition (23).

In the inflammation and metaplasia, esophagitis and Barrett's esophagus (intestinal metaplasia) are associated the microbiome change. Streptococcus is significantly decreased and the diversity also decrease in esophagitis and Barrett's esophagus (24). Patients with inflammatory diseases and diarrhea show a complex pattern with high inter individual variability in microbiome. Bacterial diversity of non-inflammatory control is significantly higher than that of Crohn's disease and ulcerative colitis patients, suggesting that reduced bacterial diversity in IBD is a disease specific feature (25). In this study, fast

stress induced the Erysipelotrichaceae sequence significantly. And the Mollicutes belong to the Erysipelotrichaceae, its increase is observed in abnormal diet intake (26). DKT maintains the diversity of microbiome and prevent the increase of Erysipelotrichaceae sequence. The exact role of this mechanism is obscure, and deserves further research.

In colon cancer (27), changing the microbiome is related the tumorigenesis through various events (e.g. infection, diet, stress, inflammation). Ginseng significantly inhibited colonic inflammation and tumorigenesis and concomitantly reduces proliferation and increased apoptosis through changing the diversity of microbiome. The epidermal growth factor receptor (EGFR) cascade is up-regulated in colonic tumors and ginseng significantly reduced EGFR activation and Cox-2 expression. Dietary ginseng altered colonic microbial diversity, and bacterial suppression with metronidazole reduced serum compound K following ginseng gavage. Furthermore, compound K significantly inhibits tumor xenograft growth. Ginseng inhibited colonic inflammation and tumorigenesis promoted by Western diet. The ginseng metabolite compound K contributes to the chemopreventive effects of this agent in colonic tumorigenesis. As DKT contains the ginseng which may relate this microbial diversity, diversity change in this study may deeply correlate to the ginseng (2).

The detail of the mechanism of the DKT for microbiome is unclear and further investigation is needed.

Fast stress induced the loss of diversity of microbiome and the DKT keeps the diversity. Unchanged microbiome characteristics associate with maintaining the intestinal activity. We suggest new mechanism of the DKT for anti-inflammatory effect is concerned with the microbiome.

CONCLUSION

DKT maintained the diversity of microbiome in fast stress model. Our data suggests the new mechanism of anti-inflammatory effect of DKT.

AUTHOR DISCLOSURES

Drs. Kozo Yoshikawa, Tomomi Kuwahara, Nobuhiro Kurita, Hirohiko Sato, Takashi Iwata, Shinya Morimoto, Tomohiko Miyatani, Hideya Kashihara, Chie Takasu have no conflicts of interest or financial ties to disclose.

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REFERENCE

1. Greenblum S, Turnbaugh PJ, Borenstein E : Metagenomic systems biology of the human gut microbiome reveals topological shifts associated with obesity and inflammatory bowel disease. Proc Natl Acad Sci 109 : 10, 2012
2. Crow JM : Microbiome : That healthy gut feeling. Nature 480 : 88-89, 2011
3. Zhu Y, Michelle Luo T, Jobin C, Young HA : Gut microbiota and probiotics in colon tumorigenesis. Cancer Lett 28 : 119-27, 2011
4. Yoshikawa K, Kurita N, Higashijima J, Miyatani T, Miyamoto H, Nishioka M, Shimada M : Kampo medicine "Dai-kenchu-to" prevents bacterial translocation in rats. Dig Dis Sci 53 : 1824-31, 2008
5. Yoshikawa K, Shimada M, Nishioka M, Kurita N, Iwata T, Morimoto S, Miyatani T, Komatsu M, Kashihara H, Mikami C : The effects of the Kampo medicine (Japanese herbal medicine) "Daikenchuto" on the surgical inflammatory response following laparoscopic colorectal resection. Surg Today 42 : 646-51, 2011
6. Chikakiyo M, Shimada M, Nakao T, Higashijima J, Yoshikawa K, Nishioka M, Iwata T, Kurita N : Kampo medicine "Dai-kenchu-to" prevents CPT-11-induced small-intestinal injury in rats. Surg Today 42 : 60-7, 2012
7. Ogasawara T, Morine Y, Ikemoto T, Imura S, Fujii M, Soejima Y, Shimada M : Influence of Dai-kenchu-to (DKT) on human portal blood flow. Hepatogastroenterol 55 : 574-7, 2008
8. Sato Y, Inoue S, Katagiri F, Itoh H, Takeyama M : Effects of Pirenzepine on Dai-kenchu-to-Induced Elevation of the Plasma Neuropeptide Levels in Humans. Biol Pharm Bull 29 : 166-171, 2006
9. Kaiho T, Tanaka T, Tsuchiya S, Yanagisawa S, Takeuchi O, Miura M, Saigusa N, Miyazaki M :

Effect of the herbal medicine Dai-kenchu-to for serum ammonia in hepatectomized patient. Hepatogastroenterol 52 : 161-165, 2004
10. Takagi A, Yagi M, Tanaka Y, Asagiri K, Asakawa T, Tanaka H, Ishii S, Egami H, Akaiwa M, Tsuru T : The herbal medicine daikenchuto ameliorates an impaired anorectal motor activity in postoperative pediatric patients with an anorectal malformation--a pilot study. Int Surg 95 : 350-5, 2010
11. Akiho H, Nakamura K : Daikenchuto ameliorates muscle hypercontractility in a murine T-cell-mediated persistent gut motor dysfunction model. Digestion 83 : 173-9, 2011
12. Tokita Y, Yamamoto M, Satoh K, Nishiyama M, Iizuka S, Imamura S, Kase Y : Possible involvement of the transient receptor potential vanilloid type 1 channel in postoperative adhesive obstruction and its prevention by a kampo (traditional Japanese) medicine, daikenchuto. J Pharmacol Sci 115 : 75-83, 2011
13. Kono T, Kaneko A, Hira Y, Suzuki T, Chisato N, Ohtake N, Miura N, Watanabe T : Anti -colitis and -adhesion effects of daikenchuto via endogenous adrenomedullin enhancement in Crohn's disease mouse model. J Crohns Colitis 4 : 161-70, 2010
14. Iwabu J, Watanabe J, Hirakura K, Ozaki Y, Hanazaki K : Profiling of the compounds absorbed in human plasma and urine after oral administration of a traditional Japanese (kampo) medicine, daikenchuto. Drug Metab Dispos 38 : 2040-8, 2010
15. Manabe N, Camilleri M, Rao A, Wong BS, Burton D, Busciglio I, Zinsmeister AR, Haruma K : Effect of daikenchuto (TU-100) on gastrointestinal and colonic transit in humans. Am J Physiol Gastrointest Liver Physiol 298 : 970-5, 2010
16. Kono T, Kanematsu T, Kitajima M : Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines : is it time yet? Surgery 146 : 837-40, 2009
17. Narita M, Hatano E, Tamaki N, Yamanaka K, Yanagida A, Nagata H, Asechi H, Takada Y, Ikai I, Uemoto S : Dai-kenchu-to attenuates rat sinusoidal obstruction syndrome by inhibiting the accumulation of neutrophils in the liver. J Gastroenterol Hepatol 24 : 1051-7, 2009
18. Wood MJ, Hyman NH, Mawe GM : The effects of daikenchuto (DKT) on propulsive motility in the colon. J Surg Res 164 : 84-90, 2010
19. Kawasaki N, Nakada K, Suzuki Y, Furukawa Y, Hanyu N, Kashiwagi H : Effect of Dai-kenchu-to on gastrointestinal motility and gastric emptying. Int J Surg 7 : 218-22, 2009
20. Kawahara H, Yanaga K : The herbal medicine Dai-Kenchu-To directly stimulates colonic motility. Surg Today 39 : 175-7, 2009
21. Kono T, Koseki T, Chiba S, Ebisawa Y, Chisato N, Iwamoto J, Kasai S : Colonic vascular conductance increased by Daikenchuto via calcitonin gene-related peptide and receptor-activity modifying protein 1. J Surg Res 150 : 78-84, 2008
22. Kataoka K, Kibe R, Kuwahara T, Hagiwara M, Arimochi H, Iwasaki T, Benno Y, Ohnishi Y : Modifying effects of fermented brown rice on fecal microbiota in rats. Anaerobe 13 : 220-7, 2007
23. Michelle S, Yatsunenko T, Manary MJ, Trehan I, Mkakosuya R : Gut Microbiomes of Malawian Twin Pairs Discordant for Kwashiorkor. Science 338 : 548-554, 2013
24. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z : Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. Gastroenterology 137 : 588-97, 2009
25. Ott SJ, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR, Timmis KN, Schreiber S : Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut 53 : 685-93, 2004
26. Fleissner CK, Huebel N, Abd El-Bary MM, Loh G, Klaus S, Blaut M : Absence of intestinal microbiota does not protect mice from diet-induced obesity. Br J Nutr 104 : 919-29, 2010
27. Dougherty U, Mustafi R, Wang Y, Musch MW, Wang CZ, Konda VJ, Kulkarni A : American ginseng suppresses Western dietpromoted tumorigenesis in model of inflammation-associated colon cancer : role of EGFR, BMC Complementary and Alternative Medicine 11 : 111, 2011