Protective effect of triphala on radiation induced acute intestinal mucosal damage in Sprague Dawley rats

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Aim of the study was to determine protective effect of triphala on radiation-induced rectal mucosal damage. Male Sprague Dawley rats (30) were divided into 5 groups. Rats in group A were sham irradiated and rats in group B underwent only irradiation. Rats in group C were administered triphala 1g/kg/day orally for 5 consecutive days before irradiation. Rats in group D and E were administered triphala 1 and 1.5 g/kg/day orally for 10 consecutive days, respectively. Rectal mucosal damage was induced by a single fraction of 12.5Gy gamma irradiation (Ir-192) on 5th day. All the rats were autopsied on 11th day and histological changes in surface epithelium, glands, and lamina propria were assessed. Proctitis showed significant improvement in surface epithelium (P<0.024), glands (P<0.000) and lamina propria (P<0.002) in group E compared to group B. Rats in group E showed significantly less change in glands (P<0.000) compared to rats in group D, All histological variables (surface epithelium, P<0.001; glands, P<0.000; lamina propria, P<0.003) compared to rats in group C. In a Tukey-b test, group E had a significantly recovered grade for glands (P<0.000) compared to groups B, C and D. Results of the present study showed that high-dose triphala improved radiation-induced damage of glands.

Keywords: Epithelium, Glands, Lamina propria, Proctitis, Radiation protection, Triphala

Radiation therapy is a useful treatment option for malignant tumors in pelvis including female genitalia, urinary tract and colorectal region. Pelvic irradiation targets primary tumor or its bed and regional lymph nodes according to the characteristics of various diseases and aims at improving survival with a local control. For prostate cancer, a radiation dose escalation showed a positive effect on prostate-specific antigen control rate and disease-free survival. However, radiation proctitis increased in a dose-dependent manner1-3. If the intestine is irradiated, acute edema and hyperemia mask normal mucosal vascular architecture often accompanied by an increase in mucus secretion4. With the passage of time, late reactions such as telangiectasia, mucosal congestion, ulceration, stricture and necrosis may occur5. In addition, it has been demonstrated in human and animal studies that severe acute reactions translate into late ones6-8.

Triphala, an herbal formulation of Ayurvedic medicine, 1:1:1 mixture of dried and powdered fruits of 3 plants, Terminalia chebula, Phyllanthus emblica and Terminalia bellerica, has been widely used in various countries including India with evidenced safety. Total body irradiation after administration of triphala (10 mg/kg/day) improved clinical symptoms and survival rates compared to saline administration plus irradiation9,10. The result may be attributed to a protective effect of triphala against free radicals including •OH, O2•-2, ABTS•+ (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) and NO•.

The present study was conducted to determine protective effect of triphala on radiation-induced rectal mucosal damage by examining histological changes in the mucosa after in vivo administration and to compare differences in mucosal histological changes in terms of dose and duration of triphala administration.

Material and Methods
Experimental animals—Male Sprague Dawley rats aged 6 weeks (150-200 g) were used in the present study. Rats were acclimatized to laboratory conditions for at least 7 days before the experiment. Rats, 3 in a

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cage, were maintained at 22 ± 1°C, and humidity at 40%-60%. During the experimental period, oral intake, performance, weight change and the absence or presence of hematochezia were assessed. If the body weight measured on the first experimental day showed a decrease by ≥20% or poor oral intake/performance persisted for ≥1 day, the rats were euthanized. Experimental protocol was approved by the Ethics Committee of Samsung Biomedical Research Institute. Care, maintenance and treatment of animals were as per the ethical policy of Laboratory Animal Research Center, Samsung Biomedical Research Institute.

**Study design**—Rats were divided into 5 groups of 6 rats. Group A: sham irradiated; Group B: irradiation alone; Group C: low-dose (1 g/kg/day) short-term triphala and irradiation; Group D: low-dose long-term triphala and irradiation; and Group E: high-dose (1.5 g/kg/day) long-term triphala and irradiation. Triphala was administered for 10 days between the 1st and 10th experimental days for long-term administration or 5 days between 1st and 5th experimental days for short-term administration. For oral administration of triphala, a 22-G catheter was inserted into stomach through oral cavity. Triphala, purchased from Sri Balaji Biotech, India, was suspended at 0.1 g/mL distilled water. Amount of distilled water in group C, D, and E was different according to each group and individual weight. In groups A, B and C in which triphala was discontinued after irradiation, 2 mL of distilled water were equally administered instead of triphala in the same manner. Irradiation was performed 1 h after administration of triphala on the 5th experimental day. Rats were put under anesthesia during irradiation by i.m injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). On the 11th experimental day, euthanasia was conducted by CO₂ gas asphyxia and intestine was dissected approximately 3 cm from anus for histological examination.

**Irradiation**—Brachytherapy was performed using high-dose-rate Ir-192 gamma radiation through an after-loading brachytherapy machine (microSelectron, Nucletron, Netherlands) which minimizes irradiation of organs other than rectum and stressful conditions due to irradiation. A source guided catheter, of which a point 2.5 cm from the tip was marked with a tape, was inserted into the rectum of rats, end of the tape was in contact with anus, and then rats and catheters were fixed to avoid any movement. Therefore, a length of catheter inserted into rectum was maintained. After catheter was connected with brachytherapy machine, the source was induced through the catheter. With the source being placed at 10, 15 and 20 mm from the tip of the catheter, a prescribed dose was irradiated to 5 mm depth from the source. Thus, the rectal mucosa between the 0.5 and 1.5 cm from anus (between the 1.0 and 2.0 cm from the tip of catheter) was irradiated evenly. The prescribed doses were determined to be 12.5 Gy through a preliminary study. Various radiation doses including sham, 5 Gy, 7.5 Gy, 10 Gy, 12.5 Gy, and 15 Gy were evaluated by same method in the present study. Group irradiated with 12.5 Gy showed significant histological changes compared to 5 Gy, 7.5 Gy, and 10 Gy. The similar histological change was examined in 12.5 Gy and 15 Gy (data not described). Therefore, 12.5 Gy has been used as an optimal prescribed dose in the present study.

**Histological examination**—Specimens were fixed with buffered formalin (10%) and 4 µm sections were stained with hematoxylin and eosin (H & E), and periodic acid Schiff (PAS) staining of goblet cells and mucin for microscopic examination. Ad hoc grading system designed by Hovdenak et al.¹¹ has been used for assessment of acute radiation-induced proctitis. It assessed histological findings in the specimens obtained 2 and 6 weeks after irradiation by using a scoring system for the surface epithelium, glands and lamina propria. It has been reported that there is a significant correlation between clinical symptoms (loose stools and abdominal pain) and the scores of histological changes at 2 weeks. Histological changes in terms of the surface epithelium, glands and lamina propria were were graded and scored. The detailed items for surface epithelium included (i) loss of cellular height/flattening of cells and (ii) cellular inflammatory infiltrates; for glands (i) luminal migration of epithelial nuclei, (ii) loss of goblet cells, (iii) mitotic activity, (iv) cryptitis (migration of segmented neutrophils through the crypt wall), (v) eosinophilic crypt abscesses, (vi) loss of glands, (vii) atrophy of glands and (viii) distortion of glands; for lamina propria (i) inflammation, (ii) edema and (iii) congestion of blood vessels. Each detailed item was rated on a 5-point scale: 0 = normal, 1 = mildly abnormal, 2 = moderately abnormal, 3 = markedly abnormal and 4 = severely abnormal. The detailed-item scores were summed up and also assessed. In addition, scores of each parameter were summed up and assessed. Histological examination was
performed by a single pathologist who was blinded to study groups.

Statistical analysis—Statistical analyses were performed using SPSS version 10.0 (SPSS Inc, Chicago, IL). Values are expressed as mean ± standard error mean. Mann-Whitney U test was used to assess the differences in histological findings between the 2 groups. Groups showing a significant difference in histological findings were tested by using Tukey-b test, a 1-way ANOVA. *P* <0.05 was considered statistically significant.

**Results and Discussion**

Out of 30 experimental rats, 3 expired, 2 in group C and 1 in group D. One rat each in groups C and D expired due to anesthesia, and 1 rat in group C expired due to complications associated with triphala administration on the 4th experimental day. The remaining rats tolerated irradiation and triphala administration until completion of experiment without significant changes in oral intake, vital signs, body weight and defecation. At the completion of the experiment, weight gain from the 1st to the 11th experimental days was 95.50 ± 4.712 g in group A, 81.83 ± 2.184 g in group B, 79.25 ± 4.003 g in group C, 69.20 ± 2.144 g in group D and 80.50 ± 2.087 g in group E. In the Tukey-b test, the weight gain was significant in group A compared to the other groups. However, there were no significant differences in weight change between groups B, C, D and E.

In irradiated groups, radiation-induced mucosal damage was observed. Regularity of surface epithelium and glands were observed in group A (Fig. 1(a)), whereas loss of cellular height of surface epithelium, severe cryptitis, loss of goblet cell, atrophy and distortion of glands, and edematous lamina propria were observed in group B (Fig. 1(b)). In group E, the histological change of flattening

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Fig. 1—Histological findings in the rectum of rats. (a): sham-irradiated rats showing normal rectal epithelium with regularity of glands and mucin vacuoles; (white arrow) (b): irradiated rats (12.5 Gy) showing marked distortion and atrophy of glands (white arrow), flat epithelium, eosinophil crypt abscess (black arrow) and absence of mucin; (c): triphala administered rats (1.5 g/kg/day) for 10 consecutive days and irradiation with 12.5 Gy showing mild flat epithelium (white arrow) and glands loss, and some reduction of mucin (black arrow). H & E stain, ×400
surface epithelium, atrophic and distorted glands and inflammation of lamina propria was milder than group B (Fig. 1(c)). Sum of scores for each histological parameter was 0.83 ± 0.477, 21.0 ± 3.1694, 22.50 ± 1.555, 19.0 ± 1.643 and 10.5 ± 0.922 in group A, B, C, D and E, respectively.

To access the degree of improvement in proctitis by administration of triphala, group B without triphala administration was compared to groups C, D and E with different doses of triphala. Proctitis was improved better in lamina propria in group D than in group B, whereas it was improved better in the surface epithelium, glands and lamina propria in group E than in group B. There was no significant difference in histological changes between groups B and C.

To access the difference according to the dose and terms of triphala, groups C, D and E were compared to each other. Proctitis was significantly improved in the glands in group E than in group D, whereas it was improved better in the surface epithelium, glands and lamina propria in group E than in group C. Proctitis was significantly improved in the surface epithelium and lamina propria in group D than in group C.

The Tukey-b test showed that the histological changes in the surface epithelium, glands and lamina propria were more severe in groups B, C, D and E than in group A. The histological changes of the surface epithelium and lamina propria were milder in group E than in groups B and C (for each), whereas those of the glands were milder in group E than in groups B, C and D (Fig. 2).

It has been reported that *Terminalia bellerica*, a component of triphala, does not show toxicity through oral administration of up to 800 mg/kg/day in Albino rats. In previous animal studies, triphala has been administered orally at 0.5 to 1.0 g/kg/day for a maximum duration of 14 days. In the present study, triphala was administered at 1.0 to 1.5 g/kg/day for 5 or 10 consecutive days, which was safe with no specific side effects. The present study, the histological changes were more remarkable at a dose of 1.5 g/kg/day than at a dose of 1.0 g/kg/day. Furthermore, the changes were more notable when triphala was administered for an additional 5 days after irradiation than when it was administered only for 5 days before irradiation.

Jageta et al. have reported that administration (i.p) of a single dose of triphala in Swiss albino mice is safe up to 240 mg/kg/day, the LD50 of triphala is 280 mg/kg and 10 Gy irradiation after 5 consecutive days administration of triphala reduces frequency of radiation sickness and prolongs survival duration. However, there is no significant correlation between the dose of triphala and the radiation-induced mortality. The discrepancy between their results and ours could not be compared because of the difference in the administration route of triphala. Sandhya et al. reported that 7.5 Gy irradiation after triphala administration to Swiss mice prevented radiation-induced damage by increasing xanthine oxidoreductase activity and a decreasing superoxide dismutase (SOD) activity. Also, it was pointed out that administration of triphala for 7 days each before and after irradiation shows better survival than that of only for 7 days before irradiation.

The dose of 1.5 g/kg/day used in the present study, a dose higher than that used in previous studies, showed the most remarkable histological changes. This implies that a higher dose than that used in previous studies is required to induce focal

![](image.png)

**Fig. 2**—Comparison of histological changes according to ad hoc grading system. In tukey-b test, change of 12.5 Gy irradiated rats mucosa for surface epithelium and lamina propria were milder in group administered with 1.5 g/kg/day triphala for 10 consecutive days compared to 1.0 g/kg/day triphala for 5 consecutive days ($P<0.000$). Change in 12.5 Gy irradiated rats mucosa for glands was improved in group administered with daily 1.5 g/kg triphala for 10 consecutive days compared to daily 1.0 g/kg triphala for 5 or 10 consecutive days and without triphala ($P<0.000$). [IR:irradiation with 12.5 Gy; LD:low dose with 1.0 g/kg/day; HD:high dose with 1.5 g/kg/day; ST: short term for 5 consecutive days before irradiation; LT: long term for 10 consecutive days before and after irradiation; TPL: triphala].
histological changes. For this reason, the future studies should use a much higher dose of triphala. In addition, ointment preparations or suppositories of triphala should be considered in future studies. Triphala administration before irradiation might be effective to scavenge free radicals that the radiation briefly produces. However, a mechanism of the continuous triphala administration after irradiation was unknown. In the experiment on full-thickness dermal wound treated with ointment, a rise of wound healing rate, increases of hydroxyproline, hexosamine and uronic acid, and a decrease of SOD were reported. This report suggested that early inflammatory phase produce large amount of reactive oxygen species that causes severe tissue damage, and triphala scavenges free radicals and reduce the oxidative stress. Although this report is not related with radiation protection or a mucosa, we can infer that a similar mechanism might be developed from the standpoint of the recovery or re-epithelialization of damaged tissue. Further studies are needed to know whether a long-term use of triphala decreases late complication.

The present study was conducted by using a modification of the method performed by ad hoc grading system. This method has been extensively used in studies on acute radiation-induced proctitis, allowing for the evaluation of detailed histological items and comparison of scores between the study groups. However, it is unclear whether individual items have similar implications. Furthermore, it is difficult to exclude subjective errors by examiners. In the present study, we minimized them by making an expert pathologist blinded to the study groups. However, it is unclear whether individual items have similar implications. Furthermore, it is difficult to exclude subjective errors by examiners. In the present study, we minimized them by making an expert pathologist blinded to the histological findings. To date, there have been numerous studies on the protective effect of various drugs on radiation-induced proctitis. Sucralfate, a compound of aluminium hydroxide and sulfated sucrose, was expected to reduce the microvasculature and radiation-induced epithelial damage by affecting basic fibroblast growth factor (bFGF). However, a phase III trial with a suppository of sucralfate showed that this substance did not affect the severity of symptoms of early and late proctitis. In addition, a double-blind trial by the oral route indicated that sucralfate did not affect bowel habits, hardness of feces, flatus and pain in patients treated with irradiation. Amifostine is reported to have a protective effect on salivary glands in patients with head and neck tumors. Montana et al. reported that although they prepared topical drugs of amifostine at various concentrations (100-450 mg) and used them as a suppository, they did not protect rectal mucosa against irradiation. Kouloulis et al. reported that administration of a suppository (1,500 mg of amifostine) is more protective against radiation-induced rectal mucosal damage than an injection of 500 mg of amifostine. However, they stated that there are some limitations in the use of a suppository in clinical practice because of the high cost of high-dose amifostine. The trial of misoprostol has been conducted, but it was found to be unsuccessful. Since recombinant human epidermal growth factor and selenium have a positive effect on the prevention of radiation-induced complication, further studies of this drug will be needed to confirm its protective effect.

Since the rats with 10-day administration of triphala (1.5 g/kg/day) showed no specific side effects, this dose is thought to be safe. Although the effect of low-dose triphala against the radiation-induced rectal mucosal damage was minimal, administering high-dose triphala (1.5 g/kg/day) for 10 days each before and after irradiation may be helpful in the recovery of acute rectal damage. More research is needed to confirm the recovery from radiation-induced early and late proctitis by the use of high-dose triphala. In addition, clinical trials should be conducted to determine whether triphala can reduce the severity and frequency of early and late complications of irradiation.

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References


