

**The modifying effects of green tea polyphenols on acute colitis
and inflammation-associated colon carcinogenesis
in male ICR mice**

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Abbreviations: ACF, aberrant crypt foci; CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; DSS, dextran sulfate sodium; EGCG, (-)-epigallocatechin-3-gallate; ELISA, enzyme-linked immunosorbent assay; GTP, green tea polyphenols; H&E, hematoxylin & eosin; IBD, inflammatory bowel disease; IL, interleukin; MIF, macrophage-migration inhibitory factor; PBS, phosphate-buffered saline; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF- α , tumor necrosis factor- α ; UC, ulcerative colitis.

Key words: green tea polyphenols, colitis, colon carcinogenesis, cytokines

Abstract. Reactive oxygen species (ROS) has been implicated as mediators of intestinal inflammation and carcinogenesis. Although green tea polyphenols (GTP) have anti-cancer property as antioxidant, however it also generates ROS *in vitro*. In this study, we investigated the modifying effects of GTP on dextran sulfate sodium (DSS)-induced acute colitis and on 1,2-dimethylhydrazine (DMH) and DSS-induced colon carcinogenesis in male ICR mice. At sacrifice of 6 days, the colon shortening induced by 2% DSS was showed similar levels by 0.1% and 0.25% GTP, but increased by 0.5% and 1% GTP-containing diet. The expression of interleukin-1 β and macrophage-migration inhibitory factor in the DSS + 0.1% GTP group were lower than the DSS alone group, while the expression levels were increased in the DSS + 0.5% GTP and DSS + 1% GTP groups when compared with the DSS alone group. In a subsequent experiment to determine the effects of 0.01-1% GTP on inflammation-associated colon carcinogenesis induced by DMH/DSS, 0.5 and 1% doses of GTP were failed to prevent multiplicity of colonic tumors, rather, they tend to increased it. Our results thus indicate that the modifying effects of GTP on DSS-induced acute colitis and DMH/DSS-induced colon carcinogenesis dependent upon its dosage and the expression of pro-inflammatory cytokines.

1. Introduction

Green tea is a popular beverage in Asian countries and it has recently become popular in Europe and North America [46]. Green tea polyphenols (GTP) has been reported to be potent antioxidant and prevent oxidative stress-related disease, due to their abilities to scavenge reactive oxygen species (ROS) and chelate metal ions, which promote ROS generation [43]. GTP has been shown to prevent inflammation and carcinogenesis in different tissues of rodents, and several mechanisms have been postulated for this activity [34,36,49,54]. However, to date, GTP and (-)-epigallocatechin-3-gallate (EGCG), the major constituent in tea catechins, have also emerged as pro-oxidants at least *in vitro* systems. For instance, EGCG and GTP generated H₂O₂ in cell-free and cell culture systems [12,20]. EGCG induced DNA damage by oxidative stress generation [14], and induced cyclooxygenase-2 and tumor necrosis factor (TNF)- α expression in macrophages [35,38]. In our previous study, EGCG enhanced pro-matrix metalloproteinase-7 production via spontaneous superoxide generation in HT-29 human colon cancer cells [26].

Colorectal cancer (CRC) is the second leading cause of death from cancer in Western countries including North America [17]. In humans, inflammatory bowel disease (IBD), including chronic ulcerative colitis (UC) and Crohn's disease, predisposes to the development of CRC [21]. Indeed, IBD, not genetic etiology, have been advanced first in importance for CRC, together with the hereditary syndromes of familial adenomatous polyposis and hereditary nonpolyposis CRC. Previous studies showed that inducible nitric oxide synthase was expressed in epithelial cells and inflammatory cells at the site of carcinogenesis in humans and animal models. Indeed,

Reinecker et al., found nitrate and oxidative DNA lesion products in inflamed colonic mucosa of rodents and IBD patients [25,42]. The effects of GTP on colon carcinogenesis are still controversial. Hirose et al., reported the enhancing effects of GTP on colon carcinogenesis in rats [18,19], although there were no proposed underlying mechanisms. Also, there has been no consistent association between the reduction of risk of CRC [24,47,51] or gastric cancer [48] and green tea consumption in previous epidemiologic studies, while green tea consumption has contributed to significantly reduced risk of breast, esophagus, kidney, liver, lung, pancreas cancers [55].

Cytokines play a key role in the pathogenesis of IBD, some of which may lead to colon carcinogenesis [4,45]. Ohkawara et al., reported that the macrophage-migration inhibitory factor (MIF) expression was increased in dextran sulfate sodium (DSS)-induced colitis in mice [37]. Interleukin (IL)-1 β also contributes to the increased severity of DSS-induced colitis [3]. Colonic mucosa contains endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and the enzymes that scavenge or decompose ROS, which generate in response to certain inflammatory stimuli [16]. In the colonic mucosa of UC patients, nitrate and oxidative DNA lesion products, 8-oxo-7,8-dihydro-2'-deoxyguanosine were increased as compared to normal tissues [25]. EGCG, the main component of GTP, had previously been shown to be the pro-oxidant *in vitro* [20].

To determine the modifying effects (enhancement or inhibition) of GTP in colitis and inflammation-associated colon carcinogenesis, we first examined the effect of GTP on DSS-induced acute colitis in ICR male mice, since this model is useful to investigate roles of oxidative stress in acute colitis. Subsequently, we determined the influence of

1 dietary GTP on inflammation-associated colon carcinogenesis using a mouse model
2 initiated with 1,2-dimethylhydrazine (DMH) and promoted by DSS [28]. In this study,
3 high doses of GTP were found to enhance acute colitis induced by DSS through
4 modification of expression of IL-1 β and MIF in the colon, while 0.1% dose of GTP
5 decreased these cytokines production. Furthermore, high doses of GTP were failed to
6 prevent inflammation-associated colon carcinogenesis by DMH and DSS. We thus
7 propose the hypothesis that GTP at excess dosage had not exerted inhibitory effects on
8 acute colitis and carcinogenesis in the inflamed colon.

9 10 **2. Materials and methods**

11 12 *2.1. Animals*

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14 Male specific pathogen-free ICR mice (4 weeks of age) were purchased from Japan
15 SLC (Shizuoka, Japan). They were housed three or five per cage and given fresh tap
16 water *ad libitum* and commercial rodent MF pelleted diet (Oriental Yeast, Co. Ltd.,
17 Kyoto, Japan), which were freshly changed every day, and handled according to the
18 Guidelines for the Regulation of Animals, as provided by the Experimentation
19 Committee of Kyoto University. The mice were maintained in a room controlled at 24 $\pm$
20 2°C with a relative humidity of 60 $\pm$ 5% and a 12 h light/dark cycle (06:00-18:00). All
21 mice were quarantined for 1 week before starting the experiments.

22 23 *2.2. Chemicals*

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GTP containing 70% total catechins and half of them is EGCG, and 3% caffeine was obtained from LKT Laboratories, Inc. (W. St. Paul, MN, USA). DMH was purchased from SIGMA-Aldrich (Tokyo, Japan). DSS with a molecular weight of 36,000-50,000 was from MP Biomedicals, LLC (Aurora, OH, USA). A rat/mouse MIF enzyme-linked immunosorbent assay (ELISA) kit was purchased from Sapporo Immunodiagnostic Laboratory, Co. Ltd. (Sapporo, Japan). Mouse IL-1 β , IL-6 and TNF- α ELISA kit were obtained from Endogen Inc. (Cambridge, MA, USA). Catalase assay kit was purchased from Calbiochem, a brand of EMD Biosciences, Inc. (San Diego, USA). All other chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless specified otherwise.

2.3. Effects of GTP on acute colitis induced by DSS

Following quarantine for 1 week, a total of 85 male ICR mice were divided into an untreated control and six experimental groups (n=12 or 13 for each group), as shown in Fig. 1. In the control group (group 1, n=12), animals were given tap water and basal diet *ad libitum*. The GTP alone group (group 2, n=12) was fed with the diet containing 1% GTP and did not receive DSS. In the DSS alone group (group 3, n=12), animals were given tap water containing 2% DSS (w/v) for 6 days and fed with a basal diet to induce acute colitis. In the GTP groups, mice were fed with diets mixed with four different concentrations of GTP (0.1% for group 4, n=12; 0.25% for group 5, n=13; 0.5% for group 6, n=12; and 1% for group 7, n=12), starting at the DSS exposure. Body weight and intakes of food and drinking water were recorded every day during the experiment. At day 6, all mice were killed by deep anesthesia with diethyl ether for determining the

effects of dietary GTP on DSS-induced acute colitis.

2.4. Isolation of colonic mucosa

At sacrifice, complete necropsy was done on all mice. All organs including colon were macroscopically inspected for the presence of lesions. As for colons, they were removed, washed in phosphate-buffered saline (PBS), and placed on filter papers. After measured the length, they were cut opened longitudinally along the main axis with surgical scissors and the contents were removed. Colons of 3 mice randomly selected from each group were fixed in Mildform[®] 10 N (Wako Pure Chemical Industries, Ltd.) and used for histopathology. Histopathological examination was performed on hematoxylin and eosin (H&E)-stained sections made from paraffin-embedded blocks. Colitis was recorded and scored according to the following morphological criteria described by Cooper et al. [11]: Grade 0, normal colonic mucosa; Grade 1, shortening and loss of the basal one-third of the actual crypts with mild inflammation and edema in the mucosa; Grade 2, loss of the basal two-thirds of the crypts with moderate inflammation in the mucosa; Grade 3, loss of all crypts with severe inflammation in the mucosa, but with the surface epithelium still remaining; and Grade 4, loss of all crypts and the surface epithelium with severe inflammation in the mucosa, muscularis propria and submucosa. Their mucosal ulcers were counted on H&E-stained sections. Colonic mucosa of the remaining mice was scrapped off using a razor before the specimens were frozen using in liquid nitrogen, until later use, according to a method previously reported by Perdue et al. [39] with slight modification.

2.5. Assay for cytokine production

The colonic mucosa obtained was minced with surgical scissors and homogenized in ice-cold PBS using a homogenizer (Hielscher-UP 50H, Hielscher, Stahnsdorf, Germany). Tissue homogenates were then centrifuged at $1900 \times g$ at 4°C for 10 min to obtain the supernatants. Total protein concentrations in the tissue supernatants were determined using a DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA), as specified by the manufacturer's protocol (dilution factor=50), with γ -globulin according to the standard. The IL-1 β , MIF, IL-6, and TNF- α concentrations were determined using each ELISA kit, according to the protocol of the manufacturer (dilution factor=5-8) respectively. The amount of cytokines was calculated as ng of MIF and pg of IL-1 β , IL-6, and TNF- α per mg of protein. Each assay was performed at least 3 times.

2.6. Measurement of SOD and catalase activities

The colonic mucosa was homogenized in ice-cold PBS using a homogenizer (Hielscher-UP 50H). The supernatants of the tissue homogenates were prepared by using the same process for the cytokine production experiment, as described above. Total SOD activity in the colonic mucosa was measured using a kit (Wako). The assay was carried out in 50 mM PBS, pH 7.8, in an incubator at 36°C . The kinetics of reduction of NBT to blue formazane was monitored through the absorbance changes with time at 560 nm. Solutions of NBT (0.24 mM) and xanthine (0.4 mM) were mixed in the plate and the reaction was started through the addition of a concentrated xanthine oxidase solution. The rate of NBT reduction was measured at 560 nm and then

calculated as a percentage of inhibition of this reaction per mg protein of colonic mucosa. The SOD activity was expressed relative to control, which was standardized to 100. The assay was performed 3 times.

The samples for measuring catalase activity were prepared by the same method described for the SOD activity assay, and the activity was measured by using a kit (Calbiochem) following the manufacturer's instructions. Each of the test samples was assayed at 500 nm and measured on a Multiskan JX (Thermo Labsystems, Vantaa, Finland). The catalase activity was expressed relative to control, which was standardized to 100. The assay was performed 3 times.

2.7. Effects of GTP on colon carcinogenesis induced by DMH and DSS

Following quarantine for 1 week, a total of 111 male ICR mice were divided into an untreated control and nine experimental groups, as shown in Fig. 5. DMH was dissolved in PBS and pH was adjusted to 6.5 by using 0.1 N NaOH. Group 1 (n=12) was served as an untreated control. Group 2 (the GTP alone group, n=6) was fed the diet containing 1% GTP for 18 weeks (from week 2 to week 20) and received no further treatment. Mice of group 3 (the DSS alone group, n=6) was received one-week treatment of 1% DSS (w/v) in drinking water (from week 2 to week 3). Group 4 (the DMH alone group, n=6) was given two weekly intraperitoneal (i.p.) injection of DMH (20 mg/kg body weight). Group 5 (n=12) was given DMH, as did for group 4 and fed with the diet containing 1% GTP for 18 weeks (from week 2 to week 20). Group 6 (n=20) was treated with DMH and followed by one-week treatment of 1% DSS (w/v) in drinking water (from week 2 to week 3). Mice in groups 7-10 (n=12 each for groups 7-9 and

n=13 for group 10) were treated with DMH and DSS and fed the diets mixed with 0.01, 0.1, 0.5, and 1% GTP for 18 weeks, starting one week after the last DMH. Body weight and intakes of food and drinking water were recorded once every week during the study. At week 20, all animals were sacrificed by deep anesthesia with diethyl ether to determine the effects of dietary GTP on colon carcinogenesis induced by DMH and DSS.

2.8. Tissue harvest

At sacrifice, all animals were sacrificed by anesthesia with diethyl ether, and complete necropsy was done on all mice. All organs were macroscopically inspected for the presence of lesions, and colon was then removed. After the length of colons was measured, they were cut open longitudinally along the main axis, and washed with PBS. The colon was placed on filter papers and fixed in Mildform[®]10 N for at least 24 h for counting aberrant crypt foci (ACF), since ACF are early biomarkers for colon carcinogenesis [2,9]. For counting ACF with a stereoscopic microscope (SMZ1000, NikonInstech Co. Ltd., Kawasaki, Japan), fixed colons were dipped in a 0.5% solution of methylene blue in distilled water for 30 s, and briefly washed with distilled water. The large intestines were macroscopically inspected and the volume of tumors was measured. The tumor volumes was calculated using the equation $V=4/3\pi r^3$, where r was the average tumor radius obtained from the three diameter measures. Histopathological examination was performed on H&E-stained sections made from paraffin-embedded blocks. Histological evaluation was done in a blind fashion by a pathologist (T. T.). Colonic neoplasms were diagnosed according to the description by Ward [50].

2.9. Statistical analysis

All measurements except for the incidences of the lesions and survivals are expressed as mean $\pm$ standard deviation (SD) of the mean. Statistically significant differences of the measures between the groups were determined using a Student's *t*-test (two-sided), Fisher's exact probability test or Chi-square test. All statistical analyses were performed with the GraphPad InStat software (version 3.05, GraphPad Software, Inc., San Diego, CA, USA). *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Effects of GTP on DSS-induced acute colitis

The DSS-treated mice (groups 3-7) exhibited symptoms of bloody feces and loss of body weight gain during the study (Fig. 2A). The mean body weights of groups 6 (DSS + 0.5% GTP) and 7 (DSS + 1% GTP, $P < 0.001$) were lower than group 3 (2% DSS alone group) at days 5 and 6, whereas the values of groups 4 (DSS + 0.1% GTP) and 5 (DSS + 0.25% GTP) were comparable to that of groups 1-3. At sacrifice, shortening of the colon was observed in the mice that received DSS (groups 3-7), as shown in Fig. 2B. The colon length of group 3 was shortened by 16% ($P < 0.05$) as compared with that of group 1 (untreated control). The shortening was evident in groups 6 and 7: shortened by 35% ($P < 0.05$) in group 6 and by 33% ($P < 0.05$) in group 7, as compared to that of group 3. The values of groups 4 and 5 did not significantly differ from group 3.

3.2. Cytokine production in colonic mucosa

The effects of GTP on DSS-induced cytokines production in colonic mucosa using ELISA were estimated. The levels of IL-1 β and MIF of group 3 (DSS alone) were significantly larger than the values of group 1 (untreated) ($P < 0.01$ for each, Fig. 3A and B). This DSS-induced increase was attenuated by 79% and 85% in the 0.1% GTP treated group, respectively ($P < 0.01$ for each, Fig. 3A and B), as compared with untreated group. However, the levels of IL-1 β in groups 6 (DSS + 0.5% GTP) and 7 (DSS + 1% GTP) were increased by 2.6-fold when compared with the DSS alone group ($P < 0.01$ for each, Fig. 3A). And the MIF levels were also elevated in these groups by 1.6- (group 6) and 1.8-fold (group 7) as compared to that of the DSS group ($P < 0.01$ for each group), whereas the 0.25% GTP (group 5) did not show significant effects (Fig. 3A and B). The IL-6 and TNF- α were variable among the groups without statistical significance (Fig. 3C and D).

3.3. Activity of antioxidant enzymes

As shown in Fig. 3E and 3F, the activities of SOD and catalase of group 2 (1% GTP alone) were significantly lowered than group 1 (untreated) ($P < 0.05$ for each value). These measures of group 3 (DSS alone) were comparable to the group 1. The treatment with GTP (0.1-1% in diet) together with DSS (2% in drinking water) did not affect these enzyme activities as compared with the DSS-treated mice.

3.4. Histopathology of colonic mucosa

Mucosal ulcerations were not observed in groups 1 (Fig. 4A) and 2 (Fig. 4B). Mucosal ulceration with marked inflammation and edema in the mucosa and submucosa was evident in groups 3 (Fig. 4C), 6 (Fig. 4F), and 7 (Fig. 4G). When compared these groups, regenerative changes of the crypt cells occurred in groups 4 (Fig. 4D) and 5 (Fig. 4E). The mean numbers of mucosal ulcer per mouse in all groups are illustrated in Fig. 5A. However, the numbers of groups 4 and 5 tend to be smaller than group 3 without statistical significance. When compared to group 3, the values of groups 6 and 7 showed similar levels of group 3. As for inflammation score, the score of each group did not reach to the significant differences.

3.5. The effects of GTP on inflammation-associated colon carcinogenesis

At week 20 the mean of body weight and colon length did not significantly differ among the groups (data not shown). Data on enumeration of ACF (insert in Fig. 7A) is given in Fig. 7A. The multiplicity of ACF formation of group 5 was the greatest, and the value was followed by group 4, and groups 6-10. ACF was not observed in groups 1-3. Colonic tumors developed in groups 6-10, but not in groups 1-5. The multiplicities of adenoma (insert in Fig. 7B) and adenocarcinoma (insert in Fig. 7C) showed a slight tendency to increase among the groups 7-10, respectively (Fig. 7B and C). The values of groups 9 and 10 tend to be larger than that of group 6 ($P < 0.16$ for each lesion), these 0.5% and 1% doses of GTP were failed to decrease colonic tumor induced by DMH/DSS. As to tumors volumes, the values of groups 7-10 have a slight tendency to be greater than that of group 6 without statistical significance (Fig. 7D). One % GTP +

DMH + DSS increased tumor incidence (78%) as compare to DMH + DSS (56%), while in the 0.01~0.5% GTP + DMH + DSS showed by 30~44% as tumor incidence (data not shown).

4. Discussion

EGCG, the main constituent in green tea, is a potential antioxidant, which serves as a metal ion chelater and ROS scavenger [33]. GTP has many favorable properties, including an effective chemopreventive effect. The properties were supported by numerous epidemiological studies in several countries [55] and their promising anti-cancer properties and a variety of action mechanisms. On the other hand, a few cohort and case-control studies have suggested the no association between green tea consumption and decreased risk of CRC development [6,8,27].

In the carcinogenesis experiment of this study, oral administration of 1% GTP in diet failed to suppress colonic tumor formation as compared to the DMH/DSS-treated mice (Fig. 7B and C). Furthermore, 1% GTP treatment after DMH exposure tend to increased ACF formation as compared with the DMH alone group (Fig. 7A). These findings are in line with those reported by Hirose et al. [18], who demonstrated that dietary GTP enhanced colon carcinogenesis in rats initiated with DMH. Moreover, feeding with 3600 ppm of GTP in diet resulted in significant increase in multiplicity of colonic tumors, when compared to the carcinogen, azoxymethane (AOM) alone group [52]. In fact, it has recently been reported that the levels of EGCG was curvilinear and plateaued between 500 and 2000 mg/kg intragastrically in the small intestine and colon [31]. Furthermore, Lambert et al. [32] reported that after oral administration of EGCG at

163.8 $\mu\text{mol/kg}$, high concentrations of EGCG were observed in the small intestine ($46.2 \pm 13.5 \text{ nmol/g}$) and colon ($7.9 \pm 2.4 \text{ nmol/g}$). And assuming that 1 g of tissue is equivalent to 1 ml, these high concentrations are in the range of those used in cell culture experiments. The results of the present study, together with previous findings, may thus suggest that oral intake of high dose of GTP is adversely affected colon carcinogenesis of certain population, who has inflamed colon. To support this notion, green tea at a high dose (6 g/day) caused toxic symptoms that included nausea, emesis, insomnia, fatigue, diarrhea, abdominal pain, and confusion in patients with androgen independent prostate cancer [22]. Also, adverse events, e.g., excess gas, upset stomach, nausea, heartburn, stomach ache, abdominal pain, dizziness, headache, and muscle pain, were reported in healthy individuals who took EGCG or polyphenon E containing EGCG (400 mg twice/day or 800 mg once/day) for 4 weeks [10]. However, all of the reported events were rated as being mild.

In the experimental animal studies, green tea catechin (GTC) significantly inhibited AOM-induced rat colon carcinogenesis when GTC was administered during the post-initiation stage at doses of 0.01-0.1% [53], which were comparable to the average intake of green tea of Japanese people [53]. In this study, when feeding with 0.01% GTP-containing diet was started at the beginning of DSS exposure, tumors multiplicity tend to decreased, but 0.5 and 1% GTP in diet were failed to decrease the frequency (Fig. 7B and C). Thus, our findings suggest that modifying (beneficial or harmful) effects of GTP on colitis-related colon carcinogenesis depend on the dose in diet.

We demonstrated for the first time that dietary GTP at dose levels of 0.5 and 1% profoundly enhances the DSS-induced acute colitis in mice presumably through increases in IL-1 β and MIF expression. IL-1 β and MIF, hallmarks of pro-inflammatory

cytokines, were significantly enhanced by feeding with 0.5% and 1% GTP, whereas 0.1% GTP in diet suppressed the expression (Fig. 3A and B). Even though, 0.25% GTP in diet had a tendency to recover ulcer and inflammation (Fig. 5A and B), however it did not showed the decreasing effects of IL-1 β and MIF production (Fig. 3A and B). It is difficult to define how 0.25% GTP in diet plays on colitis. In patients with colitis-associated colon cancer, several inflammatory mediators and cytokines were increased in their serum [7,13,23,44]. IL-1 β is a pivotal mediator in the inflammatory immune response that is characteristic of destructive inflammatory diseases [5]. The recent identification of the inflammasome, a multiprotein complex responsible for the activation of the IL-1 β converting enzyme (ICE, caspase-1), has generated new possibilities for the elucidation of the etiology and pathophysiology of IBD as a new treatment target [15]. Evidence presented above suggests that certain pro-inflammatory cytokines and enzymes play a major role in mediating tumorigenesis. Thus, elevation of inflammatory cytokines such IL-1 β and MIF may be the major factors for GTP-deteriorated colitis. Meanwhile, IL-6 and TNF- α levels in present result were no significant differences in the colon of DSS-treated groups, even GTP-, as compared with control mice. IL-6 [1] and TNF- α [41] also play important roles in colitis induced by trinitrobenzenesulfonic acid and acetic acid, respectively. However, Kwon et al. [30] reported that TNF- α showed the constitutive expression in DSS-treated colonic mucosa and present results of IL-6 and TNF- α levels may related to difference of strain and drugs.

Colonic oxidative stress is characterized by the pathogenesis in colitis through the release of inflammatory mediators and cytokines [4,29]. Therefore, ROS has potential to promote proliferation of inflammatory and epithelial cells. Concomitantly, ROS induced

the resistance to apoptosis via activating redox-sensitive transcription factors, the most well-known being nuclear factor- κ B and activator protein-1 [40]. Previous studies have shown that EGCG not only has an antioxidative property but also pro-oxidative property [12,14]. We previously reported that EGCG activates oxidative stress signaling pathways *in vitro* [26]. A major cellular defence against ROS is provided by SOD and catalase, both of which detoxify superoxide radical to water and molecular oxygen. Therefore, the activities of antioxidant enzymes in the colonic mucosa of mice given GTP were investigated in the current study. The activities of SOD and catalase were decreased in the 1% GTP alone group as compared to an untreated group, although dietary GTP did not significantly affect their activities in the DSS-administrated mice (Fig. 3E and F). These findings are inconsistent with those of cytokine production (Fig. 3A and B). At present, we are unable to explain for the contradictory results, but a time-course experiment, which is underway in our laboratory, will provide detailed insights into the redox regulation of dietary GTP in the inflamed colon of mice.

In conclusion, our results described here, together with some previous reports by others, suggest that higher doses of dietary GTP deteriorate colitis and failed to prevent colon carcinogenesis in the inflamed colon. Our findings may also indicate that excess intake of GTP or derived supplements can not be expected chemopreventive effects in certain patients who are at particular risk for developing epithelial malignancies in the inflamed large bowel.

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Figure legends

Fig. 1. Experimental protocol for determining the effects of GTP on acute colitis induced by DSS. In the untreated control group, male ICR mice were given tap water and basal diet *ad libitum*, freshly changed every day, for 6 days. In the DSS group, the mice were fed with a basal diet and 2% DSS (w/v) in tap water for 6 days to induce colitis. In the DSS + GTP groups, mice were fed with diets containing GTP (0.1, 0.25, 0.5, and 1%) for 6 days, starting the DSS exposure. The GTP alone group was fed with the diet mixed with 1% GTP and did not receive DSS (n=12-13).

Fig. 2. Effects of dietary GTP on the body weight gain (A) and colon length (B) in the DSS-induced colitis experiment. Body weights of all mice were recorded every day. Colon length was measured at sacrifice (day 6) (Values are the mean $\pm$ SD). ^aSignificantly different from group 1 of 5 days ($P < 0.01$). ^bSignificantly different from group 3 of 5 days ($P < 0.001$). ^cSignificantly different from group 1 of 6 days ($P < 0.001$). ^dSignificantly different from group 3 of 6 days ($P < 0.001$). ^eSignificantly different from group 1 ($P < 0.05$). ^fSignificantly different from group 3 ($P < 0.05$) (n=12-13).

Fig. 3. Expression levels of IL-1 β (A), MIF (B), IL-6 (C), TNF- α (D) and activities of SOD (E) and catalase (F) in colonic mucosa (Values are the mean $\pm$ SD). ^aSignificantly different from group 1 ($P < 0.01$). ^bSignificantly different from group 3 ($P < 0.01$). ^cSignificantly different from group 1 ($P < 0.05$) (n=9).

Fig. 4. Representative histopathology of the colon of mice belonging to groups 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6 (F), and 7 (G). H&E-stain. Original magnification, A-G, x40.

Fig. 5. Mean number of mucosal ulcers (A) and inflammation scores (B) of colon. (Values are the mean $\pm$ SD) (n=3-4).

Fig. 6. Experimental protocol for examining the modifying effects of GTP on DMH/DSS-induced mouse colon carcinogenesis. Group 1 was an untreated control. Male ICR mice in groups 4-10 were initiated with two weekly i.p. injection of DMH (20 mg/kg body weight). Seven days after the last DMH injection, mice in groups 3 and 6-10 were given 1% DSS (w/v) in drinking water for 1 week. In groups 7-10, 0.01, 0.1, 0.5, and 1% GTP-containing diets were given, respectively, starting at the beginning of DSS treatment, and then continued on these experimental diets for 18 weeks. To examine whether GTP promotes DMH-induced colon carcinogenesis, 1% GTP-added diet was fed after DMH treatment without DSS exposure (group 5). Group 2 was fed with 1% GTP-added diet without any further treatment for the same period. All animals were sacrificed by anesthesia with diethyl ether at 20 weeks (n=6 or 12-13 or 20).

Fig. 7. The multiplicity of ACF (A), adenoma (B), adenocarcinoma (C), and volumes of tumors (D) (n=6 or 12-13 or 20).

Fig. 1. M. Kim et al

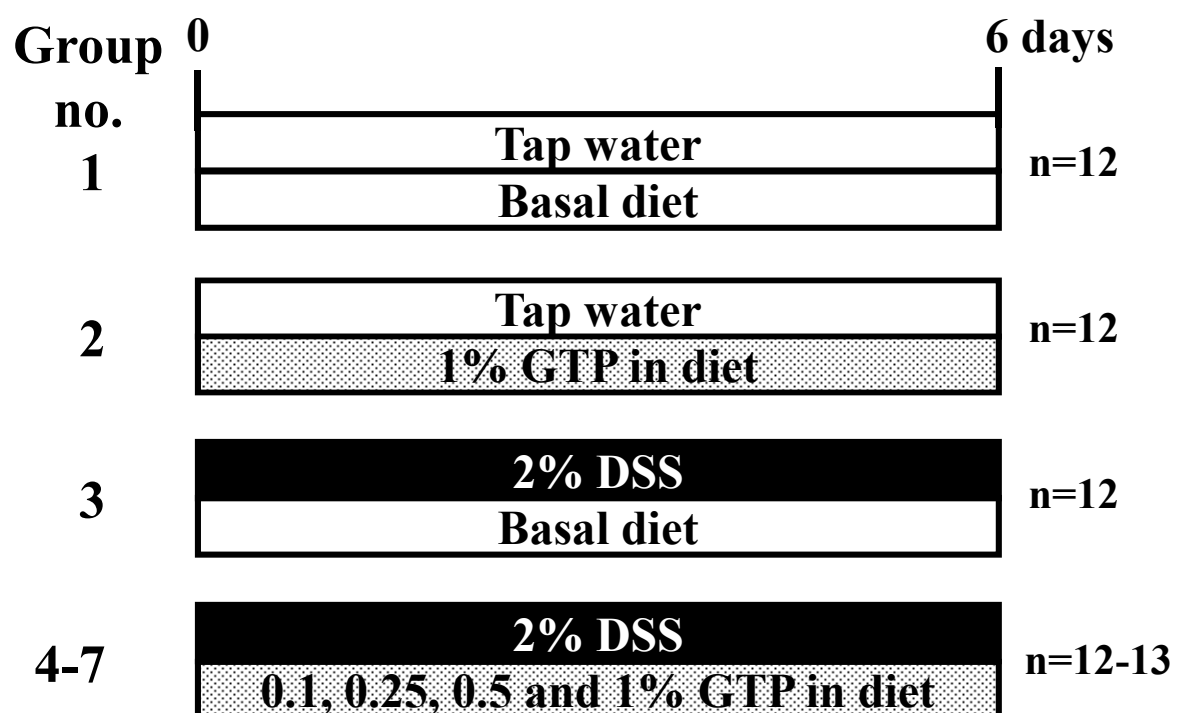


Fig. 2. M. Kim et al

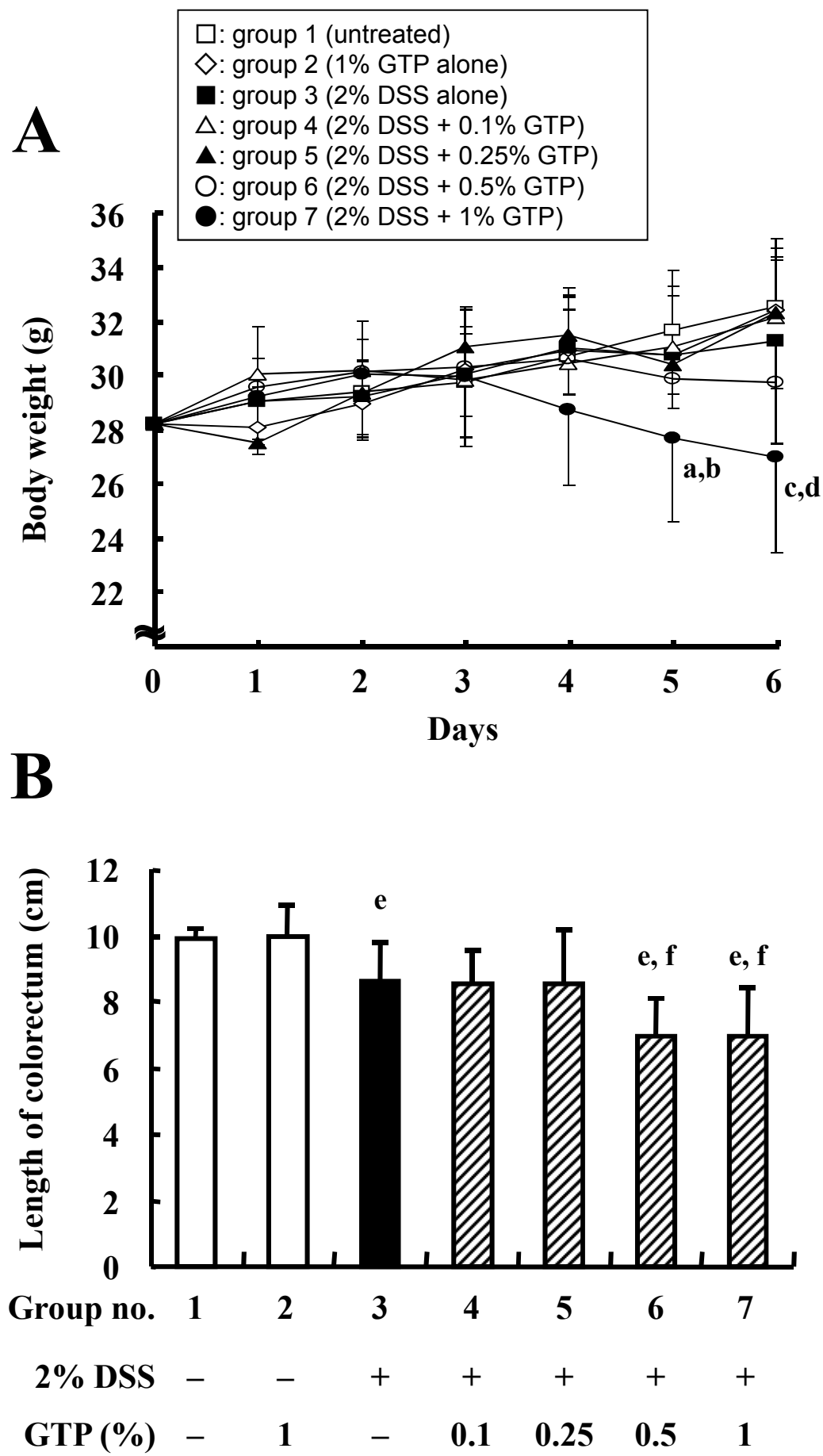


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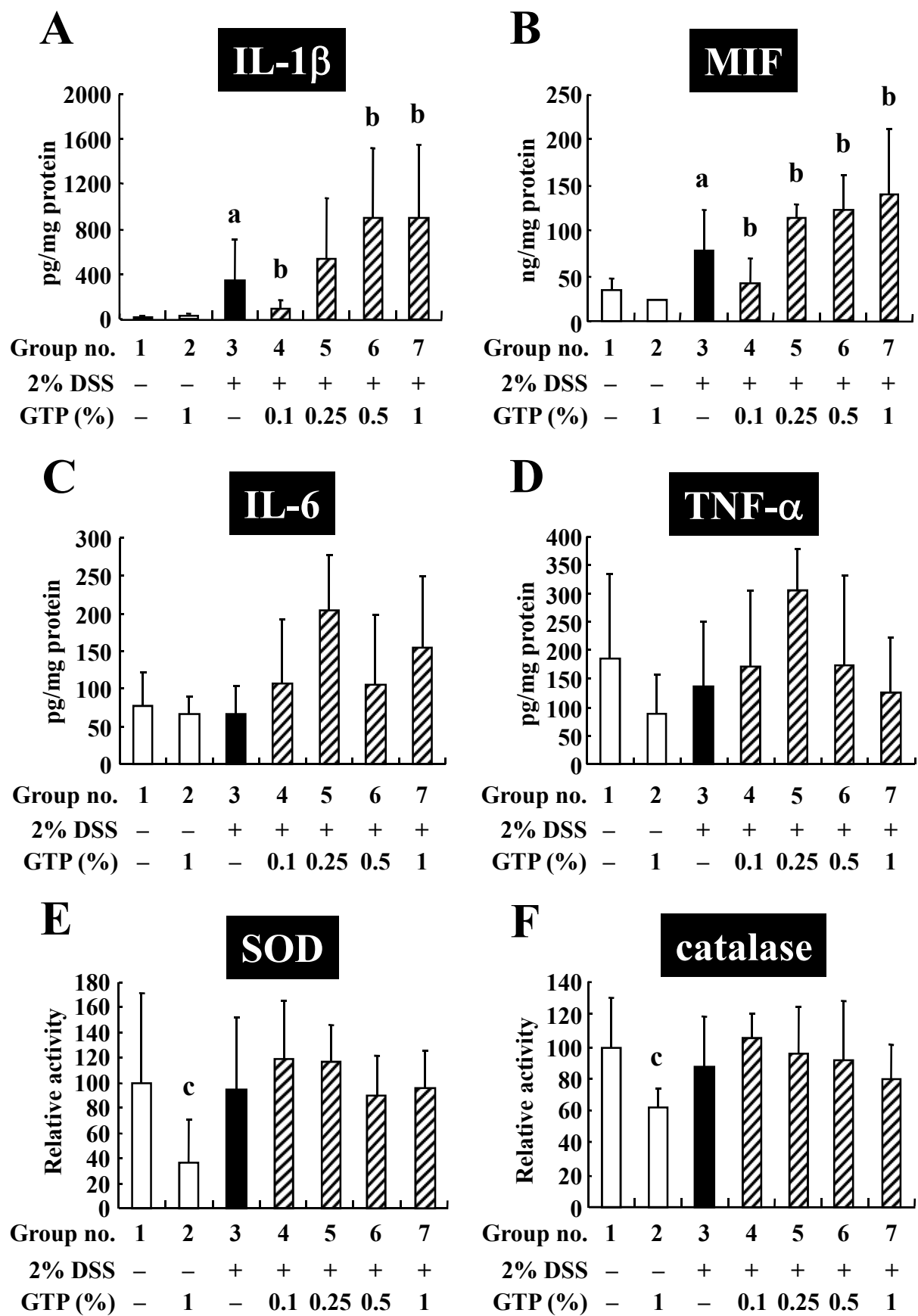


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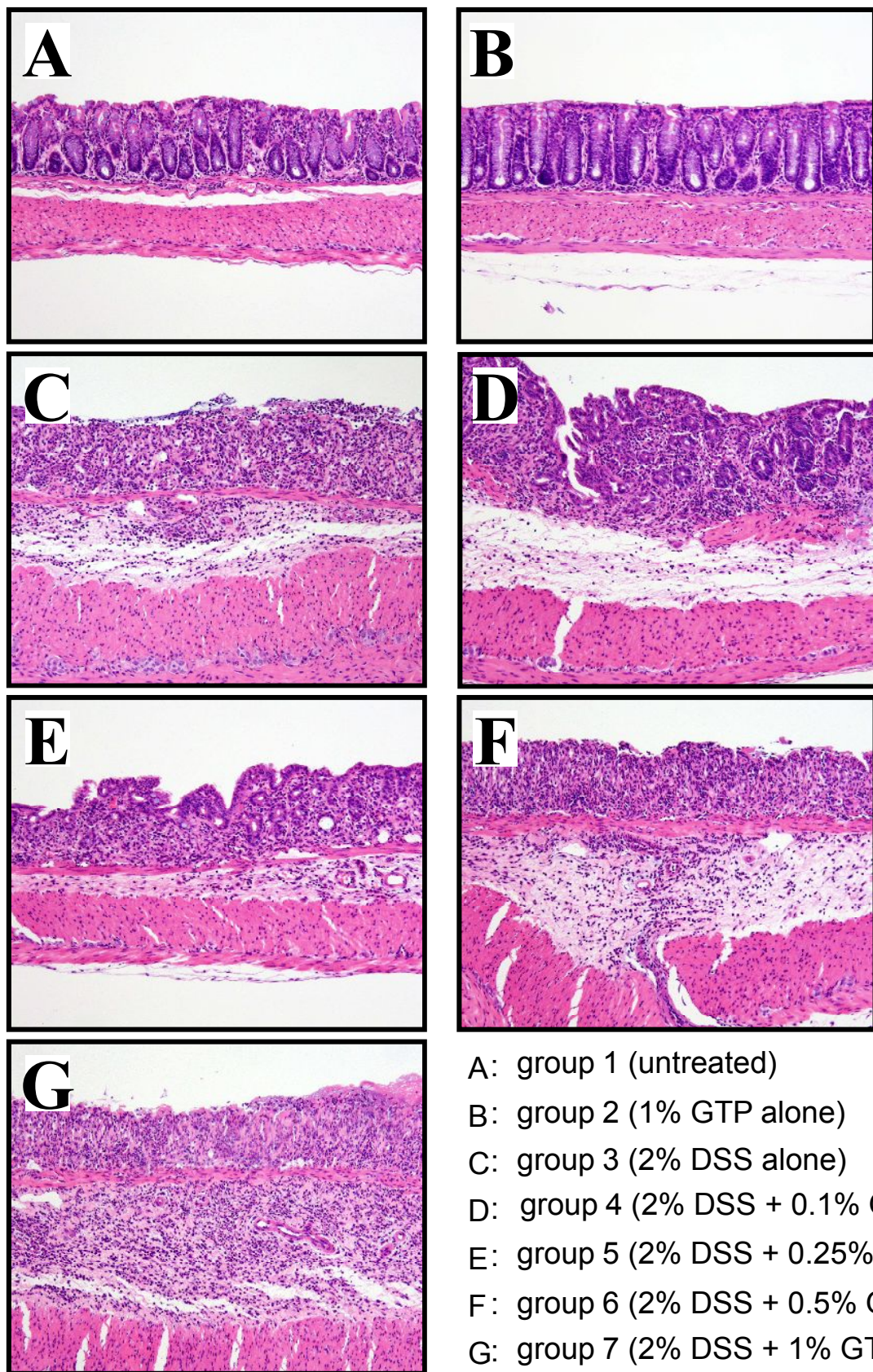


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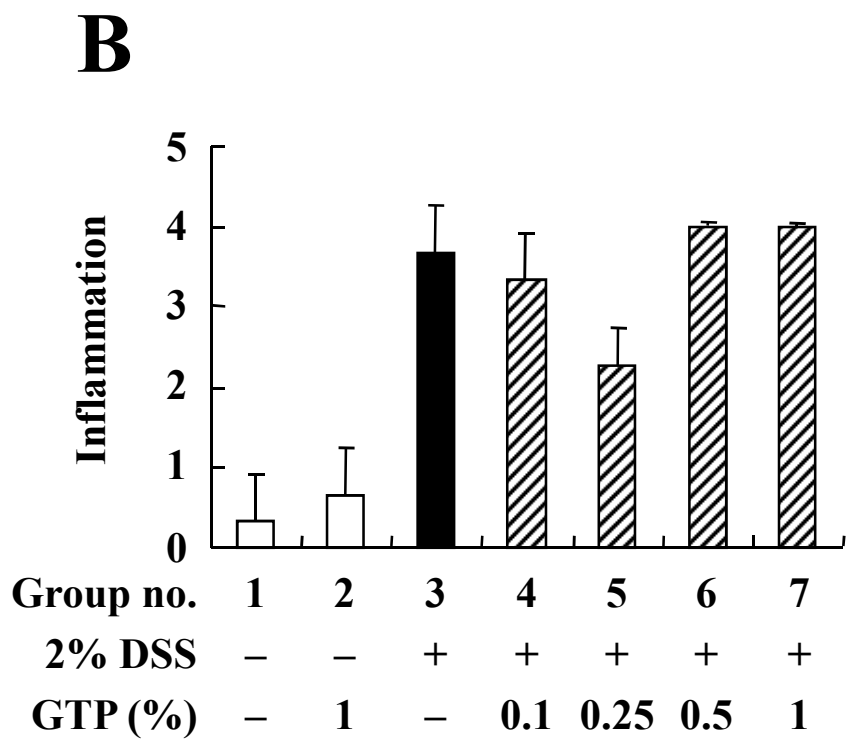
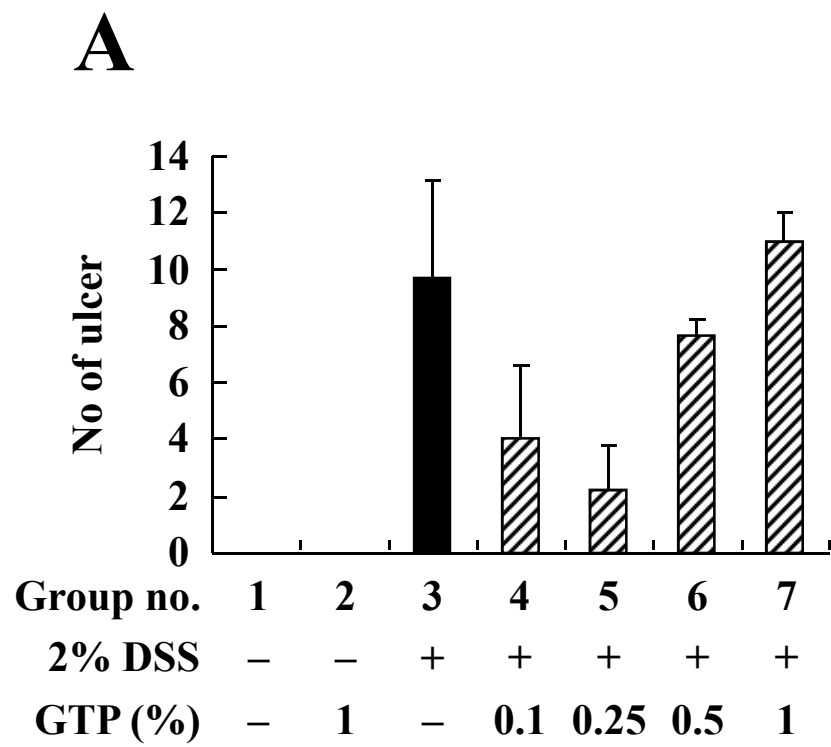


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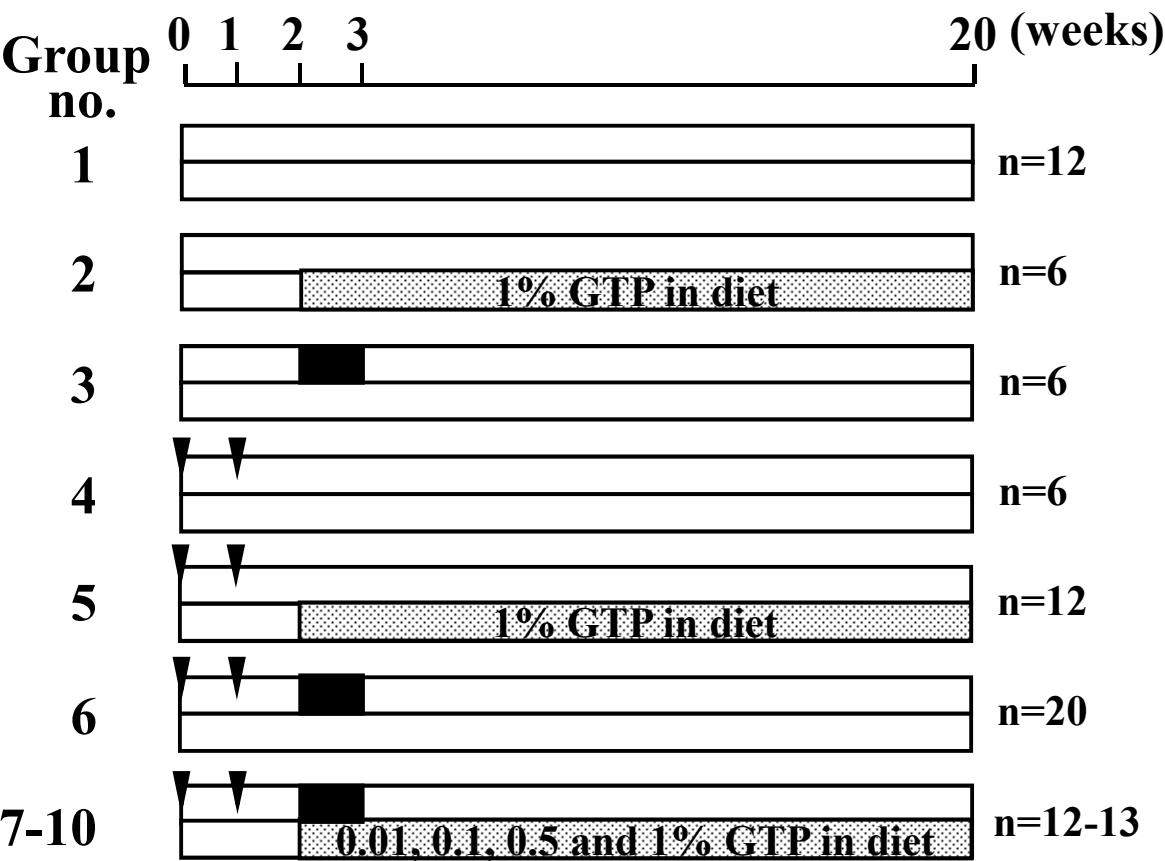


Fig. 7. M. Kim et al

