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

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

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

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

18

1 **1. Introduction**

2

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

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

1 Reinecker et al., found nitrate and oxidative DNA lesion products in inflamed colonic
2 mucosa of rodents and IBD patients [25,42]. The effects of GTP on colon
3 carcinogenesis are still controversial. Hirose et al., reported the enhancing effects of
4 GTP on colon carcinogenesis in rats [18,19], although there were no proposed
5 underlying mechanisms. Also, there has been no consistent association between the
6 reduction of risk of CRC [24,47,51] or gastric cancer [48] and green tea consumption in
7 previous epidemiologic studies, while green tea consumption has contributed to
8 significantly reduced risk of breast, esophagus, kidney, liver, lung, pancreas cancers
9 [55].

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

21 To determine the modifying effects (enhancement or inhibition) of GTP in colitis
22 and inflammation-associated colon carcinogenesis, we first examined the effect of GTP
23 on DSS-induced acute colitis in ICR male mice, since this model is useful to investigate
24 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**

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12 *2.1. Animals*

13

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*

24

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

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12 *2.3. Effects of GTP on acute colitis induced by DSS*

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

1 effects of dietary GTP on DSS-induced acute colitis.

2

3 *2.4. Isolation of colonic mucosa*

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

24

1 *2.5. Assay for cytokine production*

2

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

13

14 *2.6. Measurement of SOD and catalase activities*

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

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

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

10

11 *2.7. Effects of GTP on colon carcinogenesis induced by DMH and DSS*

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13 Following quarantine for 1 week, a total of 111 male ICR mice were divided into an
14 untreated control and nine experimental groups, as shown in Fig. 5. DMH was dissolved
15 in PBS and pH was adjusted to 6.5 by using 0.1 N NaOH. Group 1 (n=12) was served as
16 an untreated control. Group 2 (the GTP alone group, n=6) was fed the diet containing
17 1% GTP for 18 weeks (from week 2 to week 20) and received no further treatment.
18 Mice of group 3 (the DSS alone group, n=6) was received one-week treatment of 1%
19 DSS (*w/v*) in drinking water (from week 2 to week 3). Group 4 (the DMH alone group,
20 n=6) was given two weekly intraperitoneal (i.p.) injection of DMH (20 mg/kg body
21 weight). Group 5 (n=12) was given DMH, as did for group 4 and fed with the diet
22 containing 1% GTP for 18 weeks (from week 2 to week 20). Group 6 (n=20) was
23 treated with DMH and followed by one-week treatment of 1% DSS (*w/v*) in drinking
24 water (from week 2 to week 3). Mice in groups 7-10 (n=12 each for groups 7-9 and

1 n=13 for group 10) were treated with DMH and DSS and fed the diets mixed with 0.01,
2 0.1, 0.5, and 1% GTP for 18 weeks, starting one week after the last DMH. Body weight
3 and intakes of food and drinking water were recorded once every week during the study.
4 At week 20, all animals were sacrificed by deep anesthesia with diethyl ether to
5 determine the effects of dietary GTP on colon carcinogenesis induced by DMH and
6 DSS.

7

8 *2.8. Tissue harvest*

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

24

1 *2.9. Statistical analysis*

2

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

10

11 **3. Results**

12

13 *3.1. Effects of GTP on DSS-induced acute colitis*

14

15 The DSS-treated mice (groups 3-7) exhibited symptoms of bloody feces and loss of
16 body weight gain during the study (Fig. 2A). The mean body weights of groups 6 (DSS
17 + 0.5% GTP) and 7 (DSS + 1% GTP, *P* < 0.001) were lower than group 3 (2% DSS
18 alone group) at days 5 and 6, whereas the values of groups 4 (DSS + 0.1% GTP) and 5
19 (DSS + 0.25% GTP) were comparable to that of groups 1-3. At sacrifice, shortening of
20 the colon was observed in the mice that received DSS (groups 3-7), as shown in Fig. 2B.
21 The colon length of group 3 was shortened by 16% (*P* < 0.05) as compared with that of
22 group 1 (untreated control). The shortening was evident in groups 6 and 7: shortened by
23 35% (*P* < 0.05) in group 6 and by 33% (*P* < 0.05) in group 7, as compared to that of
24 group 3. The values of groups 4 and 5 did not significantly differ from group 3.

1

2 *3.2. Cytokine production in colonic mucosa*

3

4 The effects of GTP on DSS-induced cytokines production in colonic mucosa using
5 ELISA were estimated. The levels of IL-1 β and MIF of group 3 (DSS alone) were
6 significantly larger than the values of group 1 (untreated) ($P < 0.01$ for each, Fig. 3A
7 and B). This DSS-induced increase was attenuated by 79% and 85% in the 0.1% GTP
8 treated group, respectively ($P < 0.01$ for each, Fig. 3A and B), as compared with
9 untreated group. However, the levels of IL-1 β in groups 6 (DSS + 0.5% GTP) and 7
10 (DSS + 1% GTP) were increased by 2.6-fold when compared with the DSS alone group
11 ($P < 0.01$ for each, Fig. 3A). And the MIF levels were also elevated in these groups by
12 1.6- (group 6) and 1.8-fold (group 7) as compared to that of the DSS group ($P < 0.01$ for
13 each group), whereas the 0.25% GTP (group 5) did not show significant effects (Fig. 3A
14 and B). The IL-6 and TNF- α were variable among the groups without statistical
15 significance (Fig. 3C and D).

16

17 *3.3. Activity of antioxidant enzymes*

18

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

24

1 *3.4. Histopathology of colonic mucosa*

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

11
12 *3.5. The effects of GTP on inflammation-associated colon carcinogenesis*

13
14 At week 20 the mean of body weight and colon length did not significantly differ
15 among the groups (data not shown). Data on enumeration of ACF (insert in Fig. 7A) is
16 given in Fig. 7A. The multiplicity of ACF formation of group 5 was the greatest, and the
17 value was followed by group 4, and groups 6-10. ACF was not observed in groups 1-3.
18 Colonic tumors developed in groups 6-10, but not in groups 1-5. The multiplicities of
19 adenoma (insert in Fig. 7B) and adenocarcinoma (insert in Fig. 7C) showed a slight
20 tendency to increase among the groups 7-10, respectively (Fig. 7B and C). The values
21 of groups 9 and 10 tend to be larger than that of group 6 ($P < 0.16$ for each lesion), these
22 0.5% and 1% doses of GTP were failed to decrease colonic tumor induced by
23 DMH/DSS. As to tumors volumes, the values of groups 7-10 have a slight tendency to
24 be greater than that of group 6 without statistical significance (Fig. 7D). One % GTP +

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

4 5 **4. Discussion**

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

14 In the carcinogenesis experiment of this study, oral administration of 1% GTP in
15 diet failed to suppress colonic tumor formation as compared to the DMH/DSS-treated
16 mice (Fig. 7B and C). Furthermore, 1% GTP treatment after DMH exposure tend to
17 increased ACF formation as compared with the DMH alone group (Fig. 7A). These
18 findings are in line with those reported by Hirose et al. [18], who demonstrated that
19 dietary GTP enhanced colon carcinogenesis in rats initiated with DMH. Moreover,
20 feeding with 3600 ppm of GTP in diet resulted in significant increase in multiplicity of
21 colonic tumors, when compared to the carcinogen, azoxymethane (AOM) alone group
22 [52]. In fact, it has recently been reported that the levels of EGCG was curvilinear and
23 plateaued between 500 and 2000 mg/kg intragastrically in the small intestine and colon
24 [31]. Furthermore, Lambert et al. [32] reported that after oral administration of EGCG at

1 163.8 $\mu\text{mol/kg}$, high concentrations of EGCG were observed in the small intestine (46.2
2 ± 13.5 nmol/g) and colon (7.9 ± 2.4 nmol/g). And assuming that 1 g of tissue is
3 equivalent to 1 ml, these high concentrations are in the range of those used in cell
4 culture experiments. The results of the present study, together with previous findings,
5 may thus suggest that oral intake of high dose of GTP is adversely affected colon
6 carcinogenesis of certain population, who has inflamed colon. To support this notion,
7 green tea at a high dose (6 g/day) caused toxic symptoms that included nausea, emesis,
8 insomnia, fatigue, diarrhea, abdominal pain, and confusion in patients with androgen
9 independent prostate cancer [22]. Also, adverse events, e.g., excess gas, upset stomach,
10 nausea, heartburn, stomach ache, abdominal pain, dizziness, headache, and muscle pain,
11 were reported in healthy individuals who took EGCG or polyphenon E containing
12 EGCG (400 mg twice/day or 800 mg once/day) for 4 weeks [10]. However, all of the
13 reported events were rated as being mild.

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

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

1 cytokines, were significantly enhanced by feeding with 0.5% and 1% GTP, whereas
2 0.1% GTP in diet suppressed the expression (Fig. 3A and B). Even though, 0.25% GTP
3 in diet had a tendency to recover ulcer and inflammation (Fig. 5A and B), however it
4 did not showed the decreasing effects of IL-1 β and MIF production (Fig. 3A and B). It
5 is difficult to define how 0.25% GTP in diet plays on colitis. In patients with
6 colitis-associated colon cancer, several inflammatory mediators and cytokines were
7 increased in their serum [7,13,23,44]. IL-1 β is a pivotal mediator in the inflammatory
8 immune response that is characteristic of destructive inflammatory diseases [5]. The
9 recent identification of the inflammasome, a multiprotein complex responsible for the
10 activation of the IL-1 β converting enzyme (ICE, caspase-1), has generated new
11 possibilities for the elucidation of the etiology and pathophysiology of IBD as a new
12 treatment target [15]. Evidence presented above suggests that certain pro-inflammatory
13 cytokines and enzymes play a major role in mediating tumorigenesis. Thus, elevation of
14 inflammatory cytokines such IL-1 β and MIF may be the major factors for
15 GTP-deteriorated colitis. Meanwhile, IL-6 and TNF- α levels in present result were no
16 significant differences in the colon of DSS-treated groups, even GTP-, as compared
17 with control mice. IL-6 [1] and TNF- α [41] also play important roles in colitis induced
18 by trinitrobenzenesulfonic acid and acetic acid, respectively. However, Kwon et al. [30]
19 reported that TNF- α showed the constitutive expression in DSS-treated colonic mucosa
20 and present results of IL-6 and TNF- α levels may related to difference of strain and
21 drugs.

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

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

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

21

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23

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12

1 **Figure legends**

2

3 Fig. 1. Experimental protocol for determining the effects of GTP on acute colitis
4 induced by DSS. In the untreated control group, male ICR mice were given tap water
5 and basal diet *ad libitum*, freshly changed every day, for 6 days. In the DSS group, the
6 mice were fed with a basal diet and 2% DSS (w/v) in tap water for 6 days to induce
7 colitis. In the DSS + GTP groups, mice were fed with diets containing GTP (0.1, 0.25,
8 0.5, and 1%) for 6 days, starting the DSS exposure. The GTP alone group was fed with
9 the diet mixed with 1% GTP and did not receive DSS (n=12-13).

10

11 Fig. 2. Effects of dietary GTP on the body weight gain (A) and colon length (B) in the
12 DSS-induced colitis experiment. Body weights of all mice were recorded every day.
13 Colon length was measured at sacrifice (day 6) (Values are the mean \pm SD).
14 ^aSignificantly different from group 1 of 5 days ($P < 0.01$). ^bSignificantly different from
15 group 3 of 5 days ($P < 0.001$). ^cSignificantly different from group 1 of 6 days ($P <$
16 0.001). ^dSignificantly different from group 3 of 6 days ($P < 0.001$). ^eSignificantly
17 different from group 1 ($P < 0.05$). ^fSignificantly different from group 3 ($P < 0.05$)
18 (n=12-13).

19

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

24

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

3

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

6

7 Fig. 6. Experimental protocol for examining the modifying effects of GTP on
8 DMH/DSS-induced mouse colon carcinogenesis. Group 1 was an untreated control.
9 Male ICR mice in groups 4-10 were initiated with two weekly i.p. injection of DMH (20
10 mg/kg body weight). Seven days after the last DMH injection, mice in groups 3 and
11 6-10 were given 1% DSS (w/v) in drinking water for 1 week. In groups 7-10, 0.01, 0.1,
12 0.5, and 1% GTP-containing diets were given, respectively, starting at the beginning of
13 DSS treatment, and then continued on these experimental diets for 18 weeks. To
14 examine whether GTP promotes DMH-induced colon carcinogenesis, 1% GTP-added
15 diet was fed after DMH treatment without DSS exposure (group 5). Group 2 was fed
16 with 1% GTP-added diet without any further treatment for the same period. All animals
17 were sacrificed by anesthesia with diethyl ether at 20 weeks (n=6 or 12-13 or 20).

18

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

21

Fig. 1. M. Kim et al

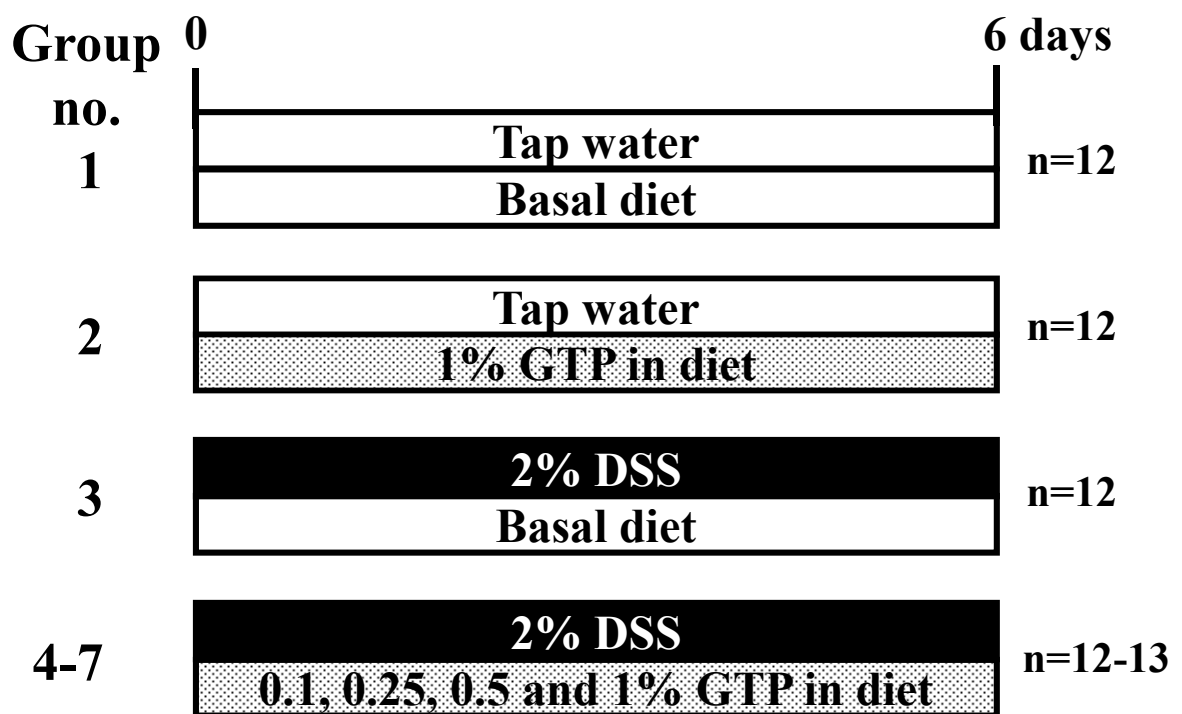


Fig. 2. M. Kim et al

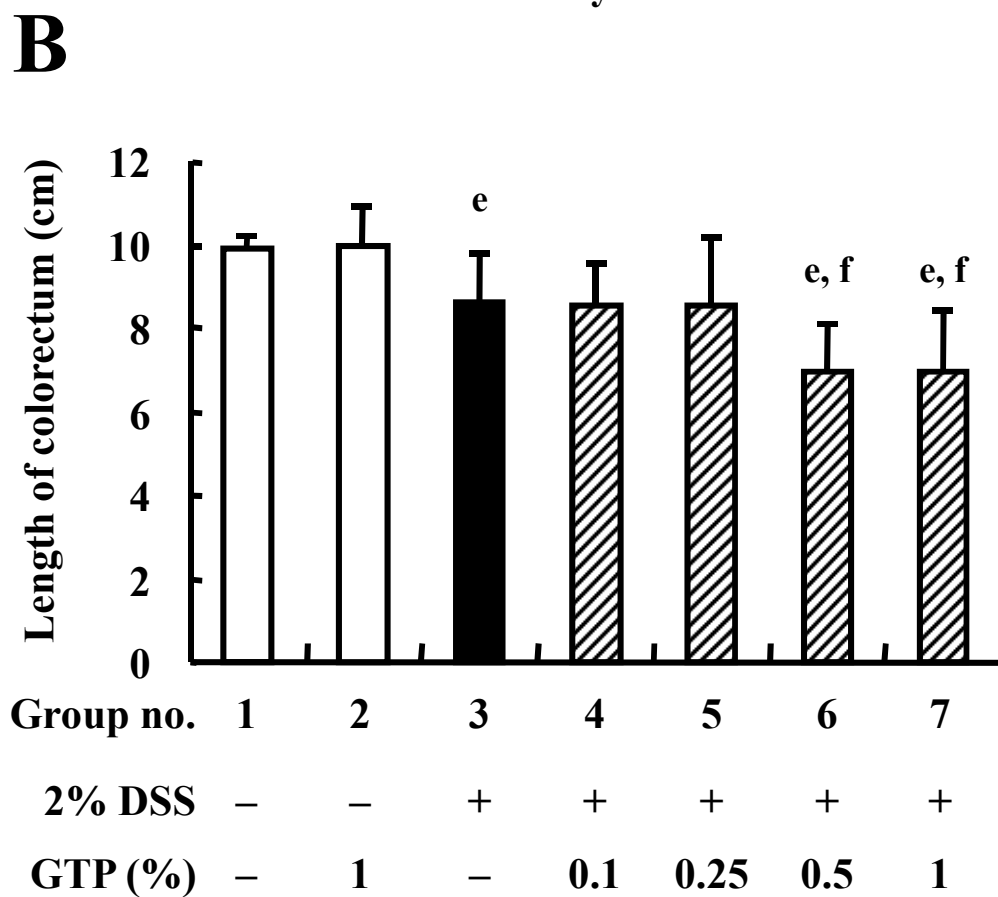
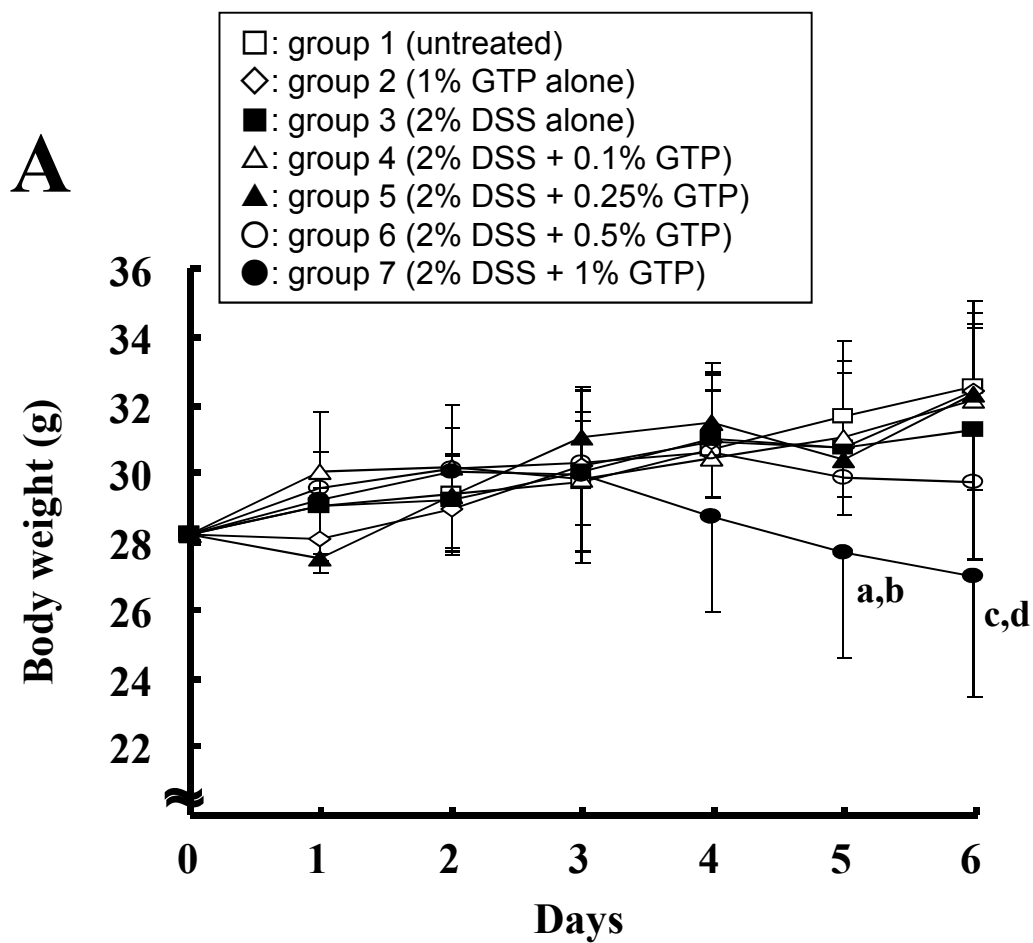


Fig. 3. M. Kim et al

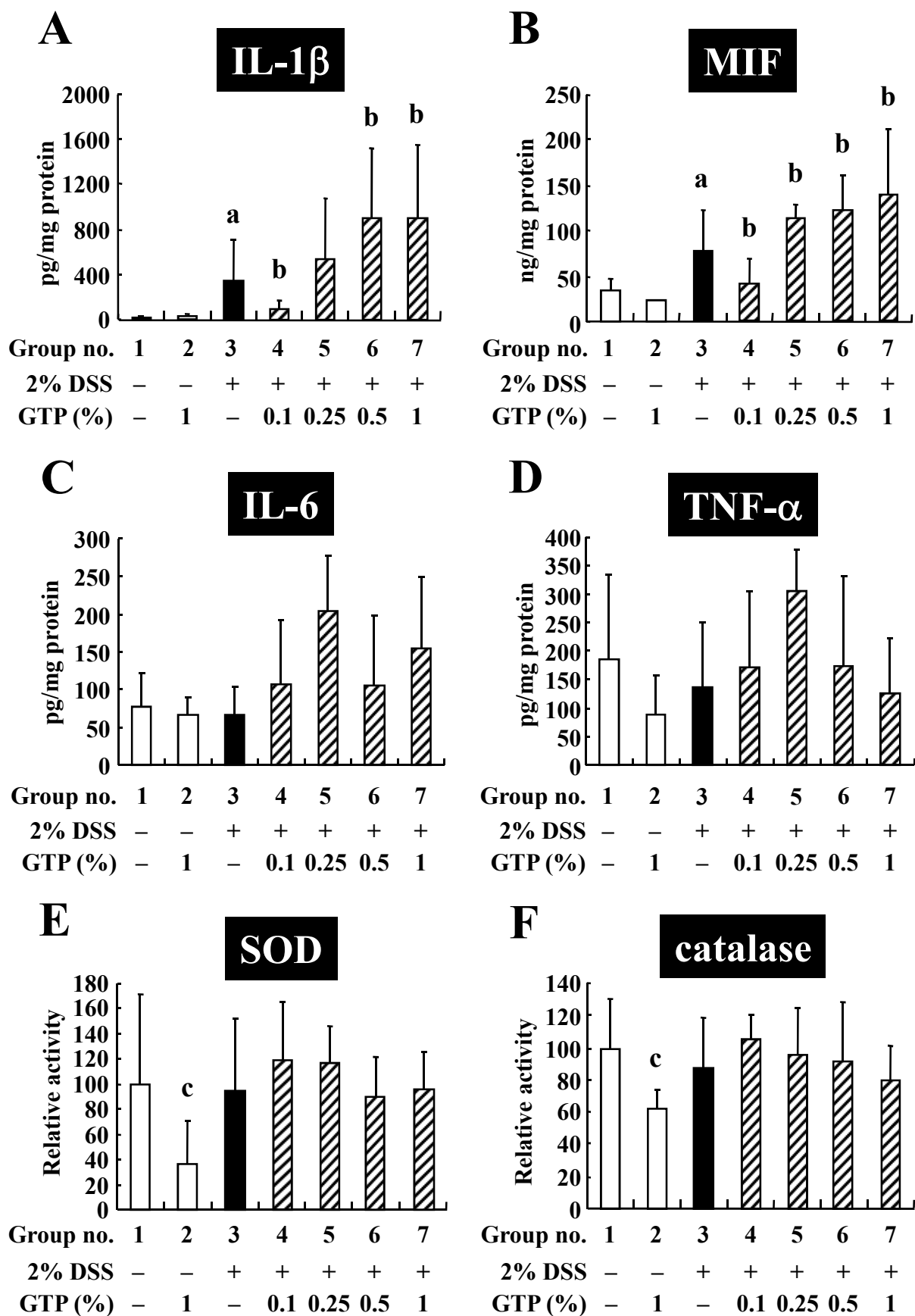


Fig. 4. M. Kim et al

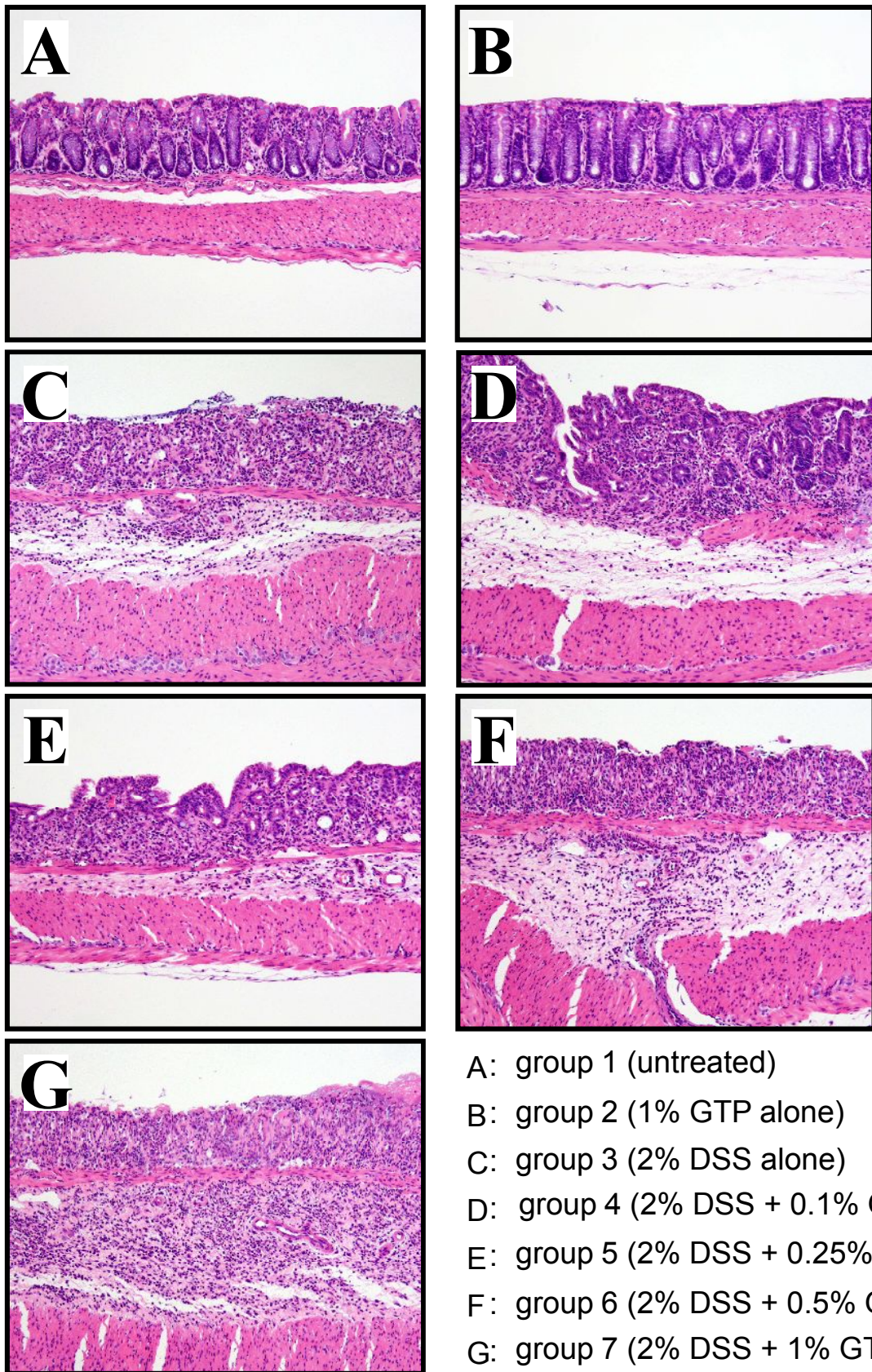
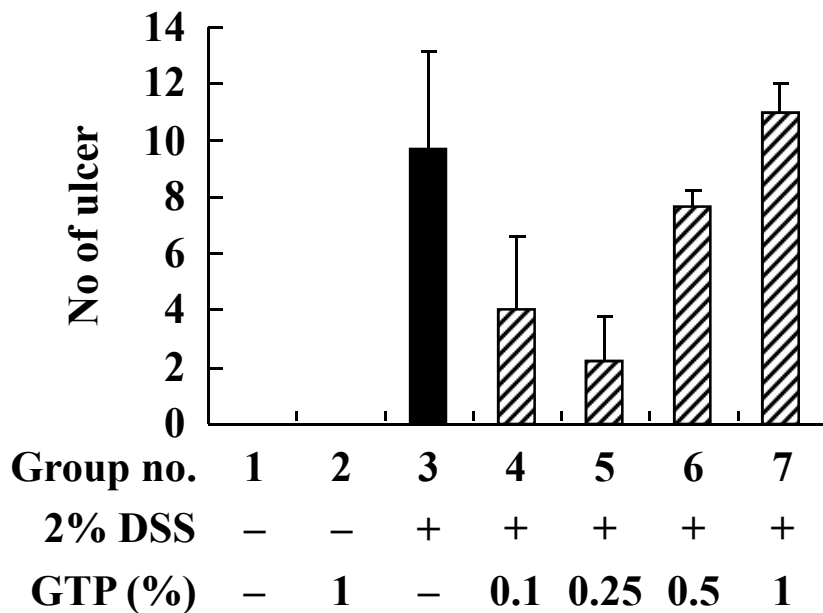


Fig. 5. M. Kim et al.

A



B

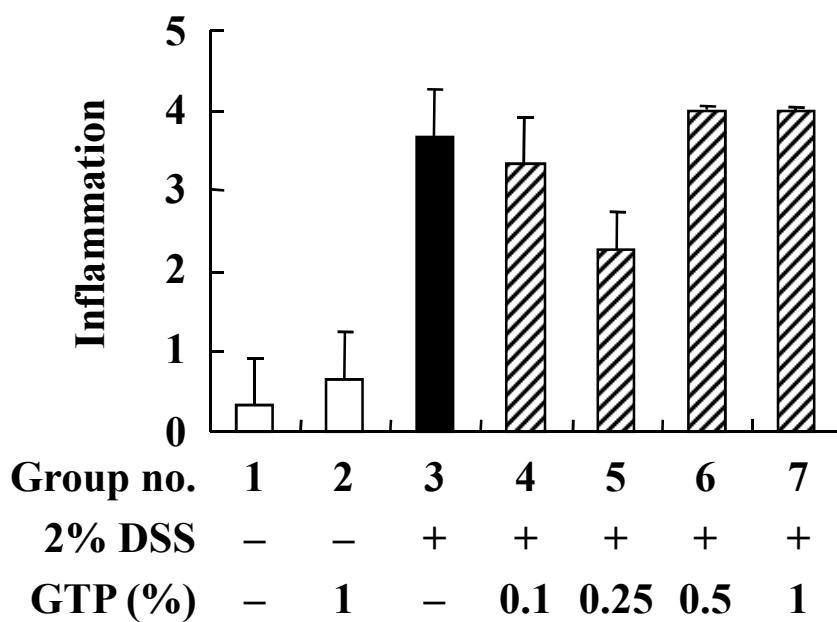


Fig. 6. M. Kim et al

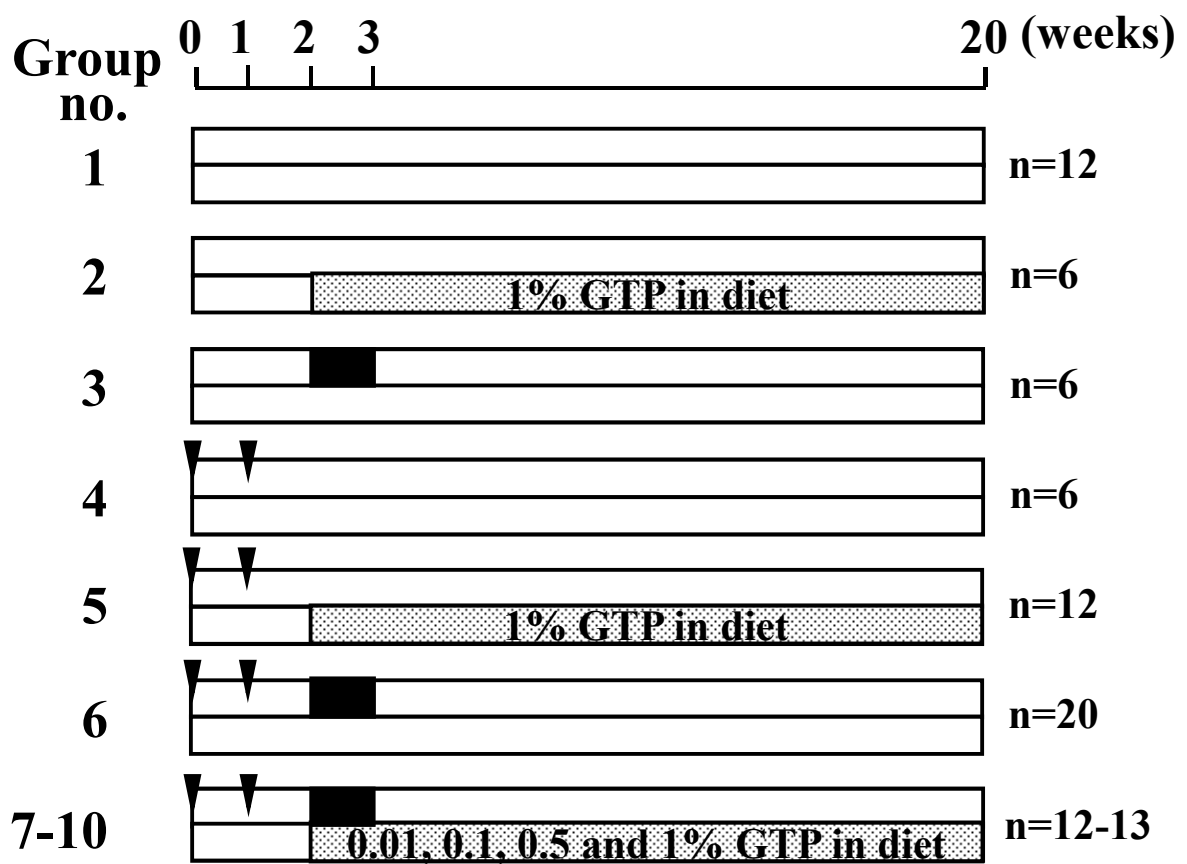


Fig. 7. M. Kim et al

