Plum polyphenols inhibit colorectal aberrant crypt foci formation in rats:

Potential role of the miR-143/AKT/mTOR axis

Nivedita Banerjee^a, Hyemee Kim^b, Stephen T. Talcott^b, Nancy D. Turner^{a,b}, David H. Byrne^d, Susanne U Mertens-Talcott^{b,c,*}

^aInterdisciplinary Program of Toxicology, Texas A&M University, College Station, TX, 77843, USA

^bDepartment of Nutrition and Food Science, Texas A&M University, College Station, TX, 77843, USA

^cDepartment of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX, 77843, USA

^dDepartment of Horticulture, Texas A&M University, College Station, TX, 77843, USA

* To whom correspondence should be addressed.

Department of Nutrition and Food Science, Texas A&M University, College Station, TX, 77843, USA. Tel: +1-979-458-1819; E-mail: smtalcott@tamu.edu

17 **Abbreviations:**

- 18 ACF; Aberrant crypt foci
- 19 AKT; protein kinase B
- 20 AOM; Azoxymethane
- 21 CAT; Catalase
- 22 COX-2; Cyclooxygenase-2
- 23 GAE; Gallic acid equivalent
- 24 GPx; Glutathione peroxidase
- 25 HIF-1 α ; Hypoxia Inducible Factor- 1 α
- 26 iNOS; Nitric oxide synthase
- 27 miRNA; microRNA
- 28 mTOR; Mammalian Target of Rapamycin
- 29 NF- κ B; Nuclear factor-kappa B
- 30 PI3K; Phosphoinositide 3-kinase
- 31 p70S6K; p70 ribosomal protein S6 kinase
- 32 ROS; Reactive oxidative stress
- 33 RPS6; Ribosomal protein s6
- 34 SOD; Superoxide dismutase
- 35 VCAM-1; Vascular cell adhesion molecule 1.

Abstract

The nutritional prevention of aberrant crypt foci by polyphenols may be a crucial step to dietary cancer prevention. The objective of this study was to determine the underlying mechanisms that contribute to the anti-inflammatory and anti-tumorigenic properties of plum (*Prunus salicina* L.) polyphenols, including chlorogenic acid and neochlorogenic acid, in AOM-treated rats. The hypothesis was that plum polyphenolics suppress azoxymethane (AOM)-induced aberrant crypt foci formation through alterations in the AKT/mTOR pathway and relative micro-RNA expressions. Sprague-Dawley rats (n=10/group) received plum beverage (1346 mg gallic acid equivalents/L) or a control beverage *ad libitum* for ten weeks with subcutaneous injections of AOM (15 mg/kg) at weeks 2 and 3. Results show that the consumption of the plum beverage decreased the number of dysplastic aberrant crypt foci by 48% ($p<0.05$) and lowered proliferation of mucosal cells by 24% ($p<0.05$). The plum beverage decreased the activity of GPx, SOD, and CAT in mucosal scrapings, as well as the SOD activity in serum. The results were accompanied by a downregulation of pro-inflammatory enzymes NF- κ B, iNOS, COX-2 and VCAM-1 mRNA. Plum inhibited the expression of AKT and mTOR mRNA, pAKT, mTOR and HIF-1 α protein levels, and the ratio of the phosphorylated/total protein expression of mTOR. Also, the plum beverage increased the expression of miR-143, which is involved in the regulation of AKT. These results suggest that plum polyphenols may exhibit a chemopreventive

57 potential against colon carcinogenesis by impacting the AKT/mTOR pathway and
58 miR-143.

59 Keywords: Plum, chlorogenic acid, colon cancer, inflammation, mTOR, miR-143,
60 rat

1. Introduction

Colorectal cancer is the third leading cause of death in the US [1]. In addition to available therapies, there is a need to develop novel therapeutic agents to prevent and treat colorectal cancer. Plant polyphenols have received attention as chemopreventive agents due to their possible antioxidant, anti-inflammatory, and anti-tumorigenic effects [2]. Recent studies have shown that several polyphenolic constituents of grapefruit, blueberry, and pomegranate suppress colon carcinogenesis via suppression of the formation of colorectal aberrant crypt foci (ACF) in rats injected with azoxymethane (AOM) [3-5].

Plums (*Prunus salicina* L.) contain a mixture of polyphenols that exert antioxidant and anti-cancer properties [6, 7]. In commercially available plums, the most predominant and therapeutically relevant compounds are flavonols (quercetin 3-glucoside), anthocyanins (cyanidin 3-glucoside, 3-rutinoside, and 3-acetylglucoside), procyanidins (dimers and trimers), phenolic acids (chlorogenic acid and neochlorogenic acid), and carotenoids [6]. Chlorogenic acid and neochlorogenic acid have been reported to exhibit antioxidant and chemopreventive activity in breast cancer, colon cancer, and U937 leukemia cells [6, 8-10]. Plum polyphenolics protect cells from oxidative stress by scavenging reactive oxidative stress (ROS) and other oxidative species [11], and suppressing

the proliferation of colon and breast cancer cells [7, 12-14]. However, the underlying mechanisms have yet to be fully described.

Literature indicates that ROS generation during carcinogenesis may activate the Phosphoinositide 3-kinase (PI3K)/ protein kinase B (AKT) signaling pathway [15]. Suppression of ROS generation by natural antioxidants could down-regulated the PI3K/AKT pathway. The PI3K/AKT pathway plays an important role in tumorigenesis, including cell proliferation and inflammation by phosphorylation of downstream targets, such as Mammalian Target of Rapamycin (mTOR), p70 ribosomal protein S6 kinase (p70S6K), and Ribosomal protein s6 (RPS6) [16-18]. The activation of mTOR downstream induced the translation of Hypoxia Inducible Factor-1 α (HIF-1 α), which controls hypoxia, vascularization, cell proliferation, and inflammation [19-21]. Previous studies have shown that polyphenols exhibit potential anti-cancer effects through suppression of the PI3K/AKT/mTOR pathway [22, 23].

Micro RNAs (miRNA) are endogenous, small, non-coding RNAs involved in the post-transcriptional regulation of gene expression by binding to the 3'-UTR promoter region of target mRNAs [24]. Reduction of the expression of miR-126, miR-143, and miR-145 has been directly linked to reduced colon tumor growth [25-27]. Also, induction of the expression of miR-126, miR-143, and miR-145 is related to decrease activities of the PI3K/AKT/mTOR pathways, which play a key roles in

colon carcinogenesis [27-29]. We previously reported that pomegranate polyphenolics induced the expression of miR-126, which occurred in parallel with suppression of the PI3K/AKT/mTOR pathway and colon carcinogenesis [5].

The hypothesis for this study was that plum polyphenolics suppress azoxymethane (AOM)-induced aberrant crypt foci formation in a rat model through alterations in the PI3K/AKT/mTOR pathway and related micro-RNA expressions. The objective of this study was to determine the underlying mechanisms that contribute to the anti-inflammatory and anti-tumorigenic properties of plum polyphenols. We investigated whether the polyphenols suppressed ACF formation, oxidative stress, and inflammation in AOM-treated rats. Further mechanistic analysis was performed with colonic mucosal samples.

2. Methods and materials

2.1. Experimental beverage

Commercial varieties of the red-fleshed plum 'Black Splendor' from California were collected at a mature firm stage. The skin and seeds were removed, and the flesh was ground in a blender. The puree was treated with pectinase while being heated in a water bath at 70°C for one hour then centrifuged. The upper aqueous beverage was stored at -20°C until use. To adjust for the

caloric content and acidity of the plum beverage (Brix 15.7 and pH 3.4), 157 g sugar and 0.5 g citric acid were added to 1L of water to prepare the control beverage (Brix 15.7 and pH 3.4). Total phenolic content in the plum beverage was measured by the Folin-Ciocalteu assay against an external standard of gallic acid [30]. Individual polyphenolics in the plum beverage were characterized using a Waters Alliance 2690 (Milford, MA) HPLC system and a Thermo Finnigan LCQ Deca XP Max mass spectrometer equipped with an ESI ion source (Thermo Fisher, San Jose, CA) [31].

2.2. *Animal treatment and tissue sampling*

The animal use protocol was approved by the Institutional Animal Care and Use Committee of Texas A&M University. 20 male Sprague-Dawley rats (3 week-old) were purchased from Harlan Teklad (Houston, TX) and distributed randomly by weight into the control and plum beverage groups (n=10/group). The rats were kept in suspended cages in a room controlled at $23\pm 2^{\circ}\text{C}$, $55\pm 5\%$ humidity, a 12 h light/dark cycle, and had free access to food pellets (Cat # 8604, Harlan Teklad, Houston, TX) and experimental beverages. All animals received two intraperitoneal injections of AOM (15 mg/kg body weight, Sigma Chemical Co., St Louis, MO). The first was performed at two weeks after starting the experimental diets, the second at three weeks. The intake of plum and the control beverage was measured every other day. Rats were euthanized in CO_2 chamber six weeks after the second AOM

treatment, and colon tissues were collected [32]. From the distal end of the colon, 1 cm sections were cut and fixed in 4% paraformaldehyde. The remainder of the colon was cut in half longitudinally, and one-half was fixed in 70% EtOH for the ACF counting, and mucosa from the other half was gently scraped for protein and RNA samples [5, 33, 34].

2.3. *ACF counting*

After 24 h of fixation in 70% EtOH, tissues were stained with 0.5% methylene blue for 1 min. The number of ACF and dysplastic ACF (foci containing 4 or more aberrant crypts) was counted under a light microscope using 40x magnification [33, 34].

2.4. *Cell proliferation assay*

For cell proliferation, Ki-67 immunohistochemistry was performed. Colon tissues were dehydrated, embedded in paraffin, then cut into four μm thick sections. The paraffin-embedded sections were stained with the primary antibody against Ki-67 (Dilution 1:50, BD Pharmingen, San Jose, CA), and incubated with biotinylated anti-mouse IgG from the ABC Elite kit (Vector Lab, Burlingame, CA). Ki-67-containing nuclei, indicative of proliferating cells, showed up as brown spots within the colonic crypt columns. Twenty-five well-formed crypt columns per rat

were identified for analysis (n=10/group). The proliferative index (%) was calculated as the percentage of Ki-67 positive cells divided by the total number of cells in one side of a crypt [35].

2.5. Measurement of antioxidant enzyme activity

Activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in mucosal samples and serum from rats were measured spectrophotometrically using kits from Cayman Chemical (Ann Arbor, MI). The microplates were read on a FLUOstar Omega plate reader (BMG Labtech Inc. Durham, NC). SOD activity was evaluated by measuring the generation rate of a chromophore at 525 nm. CAT activity was calculated at 520 nm by measuring the absorbance of quinoneimenedye. GPx was assessed at 340 nm indirectly by oxidation of NADPH to NADP⁺. The activity was normalized to protein concentration as determined by Bradford reagent (Bio-Rad, Hercules, CA) [36].

2.6. Quantitative RT-PCR

Total RNA from the mucosal scraping was isolated using the mirVana™ miRNA Isolation Kit (Invitrogen Corp., Grand Island, NY). mRNAs and miRNAs were converted to cDNA using a reverse transcription kit, and Applied Biosystems TaqMan miRNA reverse transcription kit (Invitrogen Corp., Grand Island, NY).

Primers for mRNA were purchased from Integrated DNA Technologies (Coralville, IA). Primers for miR-126, 143, and 145 were purchased from Invitrogen (Grand Island, NY). SYBR Green and TaqMan PCR Master Mixes (Applied Biosystems Inc., Foster City, CA) were used for the qPCR on Applied Biosystems 7900HT Fast Real-Time PCR system (Applied Biosystems Inc., Foster City, CA). GAPDH and 4.5S were used as the endogenous controls [31].

2.7. Western blotting

The mucosal scrapings were homogenized in a protein buffer (500 mM Tris-HCL, 1 M Sucrose, 200 mM EDTA, 100 mM EGTA, 0.4 M NaF, 10% Triton X-100, ten mM Sodium Orthovanadate, and Protease Inhibitor Cocktail), then centrifuged at 15,000 g for 20 min at 4°C. The resulting supernatant was stored at -80°C [34]. Afterward, 60µg of protein were loaded onto 10% electrophoresis gels, followed by electro-transfer onto PVDF membranes. The blots were probed with the primary antibodies against vascular cell adhesion molecule 1 (VCAM-1, Santa Cruz Biotechnology, Santa Cruz, CA), nuclear factor-kappa B (NF-κB), HIF-1α, mammalian target of rapamycin (mTOR), pAKT, pPI3K and β-actin (Cell Signaling, Beverly, MA) [31].

2.8. Multiplex Bead Assay

The protein extract (25 µg) from each mucosal tissue was used to determine the relative abundance of the phosphorylated and total protein content of AKT, mTOR, p70S6K, and RPS6 using multiplex kits (Millipore, Billerica, MA) on a Luminex L200 instrument (Luminex, Austin, TX). Data were generated by the Luminex xPONENT software. The relative level was expressed as the ratio of phosphorylated protein/total protein [37].

2.9. Statistical analyses

The sample sizes (10 rats/group) were determined according to a priori statistical power analysis ($p=0.05$, power=0.85) with the results of high multiplicity ACF counting in the previous studies [33] performed with G*Power program. Data were analyzed by Student's t-test using SAS version 9 (SAS Institute Inc., Cary, NC), and data are reported as means $\pm$ SE. Differences were considered significant if the p-value was < 0.05 .

3. Results

3.1. Chemical analysis of plum beverage and food and plum beverage intake in AOM-treated rats

The polyphenolic profile of the plum beverage is representative of plum fruit because nothing was added to the beverage at any point during the procedure. The chromatographic profiles show the presence of phenolic acids (chlorogenic acid and neochlorogenic acid) (Fig. 1A). The concentration of total soluble phenolics of plum used was 1346 mg gallic acid equivalent (GAE)/L. The plum group (313 g body weight) consumed 58.5 ml of plum beverage every day (Table 1, Fig. 1B), whereas the control rats consumed 53.2 ml of the control beverage. For the first nine days, intake of the plum beverage was lower than the control beverage, likely because of the novel taste. In the initial 48h after the AOM injection (week 2 and 3), the intake of liquid was lower in the control group compared to the plum-treated group by 30 ml and 15 ml, respectively. Food intake in the control group was lower (6 g/day) compared to the plum group (Table 1). The control group gained more weight in comparison to the plum group. The final average body weight of the control group was 43 g more than the plum group (Table 1).

3.2. Plum beverage suppressed the dysplastic ACF formation and cell proliferation in AOM-treated rats

ACF, especially dysplastic ACF (> 4 aberrant crypts/focus), are a marker for the initial stages of colon carcinogenesis. These pre-neoplastic lesions are directly linked to human and animal colon cancer incidence [38]. In this study, the number

of dysplastic ACF was reduced by 48% ($P<0.05$) in rats consuming the plum beverage (Fig. 2A). The proliferation of colon epithelial cells was reduced in rats consuming the plum beverage. In the control group, 34.2% of colonocytes in colon crypt columns were Ki-67 positive cells, compared to 25.8% of the plum group. Thus, consumption of the plum beverage induced an 8.42% reduction ($P=0.0127$) in proliferation relative to the control group (Fig. 2B).

3.3. Plum beverage decreased antioxidant enzyme activities in AOM-treated rats

Oxidative stress is linked to tumorigenesis via activation of cell inflammation and proliferation [39]. Antioxidant enzymes (GPx, SOD, and CAT) are involved in the protection against oxidative stress [40]. In the scraped colon mucosal samples GPx, SOD and CAT enzyme activities were decreased (70, 45, and 80%, respectively) in the plum group compared to the control group (Fig. 2C). In the serum, SOD activity was reduced in the plum group by 50% (Fig. 2D).

3.4. Plum suppressed the expression of inflammatory enzymes and the AKT/mTOR signaling in rats treated with AOM

We investigated the impact of plum polyphenolics on the expression of inflammatory mediators in rats treated with AOM. Plum polyphenols decreased the

expression of NF- κ B, VCAM-1, Cyclooxygenase-2 (COX-2), and Nitric oxide synthase (iNOS) mRNA (Fig. 3A), and suppressed protein expression of NF- κ B (p65) and VCAM-1 (Fig. 3C). Previous studies have shown that the AKT/mTOR signaling pathways play an important role in colon cancer [41]. Plum polyphenols decreased AKT and mTOR mRNA (Fig. 3B). The changes in mRNA levels were accompanied by decreased protein expression of pAKT, mTOR, and HIF1 α (Fig. 3C). A multiplex bead assay also showed the suppression of the ratio of the phosphorylated protein to the total protein of mTOR (Fig. 3D).

3.5. Plum increased the expression of miR-143 in AOM-treated rats

Small non-coding miRNAs are known to influence cancer progression by controlling several biological processes, such as the AKT/mTOR pathway [24]. Therefore, the impact of plum consumption on miRNA related to the AKT/mTOR pathway was determined. There were no differences in expression of miR-126 or miR-145, whereas plum induced the expression of the tumor suppressor miR-143 compared to the control (Fig. 3E).

4. Discussion

In this study, we demonstrated the anti-inflammatory and anti-tumorigenic effects of plum polyphenolics and investigated the underlying mechanisms of the

chemopreventive activities of plum polyphenolics in AOM-treated rats. It was hypothesized that plum polyphenolics may suppress AOM-induced aberrant crypt foci formation in a rat model by modulating expression and/or activation of genes, proteins, and relevant miRNA in the PI3K/AKT/mTOR pathway. Overall, results supported this hypothesis.

Plum is a rich source of chlorogenic acid, neo-chlorogenic acid, and anthocyanins, with demonstrated chemopreventive potential in breast and colon cancer cells [7, 14]. However, the molecular mechanisms underlying chemopreventive effects of plum polyphenolics have not been determined yet. The AOM-induced ACF model used in this study is a pre-clinical model of colon cancer that exhibits similar characteristics to human disease [42]. We used this animal model to explore the potential mechanisms of plum polyphenolics' influence on carcinogenesis using a daily dose of total soluble polyphenolics of 251.3 mg GAE/kg/day. By the calculation of the human equivalent amount of plum polyphenols (using the body surface area normalization method), the human equivalent dose of plum polyphenolics would be 40.7 mg GAE/kg/day (human equivalent dose (mg/kg) = rat dose (mg/kg) × rat Km (6) /human Km (37)), which equals the intake of 3 g plum polyphenols for an individual of 75kg body weight [5, 43]. Exposure to this level of polyphenols in the plum beverage was sufficient to decrease the number of dysplastic ACF as well as the percentage of proliferative cells.

Oxidative stress is a key initiator and promoter of carcinogenesis due to its ability to activate inflammatory pathways and stimulate cell proliferation of cancer cells [39]. Antioxidant enzymes are involved in the suppression of oxidative stress [40], and several polyphenolics reduce oxidative stress through up-regulation of SOD, CAT, and GPX levels [44, 45]. However, one previous study reported that extracts derived from immature plum lowered GPx, SOD, and CAT activity in benzo(α)pyrene-treated rats due to the inherent radical-scavenging abilities of the plum extracts [46]. In the current study, activities for GPx, SOD, and CAT in the mucosal tissue, and SOD in serum were lower in the plum group as compared to the control group. The beverage prepared from fully ripe plums also may have provided sufficient antioxidant capacity to reduce the need for a synthesis of endogenous antioxidant enzymes.

While conjugated native plum polyphenolics and chlorogenic acid, the predominant polyphenolic in plum, showed to have antioxidant properties [47, 48], these compounds are probably not active the compounds which have the antioxidant activities in the intestines. A significant portion of plum polyphenols might be metabolized by intestinal microbiota. Specifically, it was reported that chlorogenic acid is metabolized to m-coumaric acid, hippuric acid, 3-hydroxyhippuric acid, isoferulic acid, and ferulic acid [49]. These microbial metabolites still have the antioxidant activities but more importantly anti-

inflammatory efficacy [50, 51]. Ferulic acid, a microbial metabolite of caffeic acid has previously been shown to have potential chemopreventive effects through its scavenger activity to ROS [52]. Even though a significant portion of polyphenols might be modified by the colonic microbiota, the presented in vivo study demonstrates that these microbial metabolites appear to have significant antioxidant, anti-inflammatory and chemopreventive activities as demonstrated in previous studies [52-54].

NF- κ B plays a central role in inflammatory pathways by controlling the transcription of oncogenes, tumor suppressor genes, pro-inflammatory genes (e.g., COX-2 and iNOS), and adhesion molecules (VCAM-1) [55, 56]. The chronic inflammatory markers COX-2 and iNOS are up-regulated in colorectal cancer patients and rodents with chemically induced colon cancer. Because these proteins are associated with elevated cell proliferation and inflammation [34, 57], suppression of COX-2 and iNOS are promising targets for chemoprevention [58]. The plum beverage used in this study suppressed the expression of pro-inflammatory markers NF- κ B (p65), VCAM-1, Cox-2 and iNOS in AOM-treated rats. Consumption of the plum beverage also down-regulated the expression of components within the AKT/mTOR pathway. The AKT/mTOR pathway is linked to tumorigenesis because its stimulation leads to the induction of cell proliferation and inflammation [17, 59], making it a potential therapeutic target [60]. We observed that plum suppressed the expression of AKT and mTOR mRNA and inhibited the

phosphorylation of PI3K/AKT and the ratio of the phosphorylated/total protein of mTOR.

In this study, plum polyphenols reduced weight gain compared with the control group, even though their food intake was higher. Several reports indicate that polyphenol intake may indeed reduce weight gain in rats [61] and human [62]. It has been demonstrated that weight-loss or reduced weight gain may contribute to a reduced risk for colon cancer [63, 64]. Therefore, in this study, we hypothesized that the chemopreventive effects of plum polyphenolics were derived from a direct interaction of polyphenolics with the mTOR pathways. Additionally, it is possible that the reduced weight gain caused by plum polyphenols contributed to the anti-inflammatory activities of the study treatment. Although overall, there is a correlation between reduced body weight and reduced colon cancer risk [64], in this study, there was no correlation between weight gain and the number of dysplastic ACF ($R^2 = 0.2694$, $P = 0.2508$).

Certain study limitations will require further experimentation. The control treatment was adjusted to the caloric content and pH, however in addition to polyphenols, the plum treatment contained low levels of ascorbic acid. Previously, it was reported that high dose of vitamin C intake (i.p. 4g/kg body weight for 5-7 weeks) decreased the numbers of intestine polyps in APC/Kras mutant mice [65]. In this study, the amount of vitamin C in the plum treatment was insignificant

389 compared to the polyphenolic content. It was crucial that the study treatment is
390 representative of the natural fruit, also containing ascorbic acid. Accordingly, other
391 micronutrients were not adjusted in the control beverage. A more elaborate
392 research design might be needed to determine the chemopreventive activities of
393 each component and their respective combinations that are represented in a whole
394 fruit preparation. Also, the miR-143, an anti-oncogenic miRNA, was reported to be
395 down-regulated in colorectal cancer [29], and targets include the promoter region
396 of AKT [29, 66]. In this study, the decreased expression of pAKT was accompanied
397 by increased expression of the tumor suppressor miR-143 by the plum beverage.
398 Our findings suggest a possible role of the tumor suppressor miR-143 induced by
399 plum polyphenols as part of the underlying mechanisms for the down-regulation of
400 the AKT expression. Because we did not look at the specific relationship between
401 miR-143 and AKT expression, further *in vitro* tests are needed to elucidate the role
402 of induction of miR-143 by plum on the suppression of the AKT expression.

403
404 In conclusion, results indicated that plum polyphenols had anti-inflammatory
405 and anti-tumorigenic activities in AOM-treated rats. Interactions of plum bioactives
406 with the miR143/AKT/mTOR pathway were identified as the possible underlying
407 chemoprotective mechanisms.

408
409 **Conflict of interest:** The authors have no conflicts of interest to declare.

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Figure legends

Fig. 1. Representative chromatogram of polyphenolics in plum beverage and the daily beverage consumption by rats.

Fig. 2. Effects of plum beverage (PL) on lesion formation, colonocyte proliferation and antioxidant enzyme activities. Rats were fed control or plum beverage (1346mg gallic acid equivalents/L) ad libitum for 10 weeks with subcutaneous injections of AOM (15mg/kg) at weeks 2 and 3. All values are means $\pm$ SE (n=10/group). * indicates significant changes at $p<0.05$ (Student's t-test). (A) The number of ACF was decreased by 34% ($p=0.09$), and the number of dysplastic ACF was decreased ($p<0.05$) by 43% in plum rats compared to control rats. Visualized ACF in methylene blue-stained colon was counted. (B) Plum intake reduced cell proliferation compared to the control by 24% ($p<0.05$). The Ki-67 antibody was used to detect proliferating cells in immunohistochemistry. (C) The activities of antioxidant enzymes including GPx, SOD, and CAT in mucosa and serum were measured using ELISA kits. Plum beverage decreased the activities of GPx, SOD, CAT in mucosal samples, and (D) SOD in serum in AOM-treated rats.

Fig. 3. Effects of plum beverage (PL) on the inflammatory and the mTOR signaling pathways in AOM-treated rats. Rats were fed control or plum beverage (1346mg gallic acid equivalents/L) ad libitum for 10 weeks with subcutaneous injections of AOM (15mg/kg) at weeks 2 and 3. All values are means $\pm$ SE (n=10/group). * indicates significant changes at $p<0.05$ (Student's t-test). (A) RT-PCR assays showed that plum beverage decreased the expression of NF- κ b (p65), VCAM-1, COX-2, and iNOS mRNA, and (B) AKT and mTOR mRNA in AOM-treated rats. (C) Plum beverage decreased the expression of NF- κ B (p65), VCAM-1, pAKT, mTOR, and HIF1 α protein levels in the western blot assay. (D) Plum beverage decreased the ratio of the phosphorylated/total protein of mTOR in the multiplex assay. (E) Plum beverage increased the expression of miR-143 in AOM-treated rats.

Fig. 1.

Fig. 2.

Fig. 3.

Table 1.
Effects of plum beverage on initial and final body weight, food intake, and beverage intake

	Control	Plum
Initial Body weight (g)	79.91 ± 2.22 ^{ns}	78.02 ± 1.87
Final Body weight (g)	355.98 ± 9.49	313.30 ± 7.39*
Food intake (g/day)	10.46 ± 0.57	16.89 ± 0.40*
Beverage intake (ml/day)	53.19 ± 3.58 ^{ns}	58.49 ± 5.21

Rats were fed control or plum beverage (1346mg gallic acid equivalents/L) *ad libitum* for 10 weeks with subcutaneous injections of AOM (15mg/kg) at weeks 2 and 3. All values are means ± SE (n=10/group). Food intake was measured as the food intake per 48 h period at 6 weeks after the second AOM injection. Beverage intake was calculated as the beverage consumed for whole study. Values are statistically significant at *p<0.05 (Student’s t-test). ns means not significantly different.