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Toxicological Evaluation of *Sargassum polycystum* in Mice with Loperamide-Induced Constipation

(Penilaian Toksikologi *Sargassum polycystum* pada Tikus dengan Sembelit Akibat Loperamide)

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ABSTRACT

Constipation gradually increases in people after the age of 50 years leading to difficulty in stool passage. Until now, there have been no effective drugs without side effects. This study investigated the preventive potential and toxicity of *Sargassum polycystum* extract (SPE) against loperamide-induced constipation in mice. Daily oral administration of SPE (100, 500 and 1000 mg/kg) for 14 days significantly increased defecation frequency in constipation mice. Additionally, no significant differences in blood haematological and biochemical indices between the control, constipation and SPE-treated groups were detected. Histopathological examinations revealed non-toxic effects in liver, kidney and colon tissue samples from the SPE-treated groups compared with the control and constipation groups. Real-time polymerase chain reaction showed that the administration of SPE significantly modulated *Saa3*, *Odc1* and *Clu* expressions that promote the health of living organisms compared with the control group. Overall, this study showed that daily oral administration of *Sargassum polycystum* for 14 days could prevent constipation without any adverse effects. *Sargassum polycystum* is suitable for further use as a supplement or drug to manage constipation.

Keywords: Constipation; histopathology; *Sargassum* sp.; seaweed; toxicity

ABSTRAK

Sembelit berlaku secara beransur-ansur kepada orang berusia selepas 50 tahun yang membawa kepada kesukaran untuk membuang najis. Sehingga kini, tiada ubat yang berkesan tanpa kesan sampingan. Penyelidikan ini mengkaji potensi pencegahan dan ketoksikan ekstrak *Sargassum polycystum* (SPE) terhadap sembelit yang disebabkan oleh loperamide pada tikus. Administrasi oral harian SPE (100, 500 dan 1000 mg/kg) selama 14 hari meningkatkan kekerapan buang air besar dengan ketara pada tikus sembelit. Selain itu, tiada perbezaan ketara dalam indeks hematologi dan biokimia darah antara kumpulan kawalan, sembelit dan rawatan SPE dikesan. Pemeriksaan histopatologi mendedahkan kesan bukan toksik dalam sampel tisu hati, buah pinggang dan kolon daripada kumpulan yang dirawat SPE berbanding kumpulan kawalan dan sembelit. Tindak balas rantai polimerase masa nyata menunjukkan bahawa administrasi SPE memodulasi pengekspresan *Saa3*, *Odc1* dan *Clu* dengan ketara yang menggalakkan kesihatan organisma hidup berbanding dengan kumpulan kawalan. Secara keseluruhannya, kajian ini menunjukkan bahawa administrasi *Sargassum polycystum* secara oral setiap hari selama 14 hari dapat mengelakkan sembelit tanpa sebarang kesan buruk. *Sargassum polycystum* sesuai untuk kegunaan selanjutnya sebagai suplemen atau ubat untuk menguruskan sembelit.

Kata kunci: Histopatologi, ketoksikan; rumpai laut*; Sargassum* sp., sembelit

INTRODUCTION

Constipation is characterised by infrequent stools, difficult stool passage or both. Although not a life-threatening disease, it can cause lifestyle and economic problems. The prevalence of constipation increased after the age of 50 years and increased rapidly after 70 years (Higgins & Johanson 2004), leading to health problems in these people worldwide. Several commercial drugs are used to treat constipation, but they have several side effects. To enable the management of constipation without the side effects of conventional drugs, medicinal plants with prebiotic properties have received an increase in scientific interest because of their ability to enhance stool frequency (Sengkhim et al. 2021).

Prebiotics are naturally occurring compounds in plants. Most of these are non-digestible carbohydrates that cannot be digested by intestinal enzymes but can be fermented by gut microbiota in the lower gastrointestinal tract. The fermentation of prebiotics produces short-chain fatty acids (SCFAs) and promotes the growth of specific beneficial bacteria (Davani-Davari et al. 2019). The administration of prebiotics has beneficial effects on gut motility and can prevent gastrointestinal symptoms, especially constipation. Among the medicinal plants, seaweeds have been reported to show high prebiotic properties and high dietary fibre contents of up to 65% (de Jesus Raposo et al. 2016). It has been reported that the administration of seaweed extract can potentially prevent constipation in mice models (Sengkhim et al. 2021). Additionally, prebiotics from brown seaweed Sargassum fusiforme and Sargassum fulvellum have been shown to improve intestinal motility by increasing in faecal excretion and the intestinal transit rate (Koh et al. 2024). A possible mechanism may occur through the stretching of the colon due to accumulated dietary fibre, thereby activating the enteric nervous system in the gut wall and leading to defecation stimulation (Furness, 2000). In addition to dietary fibres, SCFAs produced during bacterial fermentation are involved in gut motility (Zheng et al. 2022) and modification of the colonic motor effect (Cherbut et al. 1998), thereby enhancing defecation. Apart from being a prebiotic property, seaweed extract also exerts various biological activities, including antioxidant (Sengkhim et al. 2021), reducing oxidative stress (Motshakeri et al. 2013), anticancer (Lee, Selvaraj & Lee 2021; Navakanitworakul et al. 2023) and anti-inflammatory (Yuvaraj et al. 2013) effects. The possible explanation for these biological activities may be due to their constituents and secondary metabolites, such as fucoxanthin, dinoxanthin, β-carotene, zeaxanthin, chlorophyll a and b and fucoidan, which are well known to exert biological activities in living organisms (Lee, Selvaraj & Lee 2021; Motshakeri et al. 2013).

In view of the potential of brown seaweed (Sargassum polycystum) to be developed as a therapeutic agent for constipation, basic evidence on safety and efficacy data are required. Although seaweed is a natural product, its consumption can have adverse effects. Hence, a toxicological evaluation of natural products is required before their development as drugs or supplements. To date, toxicological data regarding these species in living organisms are limited. Thus, this study investigated the anti-constipation effect of *Sargassum polycystum* extract (SPE) and its toxicity in constipation mice using biochemical, haematological and histopathological parameters.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Otherwise indicated, the basic chemicals used in this research were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). A TRIzol reagent and a SuperScript cDNA synthesis kit were purchased from Invitrogen (Waltham, Massachusetts, USA). Luna® Universal qPCR Master Mix was purchased from New England Biolabs (Ipswich, Massachusetts, USA).

SPE PREPARATION

Adult-stage *Sargassum polycystum* was harvested from Tean Island (9°22′0″N, 99°57′0″E), Surat Thani Province, Thailand, in January 2020. The crude extract of *S. polycystum* was prepared using an autoclave-assisted method as reported in a previous study (Khuituan et al. 2022). Briefly, dried *S. polycystum* was ground into powder and then mixed with distilled water at a 1:100 ratio. After autoclaving at 121 °C for 20 min, the mixture was filtered and centrifuged at 2,220 × g for 10 min. The supernatants were collected and freeze-dried to obtain SPE powder.

ANIMAL MODEL

Six-week-old male ICR mice were obtained from Nomura Siam International Co., Ltd., and housed at the Southern Laboratory Animal Facility, Prince of Songkla University, Thailand. All mice were acclimatised for 7 days before the experiment began. They were placed in stainless steel cages and kept under controlled conditions at 23°C–27°C and 50%–55% humidity with and a 12-h light/12-h dark cycle. The animals were given standard commercial food pellets from Perfect Companion Group Co., Ltd., Thailand, and purified water *ad libitum*.

EXPERIMENTAL DESIGN AND TREATMENT GROUPS

The mice were randomly divided into six groups (eight mice per group): control, constipation, constipation treated with 500 mg/kg lactulose (positive control), constipation treated with 100 mg/kg SPE, constipation treated with 500 mg/kg SPE and constipation treated with 1000 mg/kg SPE. SPE was orally administered once a day for 14 days. On days 12, 13, and 14, constipation was induced in all groups, except the control group, by oral administration of 5 mg/kg loperamide. The doses and times used in this experiment followed those reported in a previous study that used *Sargassum* sp. extract to prevent loperamide-induced constipation (Khuituan et al. 2022; Sengkhim et al. 2021). Body weight and food intake were recorded daily. Faecal pellets evacuated over a 4-h period were counted, and hourly averages were calculated. At the end

of the treatment period, all mice were anesthetized with thiopental sodium before collecting blood and tissue samples. All experimental procedures in this study were approved by the Animal Ethics Committee of Prince of Songkla University, Thailand (Ethical clearance MHESI 68014/2095, Ref.92/2021).

BIOCHEMICAL ANALYSIS

Blood samples were collected via cardiac puncture after anaesthesia with thiopental sodium. To determine biochemical parameters, the collected blood was immediately centrifuged at 3,000 rpm for 5 min to separate the plasma. The levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN) and creatinine were determined using a BS-20 Chemistry Analyzer (Shenzhen, China).

HAEMATOLOGICAL ANALYSIS

Blood samples collected via cardiac puncture were immediately subjected to haematological analysis. Haemoglobin (HGB), red blood cell (RBC), haematocrit (HCT), red cell distribution width, mean corpuscular volume, mean corpuscular HGB (MCH), mean corpuscular HGB concentration, platelet count (PLT), platelet distribution width, mean platelet volume, white blood cells (WBCs), lymphocytes, monocytes and granulocytes were analysed using a BC-2800Vet Auto Hematology Analyzer (Shenzhen, China).

HISTOPATHOLOGICAL STUDY

The liver, kidney and colon were excised and immediately fixed in 10% formalin overnight under constant agitation. Fixed tissues were processed and sectioned at 5 µm before staining with haematoxylin and eosin (H&E), periodic acid Schiff (PAS) and Masson's trichrome according to the standard protocol. The stained tissues were examined under a light microscope and photographed with cellSens dimension software.

REAL-TIME PCR

Liver, kidney and colon tissue samples were subjected to RNA extraction using a TRIzol reagent. Total RNA was treated with DNase before conversion to cDNA using a cDNA synthesis kit. Real-time PCR was performed using Luna® Universal qPCR Master Mix with the specific primers list in Table 1. Relative gene expression was calculated using $2^{-\Delta\Delta ct}$ method and was expressed as fold-change relative to the control.

STATISTICAL ANALYSIS

The results were presented as means ± standard error of the mean. Statistical comparison between groups was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

EFFECT OF SPE ON DEFECATION FREQUENCIES IN CONSTIPATION MICE

Defecation frequency decrease is a well-known indicator of constipation. Our results showed that the number of mice treated with loperamide was lower than that of mice in the normal control group. However, daily gavage with SPE (100–1000 mg/kg) for 14 days before loperamide treatment significantly mitigated the decrease in defecation frequency. Defecation frequency was significantly higher in all doses of the SPE pretreatment groups than in the constipation group (Table 2). However, SPE has no dose–response effect. The defecation frequencies in 100, 500 and 1000 mg/kg SPE pretreatment were high, but significant differences between doses of SPE were not detected. Possible mechanisms underlying the effects of SPE consumption on defecation frequency may be prebiotic. The digestion and fermentation of prebiotic seaweed in the gastrointestinal tract produces dietary fibres and affects microfloral diversity, which, in turn, promotes gut health benefits (Brownlee 2011; Khuituan et al. 2022). Dietary fibre accumulation in the gut lumen will stretch and activate neural cells within submucosal and myenteric plexuses, thereby increasing defecation frequency. The animal model that received prebiotics showed a decrease in colonic transit time and an increase in the number of faeces compared with the animal model with normal diet consumption (Araújo & Botelho 2022; Sengkhim et al. 2021). Additionally, secondary metabolites especially SCFAs produced from bacterial fermentation, have been reported to be involved in intestinal motility by activating G protein-coupled receptors and inhibiting histone deacetylase (Zheng et al. 2022). These SCFAs also modify the colonic motor effect via local neural mechanisms and by stimulating polypeptide YY release (Cherbut et al. 1998). Furthermore, the fermentation of algae modulates the gut microbiota by increasing the relative abundance of beneficial bacteria (*Lactobacillus–Enterococcus* and *Bifidobacteria*) while suppressing undesirable bacteria (*Eubacterium rectale* group, *Clostridium coccoides*) (de Medeiros et al. 2021; Khuituan et al. 2022). This modulation, in turn, enhances colonic function and gut motility by binding to toll-like receptors (Khuituan et al. 2022; Zheng et al. 2022). These findings indicates that SPE is a potential supplement for the prevention of constipation.

TABLE 1. Primer sequences (Fabian et al. 2011)

Primer	Sequence (5' à 3')
PCNA	Fw: CCTGTGCAAAGAATGGGGTG Rw: TCTCTATGGTTACCGCCTCC
Saa3	Fw: AATACTTCCATGCTCGGGGG Rw: GCTCCATGTCCCGTGAACTT
Oat	Fw: ACCATTAAACCAGGCGAGCA Rw: CAGGATAGCGCCCATCTTGT
Hspcb	Fw: GTCCGCCGTGTGTTCATCAT Rw: GCACTTCTTGACGATGTTCTTGC
Clu	Fw: AGCAGGAGGTCTCTGACAATG Rw: GGCTTCCTCTAAACTGTTGAGC
Gadd153	Fw:CTGGAAGCCTGGTATGAGGAT Rw:CAGGGTCAAGAGTAGTGAAGGT
Odc1	Fw:GCCACACTCAAAACCAGCAG Rw:CACTGCCTGAACGAAGGTCT
Sod1	Fw:AACCAGTTGTGTTGTCAGGAC Rw: CCACCATGTTTCTTAGAGTGAGG
Gapdh	Fw:AGGTCGGTGTGAACGGATTTG Rw: TGTAGACCATGTAGTTGAGGTCA

TABLE 2. Defecation frequency

Experiment groups	Frequency of defecation (times/h)
Control	2.75 ± 0.18
Constipation (loperamide treated)	1.92 ± 0.26
Lactulose + Constipation	3.13 ± 0.58
SPE (100 mg/kg) + Constipation	$4.06 \pm 0.40^{\#\#}$
SPE (500 mg/kg) + Constipation	$4.22 \pm 0.39^{\#\#\#}$
SPE (1000 mg/kg) + Constipation	$4.14 \pm 0.43^{\#\#}$

Mean ± SEM, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with constipation

Of note, this study calculated the defecation frequency at the end of the experiment (day 14) only. Further experiments with different time points are required to elucidate the time-response effect of SPE on constipation.

EFFECT OF SPE ON HAEMATOLOGICAL PARAMETERS IN CONSTIPATION MICE

Haematological parameter analysis showed that daily SPE consumption (100–1000 mg/kg) for 14 days had no effect on blood cell counts. All parameters listed in Table 3 were not significantly different between the normal and constipation groups. It is noteworthy that these haematological values were within the range of reference values for mice (Santos et al. 2016). These results were consistent with a previous report that *Sargassum* sp. consumption up to 1000 mg/kg for 14 days had no effect on haematological parameters in a mouse model (Tipbunjong et al. 2023). However, in some cases, *Sargassum* sp. consumption enhanced macrophage activity (Sinurat, Marraskuranto & Subaryono 2021). It is noteworthy that haematological parameters are widely used for the toxicological evaluation of naturally occurring compounds, and changes in these parameters in animal models have high predictive value for human toxicity (Olson et al. 2000). For example, *Campomanesia velutina* extract, which is widely used in Brazilian folk medicine to treat diarrhoea and reduce intestinal cramps, has been reported to show signs of toxicity at high doses. Thus, high-dose administration of *Campomanesia velutina* extract reduced RBC, HGB, HCT, WBC and PLT (Araújo et al. 2017). No significant changes were observed in any of the haematological indices, indicating that SPE did not have any adverse effects on animal health.

EFFECT OF SPE ON THE LIVER OF CONSTIPATION MICE

The gross morphological structure of the liver in all SPE doses consumed daily for 14 days had a normal appearance and did not differ from that in the normal control and

TABLE 3. Haematological parameter analysis

Parameters	control	constipation	lactulose	SPE (mg/kg)		
				100	500	1000
HGB (g/dL)	14.18±0.28	14.60±0.36	14.60±0.37	14.89±0.27	14.84±0.31	15.19±0.41
RBC (10^3/μL)	8.35±0.19	8.90±0.19	8.75±0.19	8.83±0.11	8.77±0.30	9.03±0.14
HCT (%)	42.16±1.31	44.30±0.94	44.07±1.03	44.44±0.68	43.38±1.10	44.80±0.86
RDW (%)	12.71±0.45	11.51±0.10	12.37±0.42	12.07±0.21	11.68±0.25	12.46±0.30
MCV (fL)	50.50±0.87	49.58±0.51	50.43±0.49	50.36±0.65	49.61±0.65	49.70±0.58
MCH (pg)	16.96±0.37	16.37±0.22	16.63±0.20	16.80±0.24	16.93±0.36	16.77±0.44
MCHC (g/dL)	33.73±0.92	32.90±0.22	33.08±0.13	33.46±0.23	34.20±0.39	33.86±0.60
PLT (10^3/μL)	1.55±0.15	1.32±0.08	1.39±0.10	1.58±0.24	1.32±0.11	1.29±0.18
PDW (%)	15.91±0.14	15.80±0.17	15.90±0.12	16.24±0.15	15.94±0.12	16.07±0.12
MPV (fL)	5.00±0.13	4.94±0.13	4.97±0.12	5.29±0.15	4.73±0.24	5.06±0.14
PCT	0.58±0.02	0.57±0.02	0.61±0.03	0.55±0.02	0.55±0.04	0.50±0.10
WBC (10^3/μL)	8.53±1.69	5.61±1.70	4.97±1.12	9.39±1.32	5.64±1.35	5.21±1.25
Lymphocytes (%)	69.98+5.06	61.20+5.60	48.85+6.25	65.24+6.72	66.46+4.12	59.01+5.35
Monocytes (%)	4.30+0.80	5.84+1.03	6.80+0.58	4.79+0.77	4.76+1.04	4.50+0.71
Granulocytes (%)	26.73+4.52	32.96+4.69	44.33+5.99	29.97+5.99	28.78+3.55	36.43+4.99

Mean ± SEM

constipation groups. Additionally, the colour of the liver in all SPE treatment groups was dark brownish red, similar to that in the normal control and constipation groups (Figure 1(A)). The liver-to-body weight ratio was also not significantly different between the groups (Figure 1(B)). Histological staining showed no effect on liver architecture in the SPE-treated group. Hepatocytes, hepatic cords, sinusoids, central vein and portal triad were normal without any damage, inflammation or atrophy (Figure 1(C)). The distribution of glycogen storage in liver tissue was also normal and smoothly distributed in the cytoplasm (Figure 1(D)). There was no fibrosis throughout the liver tissue, except for a small amount of collagen fibre accumulation in the portal triad, which supports the internal structures (Figure 1(E)). Moreover, a functional test based on ALP, ALT, AST, and bilirubin levels showed that liver function was normal because these parameters were not significantly changed compared with those in the normal control group (Figure 1(F)). These results are consistent with those of previous reports that *Sargassum* sp. consumption had no effect on liver histology and function (Sengkhim et al. 2021; Tipbunjong et al. 2023). It is well known that the liver is the first organ to be exposed to substances absorbed from the gastrointestinal tract and is the organ for drug metabolism. There have been many reports that several different herbs and herbal products have been implicated in hepatic injury (Teschke et al. 2013). Exposure of the liver to toxic substances has been reported to elevate liver enzyme levels, which are important markers reflecting liver function (Yang et al. 2016); increase the liver to body weight (Zhang et al. 2023); enlarge the liver, oedema, lighter colour and brittle texture (Li et al. 2021); and increase hepatocyte degeneration and necrosis, inflammatory cell infiltration and collagen accumulation (Zhang et al. 2023). Hence, the normal liver function, appearance and histology without any damage, fibrosis and inflammation in this experiment imply the safety of SPE consumption for constipation management.

EFFECT OF SPE ON THE KIDNEYS OF CONSTIPATION MICE

The gross morphological structures of the kidneys in all SPE doses consumed daily for 14 days were normal in shape, size and colour (Figure 2(A)). No signs of inflammation or haemorrhage were observed in any treatment group. The kidney-to-body weight ratio showed that daily SPE consumption for 14 days had no effect on kidney weights (Figure 2(B)). Histological examinations using H&E indicated the architecture of the glomerulus, Bowman's capsule and renal tubules were normal; no signs of damage, atrophy or inflammation were observed (Figure 2(C)). Additionally, there was no collagen deposition throughout the renal tissue confirming that there was no renal fibrosis (Figure 2(D)). Moreover, functional tests based on BUN and creatinine levels showed that SPE did not affect renal function due to these parameters were not significantly different from the normal control (Figure 2(E) and 2(F)). These results are consistent with those of previous reports that *Sargassum* sp. consumption has no effect on kidney histology and function (Sengkhim et al. 2021; Tipbunjong et al. 2023). Because kidneys are often affected by toxic substances, the kidneys of animal models exposed to toxic herbal medicines containing poisonous constituents from medicinal plants caused injury in the glomeruli, renal tubules and collecting ducts, leading to progressive interstitial renal fibrosis (Xu et al. 2020). Additionally, several pharmacological ingredients in herbal medicines have been reported to cause acute toxic kidney injury (Yang et al. 2018). Thus, the absence of changes in external morphology, histology and renal function in SPE treatment indicates that its use to relieve constipation is safe.

EFFECT OF SPE ON THE COLON OF CONSTIPATION MICE

After consumption of all SPE doses daily for 14 days, the colon tissue showed a normal external appearance, and its length was not significantly different from that of the normal control and constipation groups (Figure 3(A) and 3(B)). It has been shown that colon length decreases under inflammatory conditions (Sakena et al. 2020; Siringoringo et al. 2021). Our results indicate that SPE consumption did not cause any inflammation in the colon. Furthermore, histological studies using H&E staining showed that the epithelium of the SPE treatment group was normal, consisting of simple columnar epithelium with goblet cells (Figure 3(C)). PAS staining also showed no difference in the distribution and number of goblet cells in the epithelial layer between the groups (Figure 3(D)). These results are consistent with those of previous reports that *Sargassum* sp. consumption did not cause damage to the colon epithelium or changes in the number of goblet cells (Sengkhim et al. 2021; Tipbunjong et al. 2023). Consuming toxic contaminants causes epithelial damage and dysbiosis of the gut microenvironment and induce gut inflammation (Chen et al. 2023a). Thus, the intact colon characteristics implied that SPE did not cause any irritation to the colon

EFFECT OF SPE ON GENE EXPRESSION IN THE LIVER, KIDNEY AND COLON OF CONSTIPATION MICE

The gene groups tested in this experiment included oxidative stress (Gadd153 and Sod1), cell cycle–apoptosis regulation (PCNA and Clu), acute phase response (Saa3), metabolic processes (Odc1), detoxification enzymes (Oat) and heat-shock response (Hspcb), which have been reported to be marker genes altered during toxic insults (Fabian et al. 2011). Quantitative real-time PCR showed that daily SPE consumption for 14 days significantly modulated the expression of several genes (Figure 4). The daily SPE consumption for 14 days influenced gene

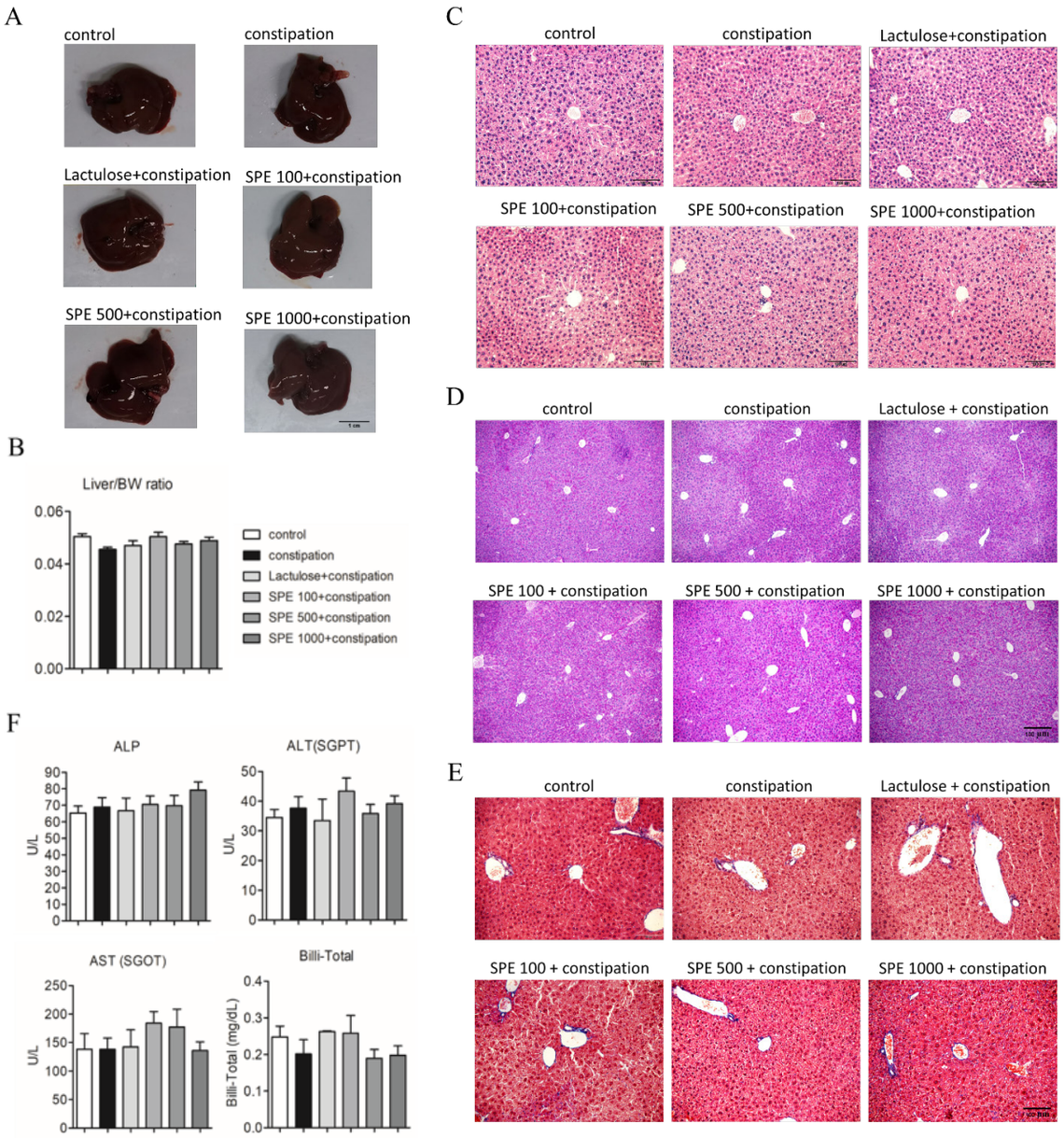

FIGURE 1. Effect of *Sargassum polycystum* extract (SPE) on the liver in mice with loperamide-induced constipation. Mice were pretreated daily with SPE (100, 500 and 1000 mg/kg) for 14 days before induction of constipation with loperamide. The liver was excised for photography (A) and weighing (B). Liver tissue was stained with haematoxylin and eosin (H&E) (C), periodic acid–Schiff (PAS) (D) and Masson's trichrome (E). Liver function enzyme levels were measured (F). Scale bars in (C)–(E) are 100 µm

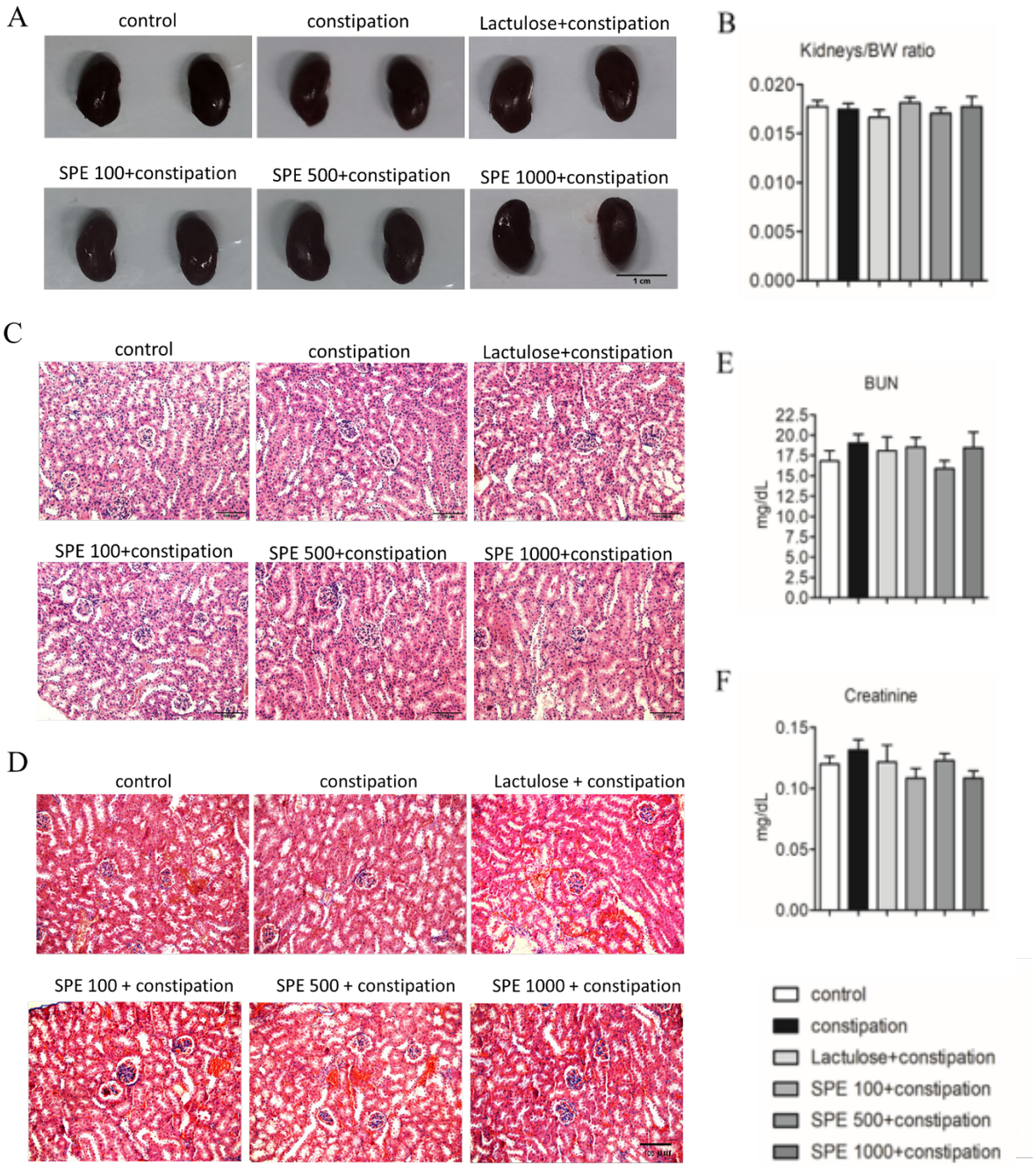

FIGURE 2. Effect of *Sargassum polycystum* extract (SPE) on the kidneys of mice with loperamide-induced constipation. Mice were pretreated daily with SPE (100, 500 and 1000 mg/kg) for 14 days before induction with loperamide. The kidneys were excised for photography (A) and weighing (B). The kidney tissue was stained with haematoxylin and eosin (H&E) (C) and Masson's trichrome (D). The renal function was measured (E, F). Scale bars in (C) and (D) are 100 μm

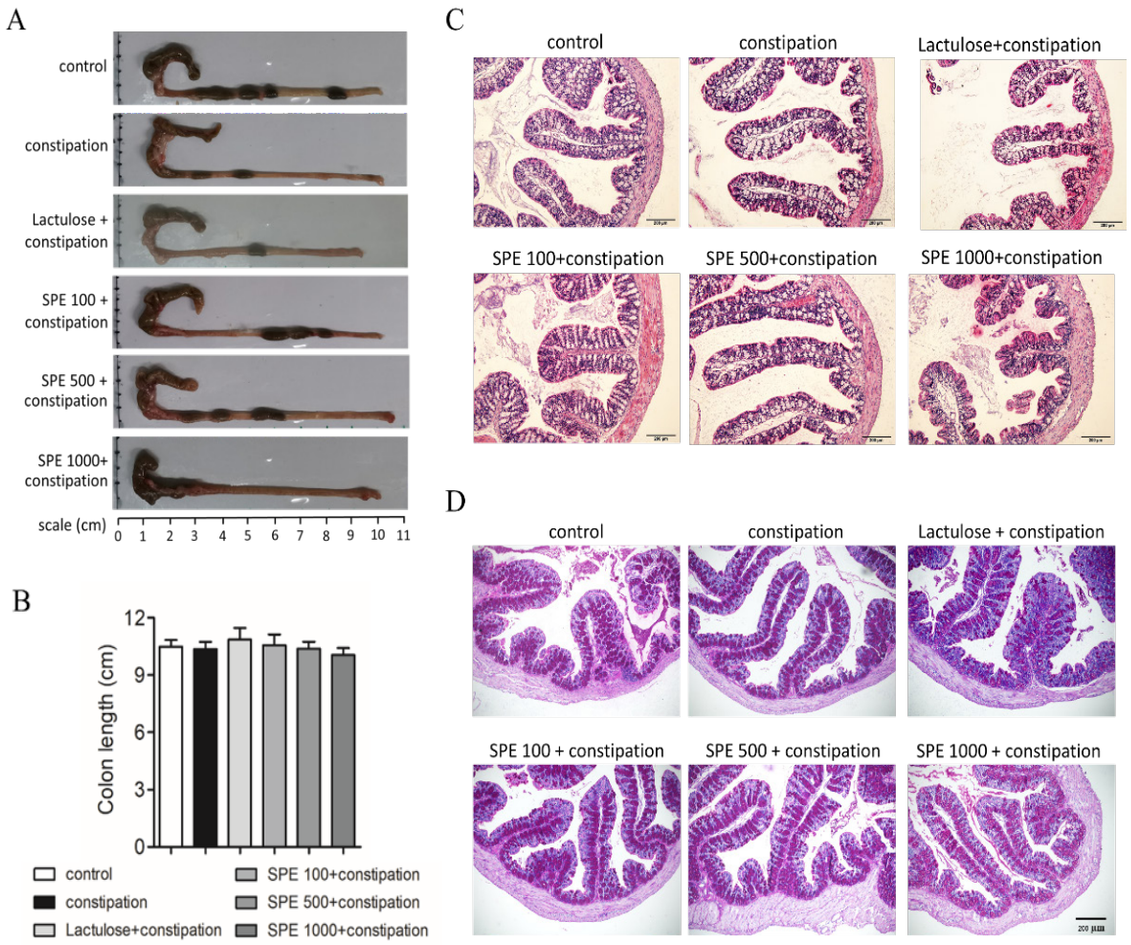

FIGURE 3. Effect of *Sargassum polycystum* extract (SPE) on the colon in mice with loperamide-induced constipation. Mice were pretreated daily with SPE (100, 500 and 1000 mg/kg) for 14 days before constipation induction with loperamide. The colon was excised for photography (A) and length measurement (B). Colon tissue was stained with haematoxylin and eosin (H&E) (C) and periodic acid–Schiff (PAS) (D). Scale bars in (C) and (D) are 200 µm

expression possibly by promoting a healthy microbiome in the host (Chichlowski et al. 2007) and subsequently modulating gene expression. Recently, Khuituan et al. (2022) reported that fermentation of prebiotics in the colon increases the counts of *Bifidobacteria*, which are beneficial for the growth of butyrate-producing bacteria, such as *Faecalibacterium*, *Eubacterium,* and *Roseburia*. These butyrate-producing bacteria induce profound changes in genes involved in energy metabolism, cell growth and proliferation and immunity (Li & Li 2006).

Odc1 expression in kidney tissue from the SPE-treated group was significantly increased compared with that in the control group. This gene has been reported to be involved in apoptosis inhibition by inhibiting mitochondrial dysfunction and reactive oxygen species (ROS) generation (Sato et al. 2020). Additionally, constipation significantly decreased *Clu* expression in kidneys compared with the control, whereas pretreatment with SPE significantly mitigated this effect. *Clu* overexpression protects against intracellular protein aggregation and cytotoxicity in neuronal cells and *Drosophila* systems (Gregory et al. 2017). These changes in *Odc1* and *Clu* expression are beneficial for cellular stress and apoptosis protection because they inhibit ROS-induced apoptosis via NF-κB and MAPK activation (Jiang et al. 2018). Moreover, the liver and kidney tissues of the constipation group showed

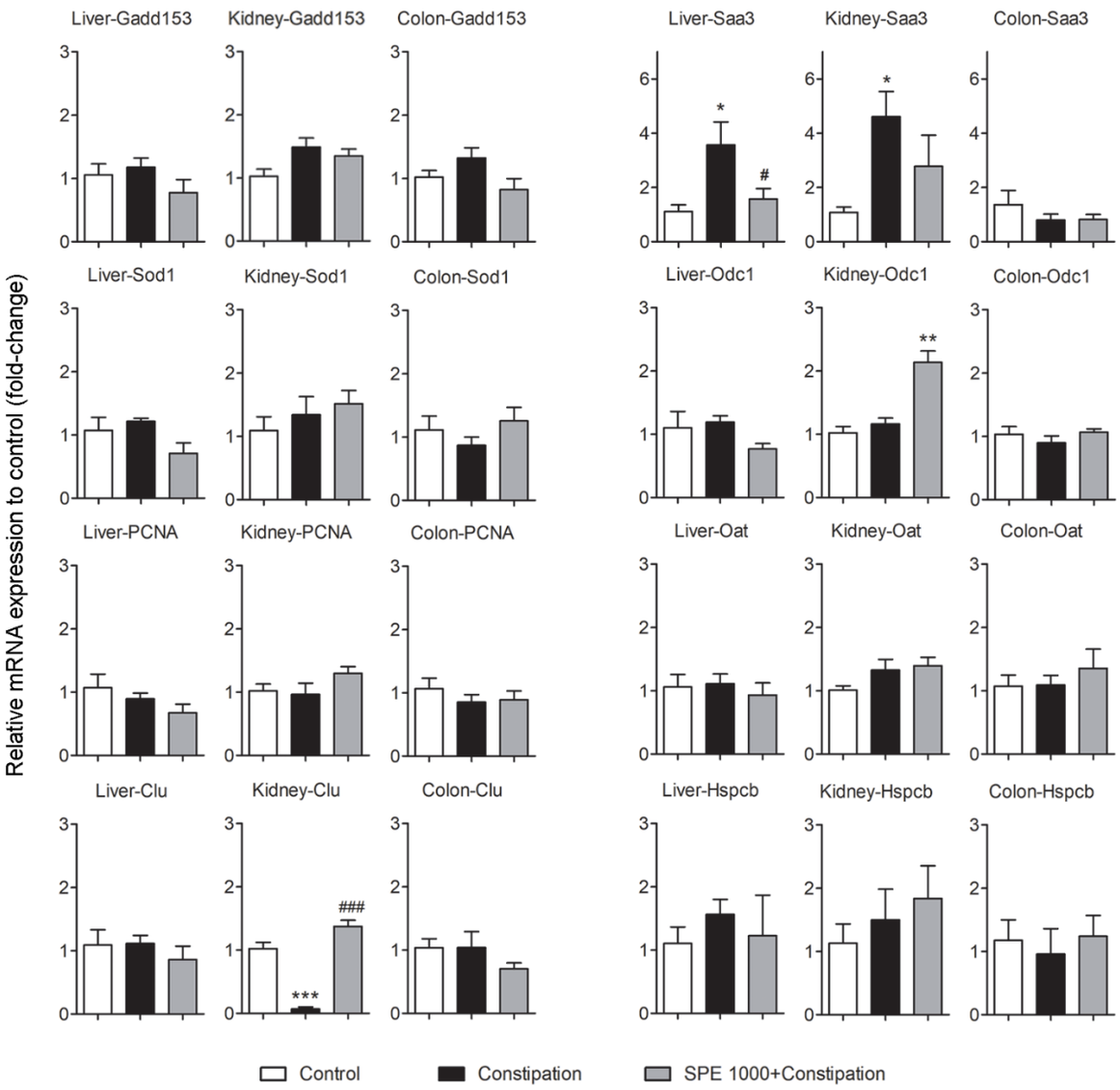

FIGURE 4. Effect of daily *Sargassum polycystum* extract (SPE) (1000 mg/kg) administration for 14 days on gene expression in the liver, kidney and colon of mice with loperamide-induced constipation. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the control, $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ compared with constipation

a significant increase in *Saa3* expression compared with the control, whereas pretreatment with SPE significantly mitigated this effect. Previously, serum amyloid A3 was present in mouse plasma following exposure to various inflammatory stimuli (Chait et al. 2020). In our study, SPE prevented the expression of *Saa3* in the liver and kidney of constipation mice, which may be due to its anti-inflammatory activity (Giriwono et al. 2019). Decreases in *Saa3* expression can prevent inflammation by suppressing the secretion of pro-inflammatory cytokines (interleukins, COX-2 and TNF-α), inhibiting macrophage infiltration and suppressing the pathogenic differentiation of CD4 T cells (Chen et al. 2023b).

Conversely, other genes in other organs being tested were not significantly different. These results were consistent with those of previous reports that SPE did not cause cell death in macrophages, dermal fibroblasts, keratinocytes and adipocytes (Lee et al. 2023; Premarathna et al. 2023); cellular oxidative stress in melanocytes

(Prasedya et al. 2022); in zebra fish (Wang et al. 2023); and cellular metabolism imbalance (Rodrigues et al. 2019). Thus, SPE consumption may exert a protective mechanism against cellular stress, apoptosis and inflammation without any adverse effects.

CONCLUSIONS

This is the first report to provide evidence of the effect of *Sargassum* extract consumption on gene expression in the liver, kidneys and colon of a mouse model. The finding of this study indicates that *Sargassum* extract can modulate the *Saa3*, *Odc1,* and *Clu* expressions, which are beneficial for the protective mechanisms against cellular stress, apoptosis and inflammation during constipation. Furthermore, these results are consistent with the lack of effects of SPE on biochemical, haematological, and histopathological parameters, indicating that SPE increased defecation frequency and relieved constipation in mice without any toxic effects on the liver, kidneys or colon. Considering the health benefits of SPE for preventing constipation and its safety and efficacy aspects, SPE is an interesting candidate supplement to manage and relieve constipation in patients.

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