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Research Article

Bisacodyl overcomes morphine-induced constipation by decreasing colonic Aquaporin-3 and Aquaporin-4 expression

Mahardian Rahmadi[1], Chrismawan Ardianto[1], Ahmad D. Nurhan[1], Rafiqa A. Chasanah[1], Dewi I. Krismonika[1], Arina D. Puspitasari[1], Budi Suprapti[1,2], Santhra Segaran[3,4], Chia-Wei Phan[5], Junaidi Khotib[1]

1 *Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, Indonesia*
2 *Department of Pharmacy, Universitas Airlangga Teaching Hospital, Surabaya 60115, Indonesia*
3 *Department of Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia*
4 *Department of Allied Health Sciences, Faculty of Health and Life Sciences, Management & Science University, Shah Alam, Selangor 40100, Malaysia*
5 *Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, University of Malaya, Kuala Lumpur 50603, Malaysia*

Corresponding author: Mahardian Rahmadi (mahardianr@ff.unair.ac.id)

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Abstract

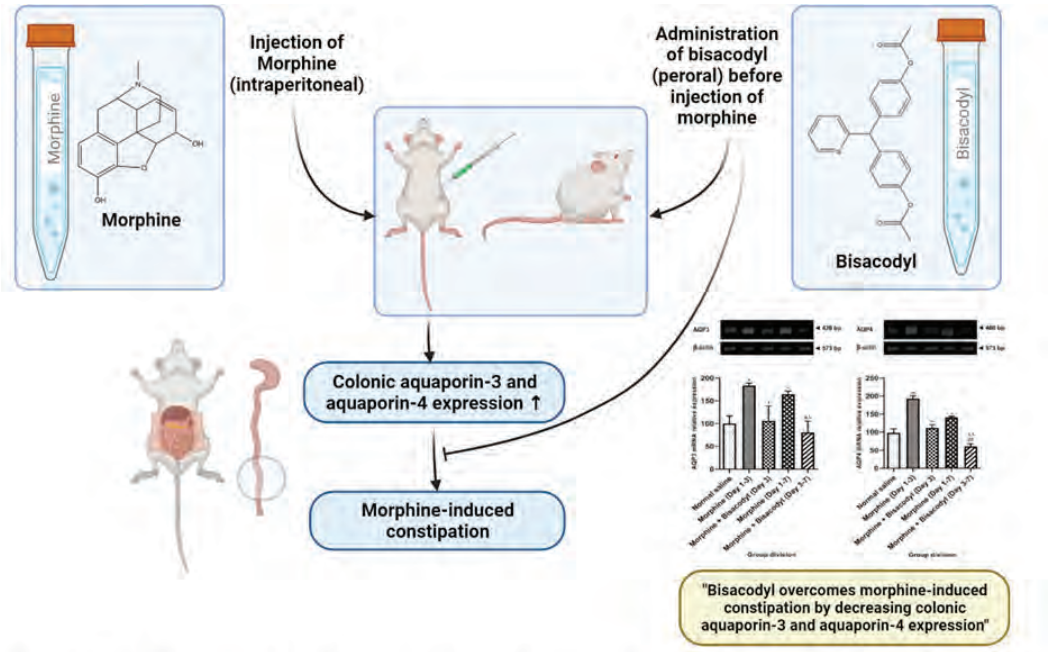
Introduction: Morphine is an opioid prescribed to treat aches and pains. However, morphine often causes opioid-induced constipation (OIC). Aquaporin (AQP) transporters, especially AQP3 and AQP4, play an essential role in mediating constipation. Bisacodyl is a common laxative used to treat constipation. To date, the effects of bisacodyl on AQP3 and AQP4 expression and the role this interaction plays in constipation are unclear. This study aimed to determine the effects of bisacodyl on AQP3 and AQP4 expression in mice after induction of constipation with morphine.

Materials and methods: The laxative effects of bisacodyl on both acute and chronic morphine-induced constipation were determined. Fecal water content, colonic bead expulsion, and colonic mRNA levels for AQP3 and AQP4 mRNA were measured.

Results and discussion: The administration of morphine to mice resulted in decreased fecal water content, longer bead expulsion times, and increased AQP3 and AQP4 mRNA levels in the colon. Meanwhile, bisacodyl administration prevented the morphine-induced changes in fecal water content, bead expulsion time, and AQP3 and AQP4 mRNA levels in the colons of mice.

Conclusion: This study suggests that bisacodyl may prevent morphine-induced constipation by preventing morphine-induced increases in AQP3 and AQP4 expression in the colon.

Graphical abstract:



Bisacodyl decreases colonic aquaporin-3, and aquaporin-4 expression is a promising pharmacological mechanism to overcome morphine-induced constipation.

Keywords

AQP3, AQP4, health risk, laxative, opioid-induced constipation

Introduction

The use of opioids for the treatment of chronic pain has increased in the last decade (Nelson and Camilleri 2016). Although opioids effectively relieve moderate to severe pain, their side effects, such as dependence, hyperalgesia, respiratory depression, nausea, vomiting, abdominal pain, and constipation, are the main reasons for discontinuation of therapy (Chou et al. 2009; Hooten et al. 2015). Opioid-induced constipation (OIC) is one of the most common side effects of opioids, occurring in up to 70% of patients receiving opioid therapy (Wan et al. 2015). Constipation is characterized by a reduction in the water content of stool, which results in a smaller volume of feces and difficulties in defecating (Kon et al. 2015; Farmer et al. 2018). Reduced frequency of defecation is also a sign of constipation (Sharma and Rao 2015; Farmer et al. 2018).

Morphine is an opioid drug used to treat moderate to severe pain (cancer) (Buenaventura et al. 2008; Rumman et al. 2016). Morphine acts as a selective μ-opioid receptor (MOR) agonist (Ninković and Roy 2013). Various studies confirmed that MOR agonists induce constipation (Ono et al. 2014). Morphine acts on enteric nerves by inhibiting the neurotransmitter acetylcholine, which decreases peristaltic activity, increases absorption of fluids and electrolytes, and increases tonal contraction resulting in constipation (Wood and Galligan 2004; Sobczak et al. 2014; Nelson and Camilleri 2015). Pharmacological therapy for constipation includes the administration of laxatives (Werth and Christopher 2021). The main laxatives to treat constipation are stimulant laxatives (Ishihara et al. 2012). Stimulant laxatives act directly on the submucosal plexus and myenteric plexus, to stimulate colonic motility and reduce water absorption from the intestine. Bisacodyl is a common stimulant laxative (Larkin et al. 2008; Corsetti et al. 2021).

The role of aquaporins (AQP) in the development of constipation is currently being explored. AQP is a canal membrane protein that transports water in the human body and is essential for water homeostasis (Ikarashi et al. 2012). There are thirteen types of AQP in humans, AQP0 to AQP12, which are expressed in various tissues, including the gastrointestinal tract (King et al. 2004). AQPs have different functions in different parts of the digestive tract (Laforenza 2012). AQP3 is predominantly expressed in colonic mucosal epithelial cells in humans and mice

(Ikarashi et al. 2011). AQP4 expression is also predominantly expressed in the colon, especially in the enteric nervous system of the mouse colon and on the basolateral membranes of epithelial cells in distal colonic villi (Thi et al. 2008).

AQP expression in the gastrointestinal tract changes over time, presumably in relation to the regulation of water homeostasis. An increase in AQP expression occurs one hour after morphine administration. Thus, giving a laxative before morphine may prevent the side effects of constipation by regulating AQP expression (Kon et al. 2015; Ikarashi et al. 2016). The present study was conducted to determine the effects of the stimulant laxative, bisacodyl, on fecal water content, colonic AQP3 and AQP4 mRNA levels, and colonic bead expulsion in mice with morphine-induced acute and chronic constipation.

Materials and methods

Experimental animals

Balb/c male mice, aged 8–12 weeks and weighing 20–30 g, in healthy condition, with normal behavior, and no visible abnormalities in the body, were used in this study. Environmental conditions of the mice were maintained at normal temperature (25±2 °C) and humidity (60±10%) and a 12-hour light/12-hour dark cycle. Food and drink were provided ad libitum. All mice were habituated for seven days before treatment. All experiments in this study were performed at the Animal Laboratory of the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. All protocols in this study complied with the Guidelines for the Care and Use of Laboratory Animals issued by the National Institute of Health revised in 1985 and approved by the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, with Certificate of ethical clearance No.684-KE of 06.04.2017.

Acute morphine-induced constipation

Thirty-six Balb/c male mice were divided into six groups. The experimental mice were injected intraperitoneally (i.p.) with 10 mg/kg of body weight (BW) morphine to induce constipation. Morphine was injected in the positive control group (morphine only) and the morphine + bisacodyl group. Meanwhile, the negative control group was injected with normal saline 10 µl/g BW i.p. Morphine and normal saline were injected 30 minutes after carboxymethyl cellulose (CMC)-Na 0.5% oral (p.o.) or bisacodyl 100 mg/kg BW p.o. administration. Normal saline, morphine, and bisacodyl were administered once a day. The treatment protocol and the group divisions of the acute morphine-induced constipation mice are shown in Fig. 1.

Assessment of acute morphine-induced constipation

To assess acute morphine-induced constipation, the weight, amount of feces, and fecal water content were measured. The weight and amount of feces were determined

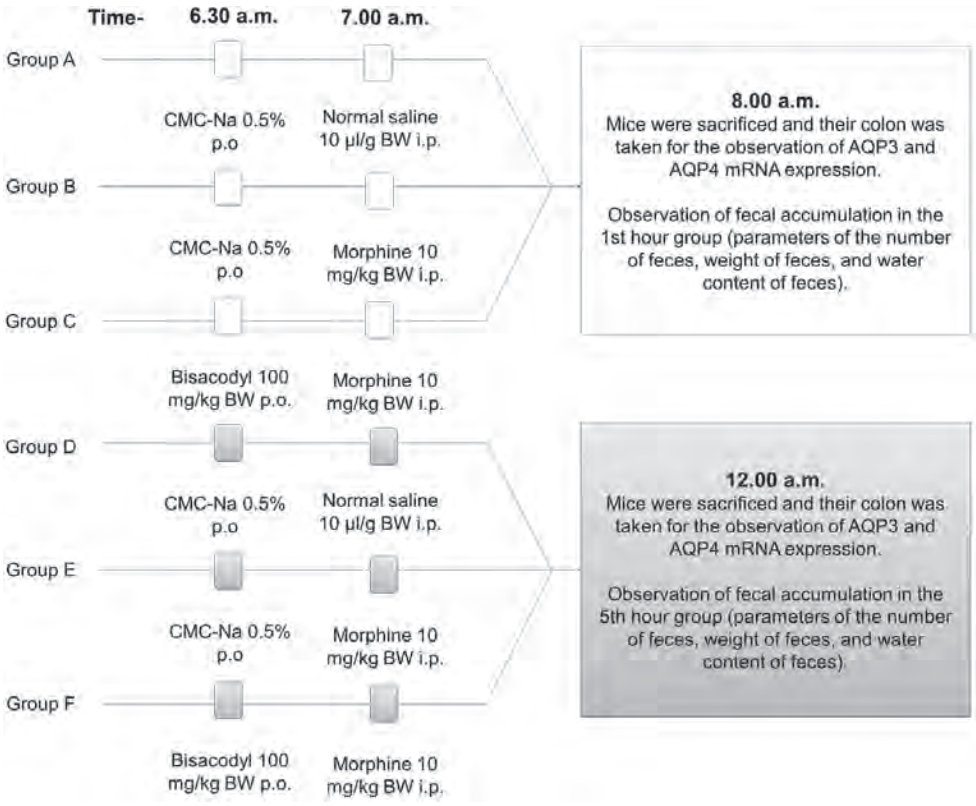


Figure 1. Treatment protocol and group division for the observation of fecal water content (%) and AQP3/AQP4 mRNA expression in mice with acute morphine-induced constipation. **Note:** BW – body weight; CMC – carboxymethyl cellulose.

based on the accumulated weight and the number of feces collected during predetermined times (first hour and fifth hour). The laxative (bisacodyl) effectiveness was determined based on the percentage of water content in the accumulated feces of mice during a predetermined time (first hour and fifth hour). Feces collection began with weighing the identified blank aluminum foil (cap and body) and writing the value as W_0. Then, the mouse feces were collected and put on the aluminum foil using the small scoop and the feces were covered with the cap part to minimize evaporation. Furthermore, the feces + aluminum foil were weighed, and the value was written as W_1. The weight value of wet feces (W_2) was W_1-W_0. The wet feces were put in the oven at 100–105 °C until a constant weight of feces was obtained. The feces + aluminum foil were weighed after drying and the value was written as W_3. The weight value of dry feces (W_4) was W_3-W_0. The water content of feces was calculated with the formula: $(W_4\text{-}W_2)/W_2$ x 100%. AQP-3 and AQP-4 mRNA expression was evaluated in colon, the mice were sacrificed at specified times (first hour and fifth hour) (Fig. 1). The mouse colons were removed and stored at -80 °C.

Chronic morphine-induced constipation

Fifty-one Balb/c male mice were divided into three treatment groups. Fecal water content, colonic bead expulsion, and reverse transcription PCR were measured. The fecal water content and colonic bead expulsion were measured in 18 mice divided into three groups (Fig. 2). AQP3 and AQP4 were measured in 15 mice divided into five groups (Fig. 3). Constipation was induced in the positive control group and the morphine + bisacodyl group. Both groups were injected with morphine 10 mg/kg BW i.p. Meanwhile, the negative control group was injected with normal saline 10 µl/g BW i.p. The injection of morphine 10 mg/kg BW or normal saline 10 µl/g BW was performed 30 minutes after the administration of bisacodyl or CMC-Na 0.5%. The bisacodyl 100 mg/kg BW p.o. or CMC-Na 0.5% p.o. was administered from day 3 of the experiment. Normal saline, morphine, and bisacodyl were administered twice a day according (Fig. 2). The treatment protocol and the group divisions of the chronic morphine-induced constipation mice are shown in Fig. 2.

Assessment of chronic morphine-induced constipation

Chronic morphine-induced constipation and the effectiveness of bisacodyl were assessed by measuring fecal water content and colonic bead expulsion. Fecal water content was expressed as the percentage of fecal water content accumulated daily, measured every 2 hours from 9 a.m. to 5 p.m. (Fig. 2). Colonic bead expulsion was assessed based on the length of time for the bead to leave the colon by monitoring the discharge of the bead from the rectum of mice. Measurements were taken from 7.00 a.m. after normal saline/morphine injection for a maximum of 2 hours per day on days 2, 3, and 7 (Fig. 2). The mice were sacrificed on day 3 (to assess the effect of short-term bisacodyl administration on AQP3 and AQP4 mRNA expression) and day 7 (to assess the effect of long-term bisacodyl administration on AQP3 and AQP4 mRNA expression) (Fig. 3). The mouse colons were removed and stored at -80 °C.

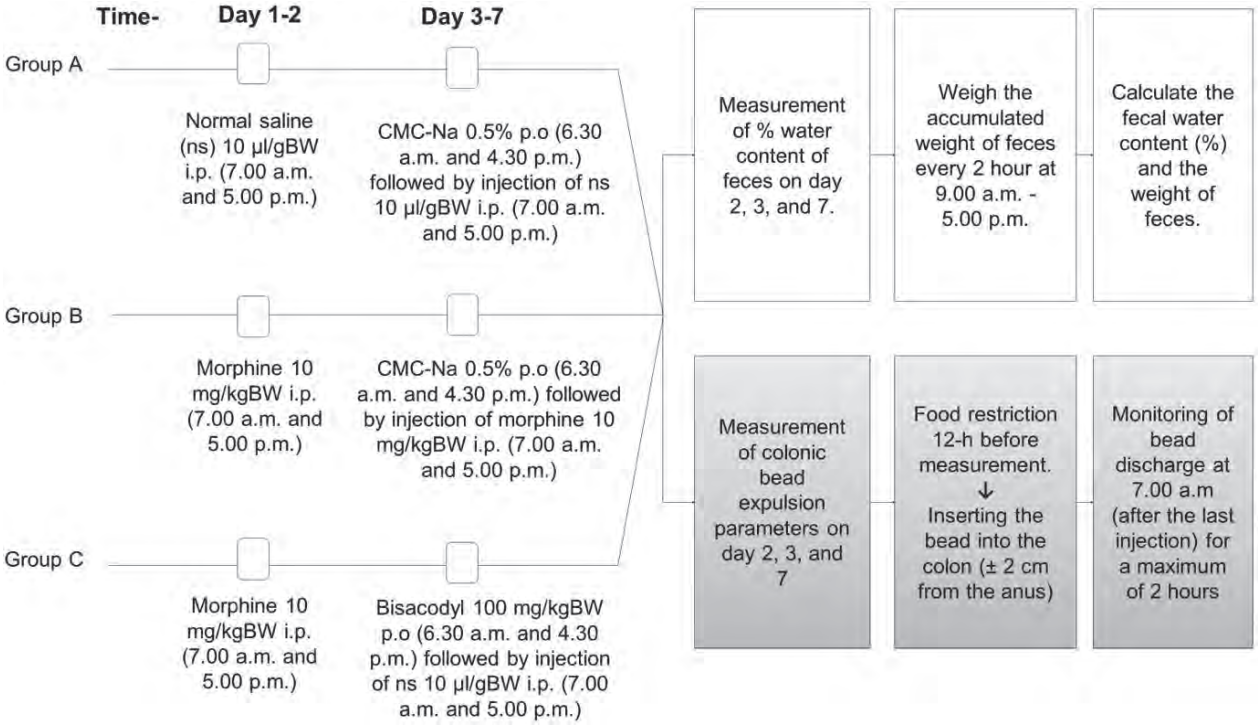


Figure 2. Treatment protocol and group division for colonic bead expulsion and fecal water content (%) in mice with chronic morphine-induced constipation. **Note:** BW – body weight; CMC – carboxymethyl cellulose.

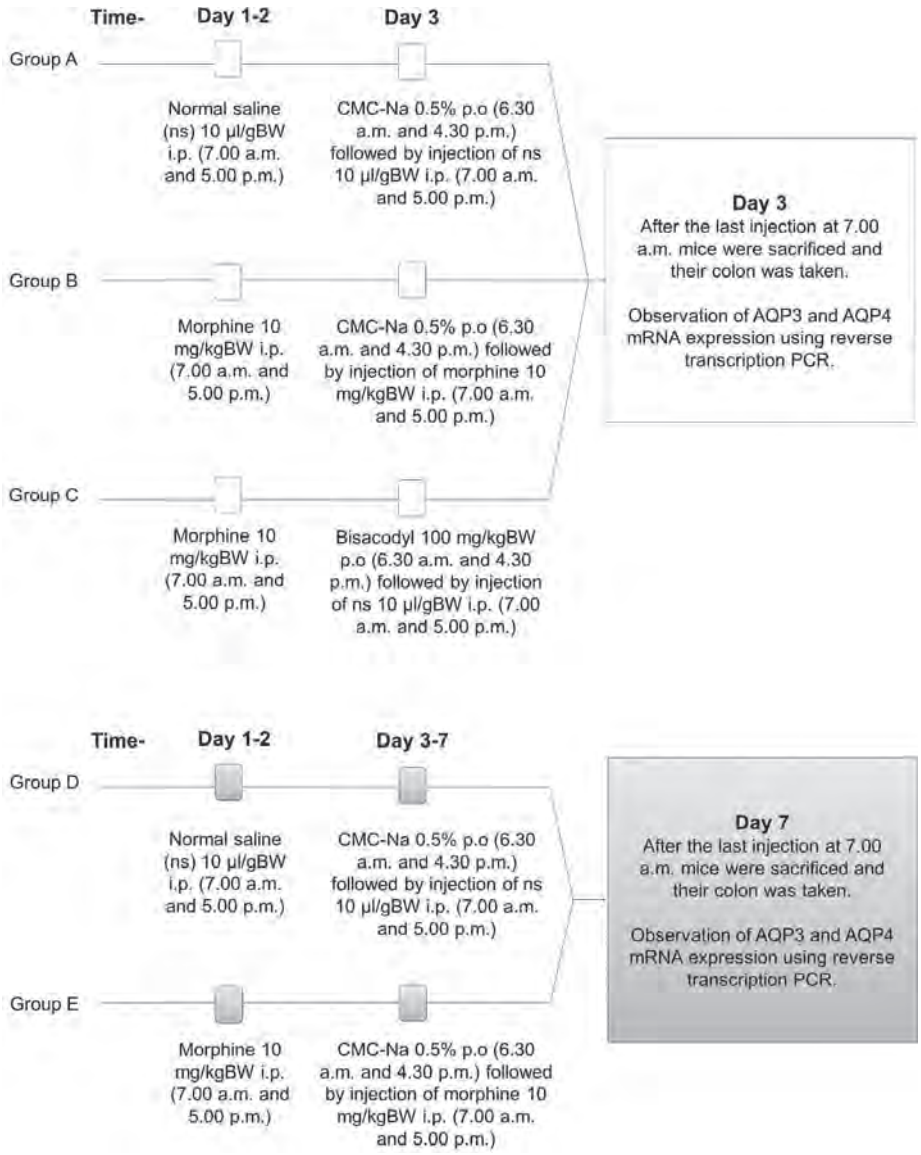


Figure 3. Treatment protocol and group division for the observation of AQP3/AQP4 mRNA expression in mice with chronic morphine-induced constipation. **Note:** BW – body weight; CMC – carboxymethyl cellulose.

Reverse transcription PCR

AQP3 and AQP4 mRNA expression levels were measured in the acute and chronic morphine-induced constipation models using reverse transcription PCR. Total RNA from mouse colons was isolated using PureLinkTM RNA Mini Kit (Life Technologies, USA). Reverse transcription was performed using the GoScriptTM Reverse Transcription System (Promega, USA) with an Oligo(dT)$_{15}$ primer. PCR was performed using GoTaq DNA Polymerase (Promega, USA). The following primers were used: AQP3 (Forward: 5'-GGGCTGTACTACGATGCAATC-3'; Reverse: 5'ACACGAAGACACCAGCGATGG-3'); AQP4 (Forward: 5'AGCCGGCATCCTCTACCTG-3'; Reverse: 5'CTGCGCGGCTTTGCTGAA-3'); and β-actin (Forward: 5'TGTTACCAACTGGGACGACA-3'; Reverse: 5'AAGGAAGGCTGGAAAAGAGC-3'). The following thermal cycler conditions were used: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 55 °C for 1 minute, extension at 72 °C for 2 minutes, and ended with a final extension at 72 °C for 5 minutes. An analysis of PCR products was carried out on 2% agarose gels (Promega, USA) after electrophoresis (Mupid-e; Advance, Tokyo, Japan). Agarose gels were stained using ethidium bromide (Sigma-Aldrich) and photographed with UV transillumination. Band intensities were determined using ImageJ software. The mean of each sample band was calculated, then the ratio of each sample to β-actin was calculated (relative expression).

Statistical analysis

Data are presented as mean±standard error of the mean (SEM). All data were statistically analyzed using two-way ANOVA with Tukey's post hoc test. All calculations were performed using the GraphPad Prism 6 Software (GraphPad, Inc., San Diego, CA, USA).

Results

Effects of bisacodyl on fecal water content (%) in mice with acute morphine-induced constipation

Data regarding fecal water content in acute morphine-induced constipation are shown in Fig. 4. In the first hour after injection, fecal water content significantly decreased in the morphine-treated group compared to fecal water content in the saline group ($p<0.01$). Bisacodyl treatment attenuated the morphine-induced decrease in fecal water content ($p<0.01$). However, fecal water content was lower in the bisacodyl/morphine-treated group compared to the saline group in the first hour ($p<0.01$). In the fifth hour after injection, fecal water content was significantly lower in the morphine-treated group compared to the saline-treated group ($p<0.05$). Bisacodyl treatment significantly attenuated the decreased fecal water content induced by morphine ($p<0.01$). Furthermore, fecal water content in the morphine-treated group in the fifth hour was significantly higher than fecal water content in the morphine-treated group in the first hour ($p<0.01$). The representative photos of feces with high and low water content are presented in Suppl. material 1.

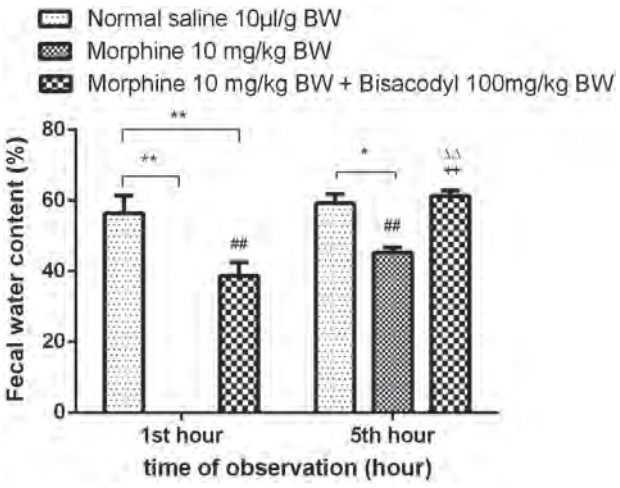


Figure 4. The percentage of fecal water content (mean±SEM) in the first and fifth hours after normal saline/morphine injection (n=6) in acute morphine-induced constipation. **Note:** ** – $p<0.01$ versus normal saline 10 µl/g BW (1st hour) group; * – $p<0.05$ versus normal saline 10 µl/g BW (5th hour) group; $^{\#\#}$ – $p<0.01$ versus morphine 10 mg/kg BW (1st hour) group; $^{++}$ – $p<0.01$ versus morphine 10 mg/kg BW + bisacodyl 100 mg/kg BW (1st hour) group; $^{\Delta\Delta}$ – $p<0.01$ versus morphine 10 mg/kg BW (5th hour) group.

Effects of bisacodyl on mice colonic AQP3 mRNA expression in acute morphine-induced constipation

The relative expression of colonic AQP3 mRNA in acute morphine-induced constipation is shown in Fig. 5. AQP3 mRNA levels increased significantly in the morphine-treated group compared to the saline group at both the first and fifth hours after injection ($p<0.01$). Bisacodyl treatment significantly attenuated the increased AQP expression in the fifth hour ($p<0.01$), but not the first hour after injection. In addition, AQP expression in the fifth hour was significantly lower than AQP expression in the first hour in the bisacodyl-treated mice ($p<0.01$).

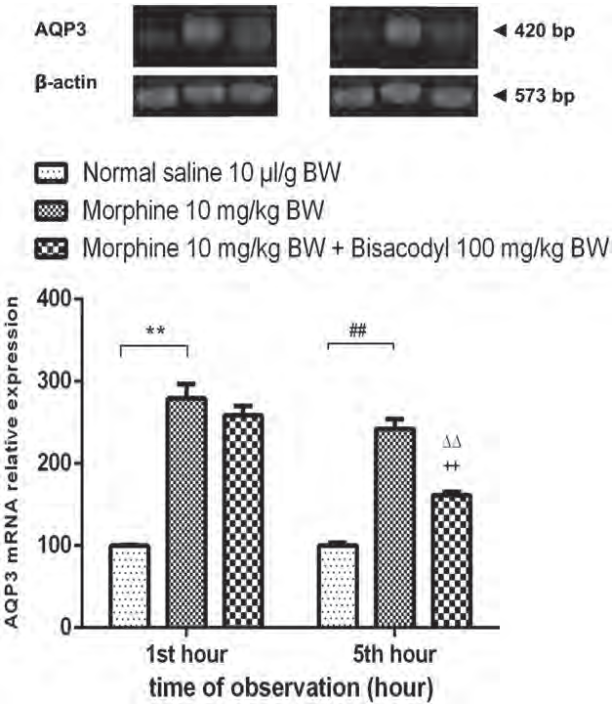


Figure 5. Mouse colonic AQP3 mRNA relative expression (mean±SEM) in the first and fifth hours after normal saline/morphine injection (n=3) in acute morphine-induced constipation. **Note:** ** – $p<0.01$ versus normal saline 10 µl/kg BW group; $^{\#\#}$ – $p<0.01$ versus normal saline 10 µl/g BW (5th hour) group; $^{++}$ – $p<0.01$ versus morphine 10 mg/kg BW + bisacodyl 100 mg/kg BW (1st hour) group; $^{\Delta\Delta}$ – $p<0.01$ versus morphine 10 mg/kg BW (5th hour) group; BW – body weight, AQP – aquaporin.

Effects of bisacodyl on mice colon's AQP4 mRNA expression in acute morphine-induced constipation

The relative colonic AQP4 mRNA expression in acute morphine-induced constipation is shown in Fig. 6. Colonic AQP4 mRNA expression did not change significantly in any group of mice with acute morphine-induced constipation in the first or fifth hours after injection of normal saline/morphine.

Effects of bisacodyl on fecal water content (%) in chronic morphine-induced constipation

Data regarding fecal water content (%) in chronic morphine-induced constipation are shown in Fig. 7. Morphine treatment significantly decreased fecal water content on days 2, 3, and 7 after injection compared to fecal water content after normal saline injection on the corresponding days ($p<0.01$). Fecal water content was also significantly lower in the bisacodyl + morphine treated group on day 2 compared to fecal water content in the normal saline group ($p<0.01$). Fecal water content in the bisacodyl + morphine group was significantly higher than in the morphine treated

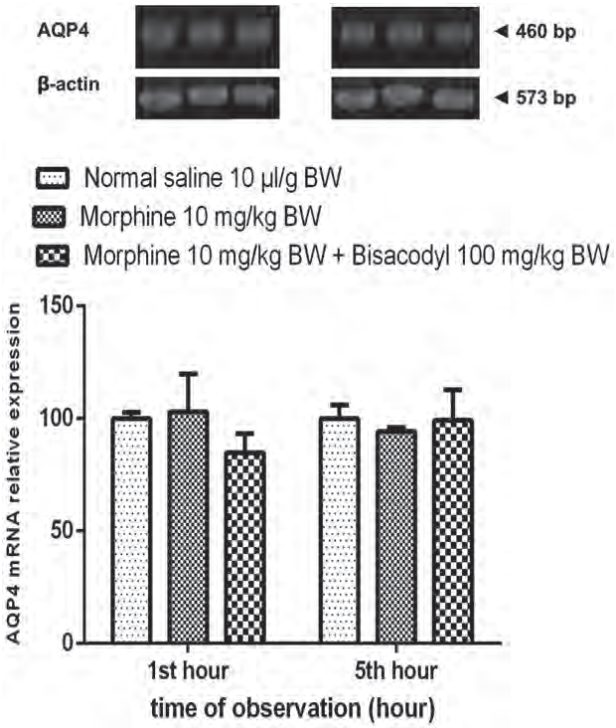


Figure 6. Mouse colonic AQP4 mRNA relative expression (mean±SEM) in the first and fifth hours after normal saline/morphine injection (n=3). **Note:** BW – body weight, AQP – aquaporin.

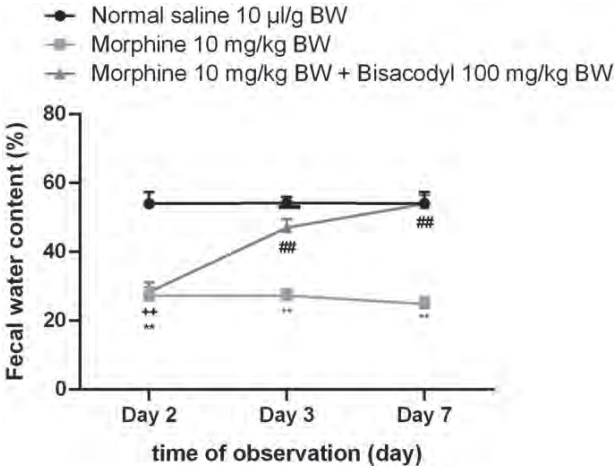


Figure 7. The percentage of fecal water content (mean±SEM) on days 2, 3, and 7 (n=6) after injection of morphine or saline. **Note:** **– p<0.01 morphine 10 mg/kg BW group versus normal saline 10 µl/g BW group; $^{++}$ – p<0.01 morphine 10 mg/kg BW + bisacodyl 100 mg/kg BW versus normal saline 10 µl/g BW group; $^{\#\#}$ – p<0.01 morphine 10 mg/kg BW + bisacodyl 100 mg/kg BW group versus morphine 10 mg/kg BW group; BW – body weight.

group on days 3 and 7 after injection of morphine (p<0.01). The representative photos of feces with high and low water content levels are presented in Suppl. material 1.

Effects of bisacodyl on colonic bead expulsion in chronic morphine-induced constipation

Data regarding bead expulsion time in chronic morphine-induced constipation are shown in Fig. 8. Morphine treatment

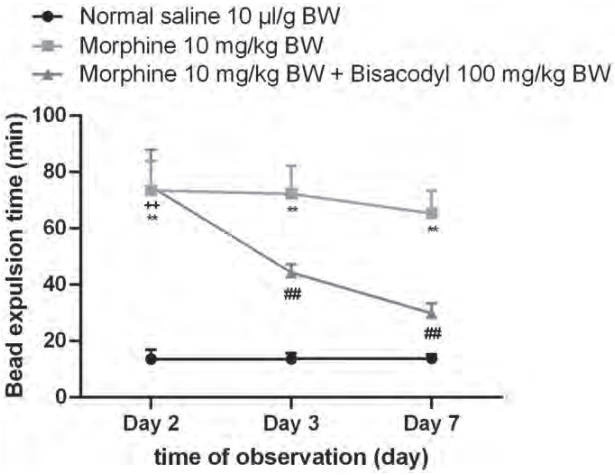


Figure 8. Bead expulsion time (mean±SEM) on days 2, 3, and 7 after normal saline/morphine injection (n=6). **Note:** ** – p<0.01 morphine 10 mg/kg BW group versus normal saline 10 µl/g BW group; $^{++}$ – p<0.01 morphine 10 mg/kg BW + bisacodyl 100 mg/kg BW versus normal saline 10 µl/g BW group; $^{\#\#}$ – p<0.01 morphine 10 mg/kg BW + bisacodyl 100 mg/kg BW group versus morphine 10 mg/kg BW group; BW – body weight.

significantly lengthened the time for bead expulsion compared with bead expulsion in the normal saline group on days 2, 3, and 7 (p<0.01). Bead expulsion time in the bisacodyl + morphine group was also significantly longer compared with bead expulsion in the normal saline groups on day 2 (p<0.01). On days 3 and 7, bead expulsion time was significantly shorter in the bisacodyl + morphine group compared with bead expulsion in the morphine group (p<0.01).

Effects of bisacodyl on mice colon's AQP3 mRNA expression in chronic morphine-induced constipation

Changes in relative colonic AQP3 mRNA expression in chronic morphine-induced constipation are shown in Fig. 9. AQP3 mRNA expression on days 1–3 in the morphine group was significantly higher than AQP3 expression in the saline group (p<0.05). The AQP3 mRNA expression in the bisacodyl + morphine on day 3 was significantly lower than AQP expression in the morphine group on days 1–3 group (p<0.05) and days 1–7 (p<0.05). In addition, AQP3 mRNA expression in the bisacodyl + morphine on days 3–7 was significantly lower than AQP expression in the morphine group on days 1–3 group (p<0.01) and days 1–7 (p<0.05).

Effects of bisacodyl on mice colon's AQP4 mRNA expression in chronic morphine-induced constipation

Changes in the relative expression of colonic AQP4 mRNA in chronic morphine-induced constipation are shown in Fig. 10. AQP4 mRNA expression in the morphine group on days 1–3 was significantly higher than AQP4 expression in the normal saline group (p<0.01). AQP4 expression in the morphine group on days 1–7 was also significantly higher than AQP4 expression in the normal saline group (p<0.05). The expression of AQP4 mRNA in the morphine + bisacodyl group on day 3 was significantly

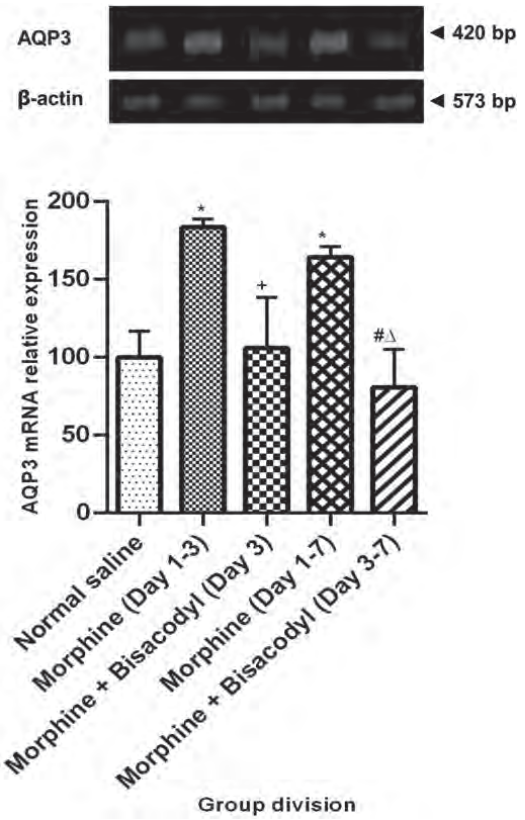


Figure 9. Mouse colonic AQP3 mRNA relative expression (mean±SEM) after normal saline/morphine injection (n=3) in chronic morphine-induced constipation. **Note:** * – p<0.05 versus normal saline 10 µl/kg BW group; + – p<0.05 versus morphine 10 mg/kg BW day 1-3 and morphine 10 mg/kg BW day 1-7 groups; # – p<0.01 versus morphine 10 mg/kg BW day 1-3 group; Δ – p<0.05 versus morphine 10 mg/kg BW day 1-7 group; AQP – aquaporin, BW – body weight.

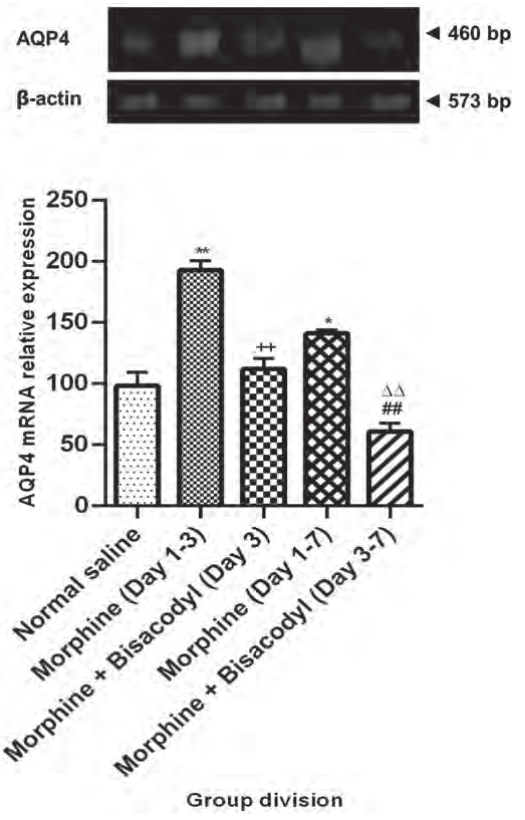


Figure 10. Mouse colonic AQP4 mRNA relative expression (mean±SEM) in after normal saline/morphine injection (n=3) in chronic morphine-induced constipation. **Note:** ** – p<0.01 and * – p<0.05 versus normal saline 10 µl/kg BW group; ++ – p<0.01 versus morphine 10 mg/kg BW day 1-3 group; ## – p<0.01 versus morphine 10 mg/kg BW day 1-3 group; ΔΔ – p<0.01 versus morphine 10 mg/kg BW day 1-7 group; AQP – aquaporin, BW – body weight.

lower compared with AQP4 expression in the morphine group on days 1–3 (p<0.01). Interestingly, AQP4 expression in the morphine + bisacodyl group on days 3–7 was significantly lower compared with AQP4 expression in the morphine group on days 1–3 group (p<0.01) and the morphine group on days 1–7 group (p<0.01).

Discussion

In the present study, the effects of bisacodyl, as a laxative agent against acute and chronic morphine-induced constipation, were investigated. The laxative effects of bisacodyl on acute morphine-induced constipation were assessed using fecal water content and AQP3 and AQP4 mRNA expression. Morphine-induced changes in fecal water content in our study were in agreement with several previous studies that showed lower fecal water content in response to short-term morphine administration (Fig. 4) (Suo et al. 2014). Low fecal water content occurs when morphine binds to the µ-opioid receptor, increasing fluid absorption and, thereby, increasing fecal contact time in the colon, which stimulates colonic mucosal receptors to activate reflex fluid absorption resulting in constipation (Camilleri 2011; Kon et al. 2015). The morphine group that had received bisacodyl showed a higher percentage of fecal water content compared to the morphine-only group (Fig. 4). These changes in fecal water content indicate that acute morphine-induced constipation developed in our model and bisacodyl was capable of overcoming this morphine-induced constipation. Bisacodyl acts by disrupting the enteric nervous system and increasing intestinal motility while preventing excessive water absorption (Twycross et al. 2012).

AQP is a water transporter that supports water homeostasis and water absorption in the intestines. Under constipation conditions, the amount of AQP increases so that more water is transported from the luminal side to the blood vessels, resulting in drier harder feces, which is difficult to excrete. Thus, changes in AQP expression are directly related to fecal water content (Ikarashi et al. 2012; Ikarashi et al. 2016). Our study found that AQP3 mRNA expression increased within one hour after morphine injection and remained high until at least 5 hours after morphine administration compared to that in the mice which had been injected with saline only (Fig. 5). This result is in agreement with previous studies, which found an increase in AQP3 expression one hour after morphine administration, which correlated with constipation in experimental animals (Camilleri 2011). The

morphine group that had received bisacodyl (first hour) showed no significant difference in AQP3 expression compared to the morphine-only group (first hour) (Fig. 5). This result indicates that mice are still constipated at the one-hour time point. Interestingly, bisacodyl treatment significantly decreased AQP3 expression 5 hours after morphine injection compared to the morphine-only group (fifth hour) (Fig. 5). These results are consistent with several previous studies indicating that bisacodyl decreases AQP3 expression on colonic mucosal epithelial cells, resulting in decreased water transport from the luminal side to the vasculature (Ikarashi et al. 2016). Bisacodyl reduces AQP3 expression by activating macrophages and increasing the production and secretion of PGE2. Furthermore, PGE2 affects mucosal epithelial cell function in the colon in a paracrine manner to reduce AQP3 expression. The decreased AQP3 expression causes a decrease in the absorption of water, resulting in increased fecal water content, which makes the defecation process easier (Ikarashi et al. 2011; Ikarashi et al. 2016). Regarding the effects of bisacodyl on AQP4 mRNA expression, we did not find any significant differences in AQP4 expression in the mice treated with morphine + bisacodyl compared with the mice receiving morphine only during the development of acute constipation (Fig. 6). As far as we know, no previous studies have explored the effect of bisacodyl on AQP4 expression in constipation. AQP4 is widely expressed in the intestinal mucosa and regulates water homeostasis, especially in mouse models (Thi et al. 2008). The absence of differences in AQP4 expression may be due to the short treatment time.

In addition to acute constipation, we examined the effects of bisacodyl on the development of chronic morphine-induced constipation. Chronic constipation was assessed using fecal water content, colonic bead expulsion, and AQP3 and AQP4 mRNA expression. Fecal water content decreased in mice in response to morphine. Thus, morphine induces both acute and chronic constipation. On the third and seventh day of morphine treatment, fecal water content was significantly higher after bisacodyl treatment compared with fecal water content after treatment with morphine only (Fig. 7). These results confirm the effectiveness of the bisacodyl laxative; bisacodyl provides significant therapeutic effectiveness even after the first administration, based on the fecal water content.

Bisacodyl also impacted colonic bead expulsion in mice with chronic morphine-induced constipation. The mean bead expulsion time in the mice which had received morphine was longer than expulsion time in the mice receiving saline only (Fig. 8). These results agree with previous studies, which revealed that morphine causes constipation, as exhibited by longer bead expulsion time in the morphine-treated mice (30–120 minutes) compared to the mice that did not receive morphine (5–15 minutes) (Mori et al. 2013). In our study, after the third and seventh day of morphine and bisacodyl therapy, the mean bead expulsion time was significantly faster compared to bead expulsion time after treatment with morphine only. These results indicate that bisacodyl provided a significant laxative effect after the first treatment, reflected by shorter bead expulsion times. Bisacodyl stimulates intestinal peristalsis and intestinal contractions so feces are excreted faster (Manabe et al. 2009).

On the third and seventh days of morphine treatment, AQP3 mRNA expression was significantly higher compared to that in the mice treated with saline only (Fig. 9). These results demonstrate that both short and long-term morphine administration results in constipation, which is characterized by increased AQP3 expression. AQP3 mRNA expression decreased significantly in the mice treated with bisacodyl and morphine compared to AQP3 expression in the mice treated with morphine only, three days after the beginning of treatment. These results confirm the effectiveness of bisacodyl in preventing morphine-induced constipation; bisacodyl decreased AQP3 mRNA expression, in both acute and chronic morphine-induced constipation (Ikarashi et al. 2011; Farmer et al. 2018).

Bisacodyl also prevented the increased AQP4 mRNA expression in chronic morphine-induced constipation. AQP4 mRNA expression increased in the mice receiving morphine on days 1–3 and on days 1–7 compared with AQP4 expression in the mice receiving saline only (Fig. 10). These results indicate that changes in the expression of the AQP transporters, especially AQP4, depend on the intensity and/or time course of morphine induction. Elevated AQP4 expression in the colon results in more water absorption in the intestine (Ikarashi et al. 2016; Ly et al. 2021). Increased water absorption decreases fecal water content, leading to constipation. On the third and seventh day of treatment, bisacodyl treatment attenuated morphine-induced increases in AQP4 mRNA expression (Fig. 10). These results indicate that the laxative effect of bisacodyl is closely associated with the modulation of AQP4 mRNA expression in the colon in the mice with morphine-induced constipation.

Taken all together, this is the first study that has successfully explored the effectiveness of bisacodyl in treating morphine-induced constipation through decreased expression of AQP3 and AQP4. Our end-point PCR band data obtained in this study are very convincing either visually and semi-quantitatively that morphine increases the expression of AQP3 and AQP4, and bisacodyl decreases the expression of AQP3 and AQP4 induced by morphine. Of course, the findings of our present studies serve as the basis for further exploration.

Conclusions

The present findings indicate that colonic AQP3 expression is increased in acute morphine-induced constipation and both AQP3 and AQP4 expressions are increased in the colon in chronic morphine-induced constipation. Increased AQP3 and AQP4 mRNA levels are associated with decreased fecal water content. Meanwhile, administration of bisacodyl exerts a laxative effect by inhibiting water transport from the intestinal tract to the vascular side of the intestinal epithelial cells via decreased colonic AQP3 and AQP4 expression. Thus, further exploration of signaling pathways involved in AQP3 and AQP4 upregulation is important in developing pharmacological therapies to modulate AQP3 and AQP4 transporters for the treatment of constipation.

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Supplementary material 1

The representative photos of feces with high water content and low water content

Author: Mahardian Rahmadi, Chrismawan Ardianto, Ahmad D. Nurhan, Rafiqa A. Chasanah, Dewi I. Krismonika, Arina D. Puspitasari, Budi Suprapti, Santhra Segaran, Chia-Wei Phan, Junaidi Khotib

Data type: figure (docx file)

Explanation note: The representative photos of feces with high water content and low water content.

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Author Contributions

- **Mahardian Rahmadi**, PhD in Pharmacology, Associate Professor, Faculty of Pharmacy, e-mail: mahardianr@ff.unair.ac.id, **ORCID ID** https://orcid.org/0000-0002-7256-2446. The author created the concept and the methodology of the study and obtained the funding for the study.

- **Chrismawan Ardianto**, PhD in Pharmacology, Associate Professor, Faculty of Pharmacy, e-mail: chrismawan-a@ff.unair.ac.id, **ORCID ID** https://orcid.org/0000-0003-3713-7900. The author created the methodology of the study and revised the manuscript.

- **Ahmad Dzulfikri Nurhan**, M. Pharm in Biomedical Pharmacy, Research Assistant, Faculty of Pharmacy, e-mail: ahmad.dzulfikri.nurhan-2019@ff.unair.ac.id, **ORCID ID** https://orcid.org/0000-0001-9584-0667. The author drafted and revised the manuscript and conducted a formal analysis of the data.

- **Rafiqa Amalia Chasanah**, B. Pharm, Apothecary Professional Student, Faculty of Pharmacy, e-mail: rafiqa.amalia.chasanah-2016@ff.unair.ac.id. The author validated the experimental study and collected the relevant data.

- **Dewi Islamiah Krismonika**, B. Pharm, Apothecary Professional Student, Faculty of Pharmacy Universitas Airlangga, Surabaya, Indonesia; e-mail: dewi.islamiah.krisimonika-2016@ff.unair.ac.id. The author collected the relevant data and visualized the collected data.

- **Arina Dery Puspitasari**, M. Clin. Pharm Assistant Professor, Faculty of Pharmacy, e-mail: arinadery@ff.unair.ac.id. The author validated and monitored the experimental study.

- **Budi Suprapti**, Professor in Clinical Pharmacy, Faculty of Pharmacy Universitas Airlangga, Surabaya, Indonesia, and Department of Pharmacy, Universitas Airlangga Teaching Hospital, e-mail: budi-s@ff.unair.ac.id. The author provided the resources and monitored the experimental study.

- **Santhra Segaran**, M.Sc in Biomedicine, PhD student in Department of Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia, and Lecturer in Department of Allied Health Sciences, Faculty of Health and Life Sciences, Management & Science University, e-mail: santhra@msu.edu.my, **ORCID ID** https://orcid.org/0000-0002-9756-3074. The author conducted a formal analysis of the data and obtained the funding of the study.

- **Chia-Wei Phan**, PhD in Biotechnology, Senior Lecturer, Department of Pharmaceutical Life Sciences, Faculty of Pharmacy, e-mail: phancw@um.edu.my, **ORCID ID** https://orcid.org/0000-0003-1373-1374. The author created the methodology of the study.

- **Junaidi Khotib**, Professor in Biopharmaceutics, Faculty of Pharmacy, e-mail: junaidi-k@ff.unair.ac.id, **ORCID ID** https://orcid.org/0000-0002-8468-8441. The author created the concept of the study.