MS Editions

Blacpma

BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
19 (6): 555 - 568 (2020)
© / ISSN 0717 7917 / **www.blacpma.ms-editions.cl**

Articulo Original / Original Article

Glabridin alleviates ischemia/reperfusion-induced functional failure of smooth muscle of rat ileum by upregulating the cAMP

[Glabridin alivia la falla funcional inducida por isquemia/reperfusión del músculo liso de ileum murino regulando el incremento de cAMP]

Ali Parlar[1], Saliha Ayşenur Çam[2] & Seyfullah Oktay Arslan[2]

[1]***Department of Pharmacology, Faculty of Medicine, University of Adiyaman, Turkey***
[2]***Department of Pharmacology, Faculty of Medicine, University of Ankara Yildirim Beyazit, Turkey***
Contactos | Contacts: **Ali PARLAR** - ***E-mail address:*** **aparlar@adiyaman.edu.tr**

Abstract: Despite the development of modern medicine, alternative medicine, which has not lost its timeliness, remains attractive for the treatment of various diseases. Glabridin, a major flavonoid of *Glycyrrhiza glabra*, is known for its antioxidant and anti-inflammatory activity. The aim of this study was: 1) to determine the possible protective role of glabridin against ischemia/reperfusion (I/R) injury of the intestine; 2) to evaluate the *in vitro* contractile responses of ileum smooth muscles to acetylcholine after an intestinal I/R; and 3) to explain the underlying molecular mechanism of its effect. Rats were assigned to groups of six rats each; 1) I/R, 2) gla10, 3) gla20, 4) gla40, 5) N5-[imino(nitroamino)methyl]-L-ornithine, methyl ester monohydrochloride (L-NAME)+gla40, and 6) Sham group. The healing effect of glabridin was abolished by L-NAME. Glabridin did not cause contractility of the smooth muscles to acetylcholine-induced contractile responses in intestinal I/R. Yet, it increased to spontaneous basal activity.

Keywords: Glabridin; Ileum contractility; Intestinal ischemia/reperfusion; cAMP; cGMP; Oxidative injury.

Resumen: A pesar del desarrollo de la medicina moderna, la medicina alternativa, sin perder su vigencia, sigue siendo atractiva para el tratamiento de varias enfermedades. Glabradina, el flavonoide mayoritario de *Glycyrrhiza glabra*, es conocido por su actividad antioxidante y antiinflamatoria. Los propósitos de este estudio fueron: 1) Determinar el posible rol protector de glabradina ante daños intestinales por isquemia/reperfusion (I/R) 2) Evaluar *in vitro* las respuestas de contracción de los músculos lisos del ileum ante acetilcolina después de I/R intestinal; y 3) Explicar el mecanismo molecular subyacente de este efecto. Se asignaron grupos de seis ratas: 1) I/R, 2) gla10, 3) gla20, 4) gla40, 5) N5-[imino(nitroamino)metil]-L-ornithina, metil ester monohidrochloruro (L-NAME)+gla40, y 6) Grupo testigo. El efecto curativo de glabridina fue abolido por L-NAME. Glabridina no causó contracción en el músculo liso como respuesta acetilcolina-inducida I/R. Además, incrementa la actividad basal expontánea.

Palabras clave: Glabridina; Contractilidad de ileum; Isquemia/Reperfusión intestinal; cAMP; cGMP; Daño oxidativo

Recibido | Received: December 21, 2019
Aceptado | Accepted: March 20, 2020
Aceptado en versión corregida | Accepted in revised form: April 6, 2020
Publicado en línea | Published online: September 30, 2020
Este artículo puede ser citado como / This article must be cited as: A Parlar, SA Çam, SO Arslan. 2020 Glabridin alleviates ischemia/reperfusion-induced functional failure of smooth muscle of rat ileum by upregulating the cAMP. **Bol Latinoam Caribe Plant Med Aromat** 19 (6): 555 – 568. **Https://doi.org/10.37360/blacpma.20.19.5.39**

INTRODUCTION

Small bowel ischemia/reperfusion (I/R), a pathophysiological condition which results in cell integrity deterioration may occur after an abdominal aortic aneurysm, trauma, the shock of hemorrhage or trauma, acute mesenteric ischemia, and small bowel transplantation (Deitch, 1992). The temporary disruption of blood flow is called ischemia, followed by reperfusion, which is the main source of injury to the tissues as a result of disruption of the mucosal barrier when the plasma proteins escape the vessel, causing a decrease in intestinal contractility and motility (Zheng *et al*., 2012). I/R stimulates immune system cells to produce chemokines, reactive oxygen species (ROS), and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, which cause the disruption of the intestinal mucosa following the leakage of neutrophils from the plasma into the inflamed tissue (Kahkhaie *et al*., 2019). This results in increased levels of myeloperoxidase (MPO) and malondialdehyde (MDA) and decreasing glutathione (GSH) content (Bayram *et al*., 2019).

Moreover, ROS metabolites contribute to the destruction of cells and the inability of the intestinal tissue to function (Wiegman *et al*., 2015). Arab et al. report that activation of several transcription factors such as nuclear factor kappa B (NF-kB) and cyclo-oxygenase-2 (COX-2) is due to excessive ROS and cytokine production (Arab *et al*., 2014). In addition, previous studies showed that inflammation is associated with decreased intracellular cyclic adenosine monophosphate (cAMP) levels, which is the dominant mediator of smooth muscle relaxation and decreased cyclic guanosine monophosphate (cGMP) levels, which is another mediator of smooth muscle relaxation by nitric oxide (NO) and increased phosphodiesterase enzyme activity (Parlar *et al*., 2020). Therefore, phosphodiesterase (PD) enzyme inhibitor drugs are used in the treatment of diseases such as erectile dysfunction, pulmonary hypertension, acute refractory cardiac failure, intermittent claudication, and chronic obstructive pulmonary disease (Carvajal *et al*., 2000; Maurice *et al*., 2014).

Antispasmodic drugs used in cases such as gastrointestinal, billiard, or genitourinary system smooth muscle cramps are used in the symptomatic treatment of these cramps originating from smooth muscles (Leri *et al*., 1997). However, the discovery of new molecules of natural origin is an important target for the pharmaceutical industry, not only because of the increasing human demand for natural products, but also because of the adverse effects of synthetic antispasmodic drugs such as neurotoxicity (Heghes *et al*., 2019). In recent years there has been increasing interest in experimental and clinical studies of glabridin. It is an isoflavan obtained from *Glycyrrhiza glabra*. It has been used as a medicine in Asian countries, especially in China, for more than 4000 years, and consists of about 30 species (Tian *et al*., 2008). They are used in the treatment of certain diseases, detoxification, and treatments for injuries (Hoffmann *et al*., 2016).

Glabridin exhibits various biological activities, including antioxidant and estrogen hormone-like activity (Aoki *et al*., 2005). Glabridin has been shown to treat liver steatosis in obese rats (Ahn *et a*l., 2013). It has been reported to exhibit multiple pharmacological effects in humans against gastric cancer cells which inhibit proliferation and kill harmful bacteria (against *Helicobacter pylori*) that treat stomach disease, treat diabetes, and improve cognitive impairment (Hasanein, 2011; Wu *et al*., 2013; Zhang *et al*., 2018).

Despite the data in hand, the role of glabridin in I/R damage remains controversial. Therefore, the aim of this study is to explain the possible protective effects of glabridin against inflammation caused by I/R injury and its antispasmodic effects against intestinal smooth muscle cramps, as well as its underlying molecular mechanism.

MATERIALS AND METHODS

Chemicals

Dimethyl Sulfoxide (DMSO, for vehicle), $NaHCO_3$, potassium chloride (KCl), $MgSO_4$, KH_2PO_4, $CaCl_2$, NaCl, glucose, phosphate buffered saline (PBS), trichloroacetic acid (TCA), hexadecyltrimethy-lammonium bromide (HETAB), o-dianisidine, thiobarbituric acid (TBA), dithiobisnitrobenzoate (DTNB), N-(1-naphthyl) ethylenediamine dihydro-chloride (NEDA), acetylcholine chloride (Ach), and butylated hydroxy toluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA), N^5-[imino(nitroamino)methyl]-L-ornithine, methyl ester monohydrochloride (L-NAME) and glabridin were obtained from Cayman Chemical (Michigan, USA) and Xi'an ZB Biotech Co., Ltd. (Shaanxi, China), respectively. All chemicals used in this study are 98% pure. Especially, the purity of glabridin was confirmed by High-performance liquid chromatography (HLPC), in the laboratory of the

Faculty of Pharmacy of Adiyaman University (data not shown).

Animals

Male Wistar rats weighing 180-250 g were used in the study. The animals were taken from Adiyaman University Experimental Animals Production Center after obtaining permission from the Adiyaman University Local Ethics Committee (2019/0011). The rats were randomly divided into six groups (according to treatment) and kept in transparent cages for ten days to accommodate to the environment and provide free access to water and a standard pellet diet until one day prior to the experiment. Groups were named: 1) I/R group (2% dimethyl sulfoxide in 0.9% NaCl, 2 ml volume, intraperitoneal [i.p], once daily for five consecutive days), 2) gla10 (glabridin 10 mg/kg, i.p) group, 3) gla20 (glabridin 20 mg/kg, i.p) group, 4) gla40 (glabridin 40 mg/kg, i.p) group, 5) L-NAME + gla40 (glabridin 40 mg/kg, i.p + 10 mg/kg L-NAME, tail vein, once daily for five consecutive days), and 6) the sham group (The animals in this group were incised under anesthesia without the I/R protocol, the mesenteric artery was observed, and the abdominal walls were closed with silk suture) (n=6).

Induction of ischemia and reperfusion of ileum

Intestinal I/R was performed as previously described (Arslan *et al*., 2005). Under ketamine and xylazine anesthesia, rats in all groups underwent midline laparotomy and the small intestines of rats in the sham group were observed, while the superior mesenteric artery of the rats in the other groups was occluded with bulldog clamps for 30 minutes. To prevent dehydration, the intestines were covered with humid sterile gauze. Following ischemia, the clamp was removed and the abdominal wall was sutured with 3.0 silk suture. After 150 minutes, the abdominal wall underwent a re-laparotomy. The dose of glabridin was based on the study of El-Ashmawy *et al*. (2018), while the dose of L-NAME was based on the study of Sayan *et al*. (2008).

After the animals were sacrificed with a high dose anesthesia, ileum tissue (approximately 10 cm behind ileocecal valve) was taken immediately at 2 cm, pre-oxygenated, and heated to 37°C Krebs solution ($NaHCO_3$ 24.88, KCl 4.7, $MgSO_4$ 1.16, KH_2PO_4 1.18, $CaCl_2$ 2.52, NaCl 118, and glucose 11.1 in mM) and placed in an isolated organ bath. The remaining parts of the ileum (5 parts) were stored at -80°C until analyzed for biochemical studies.

Ileum muscle contractility in vitro

Ileum smooth muscle were calibrated for 60 min under 2 g in a four-channel tissue bath (Commat Ltd, Ankara, Turkey, donated with Biopac Systems, Inc) with Krebs solution that was regularly fed with a gas mixture of 5% CO_2 and 95% O_2 at 37°C. Following the 2 g tension equilibration, spontaneous contractions of the rat ileum smooth muscles against Ach (10^{-8} to 10^{-3} M) were recorded in the I/R, glabridin (10, 20 and 40 mg/kg doses) and L-Name + gla40 groups. In addition, the cumulative responses of acetylcholine rat ileum smooth muscles were recorded in the sham group. The reactions of rat ileum smooth muscles to 30 mM KCl in the whole group were recorded. The 10^{-6} M of Ach was found to have an EC_{50} value where EC_{50} value of acetylcholine concentrations ranging from 10^{-8} to 10^{-3} was calculated only for the sham group.

Measurement malondialdehyde and glutathione levels in ileum tissue

MDA and GSH levels were measured by the method described by Casini *et al*. (1986) and the Ellman method (Parlar & Arslan, 2019), respectively, with minor changes. 50 mg of ileum tissue was homogenized and centrifuged in ice-cold TCA. To measure the MDA level, BHT and TBA were added to the supernatant, while to measure the GSH level, Na_2HPO_4 and DTNB were added to the supernatant. They were measured on a spectrophotometer at a wavelength of 535 and 412 nm and were expressed as µmol/mg tissue.

Measurement of myeloperoxidase activity in ileum tissue

The amount of neutrophils that migrated to the inflamed tissue was determined by measuring the level of the MPO enzyme as described by Bradley *et al*. (1982). Briefly, homogenized tissue was suspended in PBS and HETAB; then, the homogenized tissues were centrifuged at 40,000 g for ten minutes. The supernatant was added to a mixture of o-dianisidine and H_2O_2 solutions. It was measured on a spectrophotometer at a wavelength of 460 nm, and was expressed as U/mg tissue.

TNF-α, IL-1β, cAMP, and cGMP activities in ileum tissue

Tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, cAMP, and cGMP levels in intestinal tissue were measured by the ELISA method according to the manufacturer's guideline for users (Abcam ab46070, ab100768, ab234585, and ab133052, respectively, Cambridge, United Kingdom). After weighing 50 mg of tissue, it was homogenized in PBS to measure TNF-α and IL-1β and in-cell lysis buffer to measure cAMP and cGMP. They were expressed as pg/g tissue, pg/g tissue, pmol/mg protein, and pmol/mL, respectively. A Bicinchoninic Acid (BCA) Assay kit (Thermo Scientific) was used to determine the amount of protein in the tissue.

Measurement of NO levels in ileum tissue

L-NAME, a non-specific NO inhibitor, was given to test whether cGMP was involved in the muscle relaxant mechanism of glabridin. As previously determined by Pal *et al*. (2019), the amount of NO was determined by measuring the level of nitrate and nitrite, the metabolites of NO in the ileum homogenate. The number of metabolites in the homogenate was determined using a Griess reagent prepared by mixing 0.1% NEDA and 2% sulfanilamide in an equal ratio. It was measured on a spectrophotometer at a wavelength of 540 nm and was expressed as pmol/mg tissue.

Statistical analysis

The EC_{50} value, defined as the concentration of Ach, which induces 50% of maximal contraction, and other tests were determined using GraphPad Prism software (version 7.01) One-way ANOVA with the Bonferroni test was used for statistical analysis using GraphPad Prism software; however, for concentration-response curves of Ach (from 10^{-8} to 10^{-3} M), a two-way ANOVA with multiple comparisons and a Bonferroni test was used. Significance was evaluated when the $p<0.05$, and data were expressed as mean ± standard deviation (SD).

RESULTS

Responses of ileum muscle contractility in vitro

According to the sham group (0.72 ± 0.05 g), KCl (30 mM) did not cause the contraction of ileum smooth muscles in the intestinal I/R group (0.32 ± 0.06 g, $p<0.001$). When comparing the intestinal I/R group with L-NAME+gla40 (0.35 ± 0.06 g), gla10 (0.33 ± 0.05 g), gla20 (0.31 ± 0.06 g), and the gla40 (0.33 ± 0.05 g) groups, there was no statistical significance (Table No. 1).

Table No. 1
The cumulative effects of Ach and KCl in isolated rat ileum

Ach Con. (Log M)	I/R	gla10	gla20	gla40	L-NAME+gla40	sham	P Value
10^{-8}	0.67±0.15	0.75±0.37	0.78±0.17	0.85±0.31	0.97±0.14	0.94±0.11	$p>0.999$
10^{-7}	0.88±0.20	0.93±0.19	1.06±0.13	1.15±0.14	1.23±0.09	1.94±0.35***	$p<0.001$
10^{-6}	1.07±0.41	1.17±0.35	1.19±0.19	1.27±0.18	1.54±0.16	2.98±0.55***	$p<0.001$
10^{-5}	1.23±0.39	1.25±0.26	1.27±0.33	1.34±0.34	1.77±0.31	3.70±0.44***	$p<0.001$
10^{-4}	1.31±0.18	1.37±0.30	1.30±0.36	1.39±0.36	1.83±0.32	4.16±0.43***	$p<0.001$
10^{-3}	1.35±0.14	1.44±0.15	1.28±0.50	1.32±0.37	1.32±0.37	4.24±0.39***	$p<0.001$
KCl (30mM)	0.32±0.06	0.33±0.05	0.31±0.06	0.33±0.06	0.35±0.06	0.72±0.05***	$p<0.001$

Response of rat ileum smooth muscle against cumulative concentrations of Ach (10^{-8} – 10^{-3} M) in the I/R, sham and L-NAME+gla40 (glabridin 40 mg/kg i.p., and L-NAME 10 mg/kg, tail vein, once daily for five consecutive days) and spontaneous basal activity of rat ileum in isolated tissue baht. For basal activity and KCl, One-way ANOVA followed by Bonferroni's post hoc test was used to determine statistical differences. For cumulative concentrations of Ach, Two-way ANOVA followed by Bonferroni's post hoc test was used to determine statistical differences. ***$p<0.001$ vs I/R group. Data are expressed as mean ± SD (n=6)

As shown Table No. 1, when the sham and I/R group were compared, the statistical significance was found at concentrations of 10^{-7} to 10^{-3} M of Ach. It was found that both pre-treatment with glabridin (10, 20, and 40 mg/kg, once daily for five consecutive days) and co-administration of glabridin

(40 mg/kg) with L-NAME did not show a statistical significance as compared to the I/R group. EC_{50} and R^2 values were calculated as $10^{-6.128}$ M and 0.9172, respectively, and are shown in table 1.

As illustrated with original traces in Figure No. 1, the cumulative dose of acetylcholine (10^{-8}-10^{-3} M) in isolated rat ileum produced concentration-dependent contraction. With a 10^{-6} M concentration of Ach, in the sham group, the contraction caused about 4 g of tension, while in the I/R group and the pre-treatment of glabridin (10, 20, and 40 mg/kg, once daily for five consecutive days) groups, the tension was about 1 g. Moreover, co-administration of L-NAME with glabridin did not cause any contraction of the ileum smooth muscles (Table No. 1).

Intestinal I/R (0.14 ± 0.05 g) caused a significant decrease in spontaneous basal activity of ileum by 207% compared to the sham group (0.29 ± 0.05 g, p<0.001). Pre-treatment with the middle and highest dose of glabridin significantly increased the spontaneous basal activity of the ileum by 164% and 178% (0.23 ± 0.02, 0.25 ± 0.03 g, p=0.05, p=0.07, respectively), while both pre-treatment with the lowest dose of glabridin and co-administration L-NAME with glabridin did not cause any change in the spontaneous basal activity of ileum as compared to the I/R group (0.19 ± 0.06, 0.16 ± 0.04 g, respectively) (Figure No. 2a).

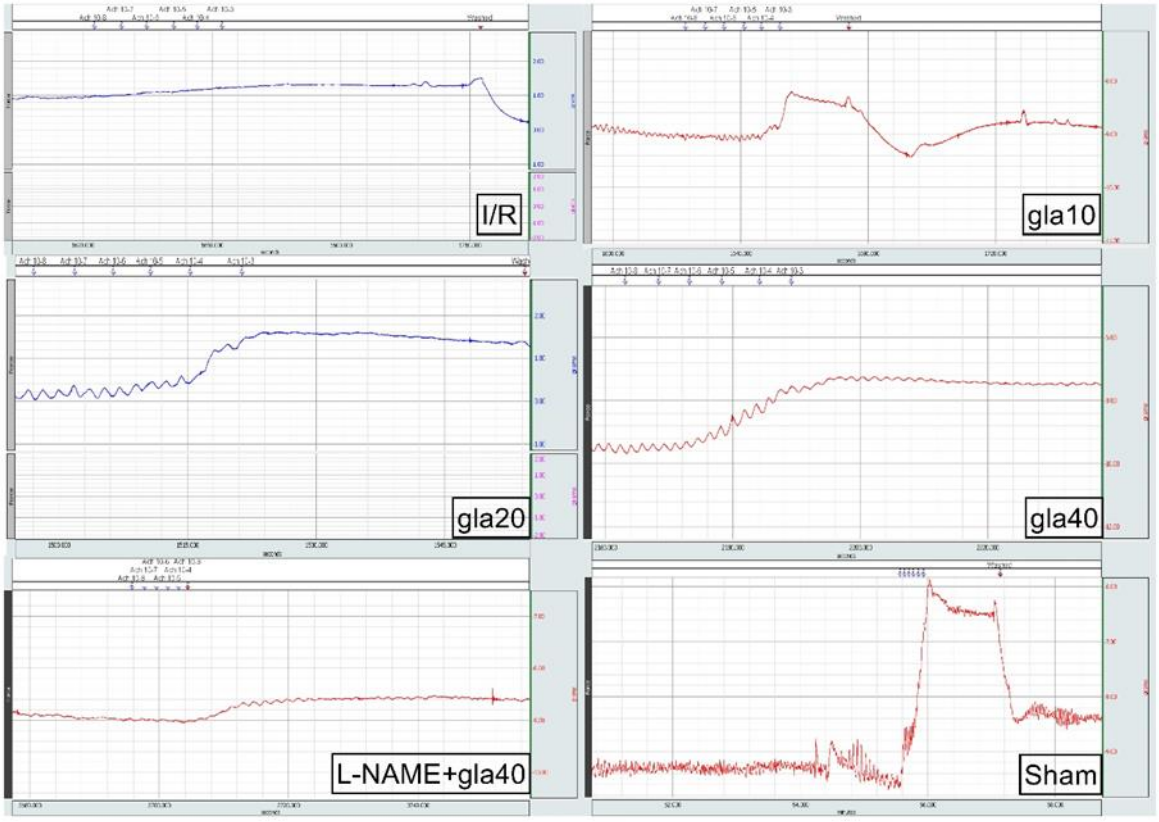

Figure No. 1
Original trace samples. Response of rat ileum smooth muscle against cumulative concentrations of Ach (10^{-8} – 10^{-3} M) in the I/R, gla10, gla20, gla40, L-NAME+gla40 and sham samples in isolated tissue baht.

Malondialdehyde levels in ileum tissue

I/R (75.03 ± 13.04 µmol/mg tissue) caused a significant increase in the MDA level by 175% (42.87 ± 7.12 µmol/mg tissue, p<0.001) compared to the sham group. However, co-administration of L-NAME with glabridin caused a decrease in MDA

content by 21.75% (58.71 ± 7.63 µmol/mg tissue) as compared to the I/R group (p=0.049). In addition, pre-treatment with the middle and highest dose of glabridin significantly decreased the MDA level by 22.45% and 27.95% (58.18 ± 7.44, 54.06 ± 7.06 µmol/mg tissue, p=0.041, p=0.005, respectively), while this effect of glabridin reducing MDA level was not observed at the lowest dose (72.67 ± 9.13 µmol/mg tissue) as compared to the I/R group (p=0.039, =0.05, and >0.99, respectively) (Figure No. 2b).

GSH activity in ileum tissue

As illustrated in Figure No. 2c, I/R caused a significant decrease in GSH content by 218% (1.75 ± 0.37 µmol/mg tissue) compared to the sham group (3.83 ± 0.45 µmol/mg tissue, p<0.001). However, the co-administration of L-NAME with glabridin caused an increase in GSH content of 149% (2.61 ± 0.32 µmol/mg tissue) as compared to the I/R group (p=0.003). In addition, pre-treatment with the middle and highest dose of glabridin significantly increased the GSH level by 150% and 157% (2.64 ± 0.29, 2.75 ± 0.38 µmol/mg tissue, respectively), while this effect of glabridin increasing GSH level was not observed at the lowest dose (10 mg/kg, 1.79 ± 0.21 µmol/mg tissue) as compared to the I/R group.

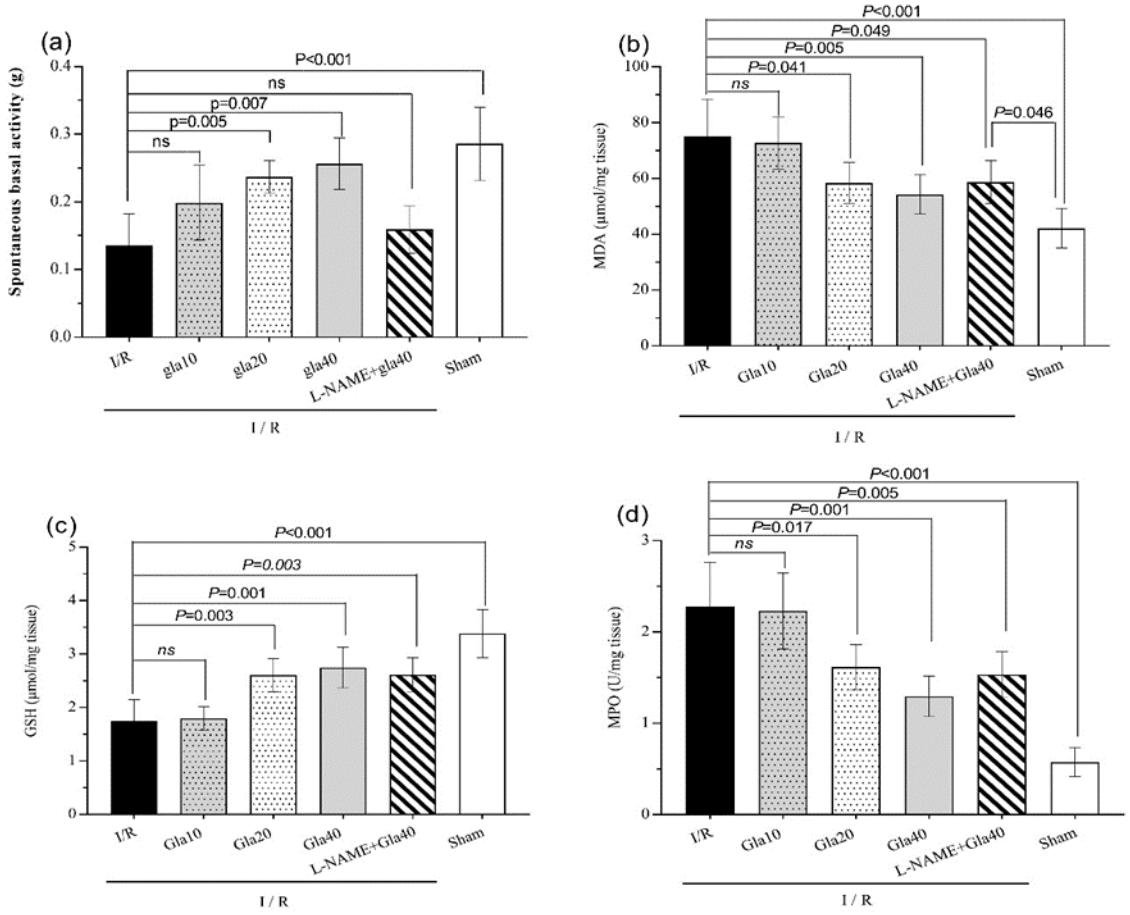

Figure No. 2

The effects of glabridin on spontaneous contraction of the ileum smooth muscle and MDA, GSH and MPO levels in ileum tissue. The effects of glabridin on spontaneous activity of the ileum (a), on MDA (b) activity, GSH (c) and MPO (d) levels in intestinal I/R injury in rats. Data are expressed as mean ± SD (n=6). One-way ANOVA followed by Bonferroni's post hoc test was used to determine statistical differences

MPO Activity in Ileum Tissue

As seen in Figure No. 2d, I/R caused a significant increase in MPO activity by 393% (2.28 ± 0.49 U/mg tissue) compared to the sham group (0.58 ± 0.16 U/mg tissue, $p<0.001$). However, the co-administration of L-NAME with glabridin caused a decrease in the MPO level of 32.89% (1.53 ± 0.25 U/mg tissue) as compared to the I/R group ($p=0.005$). In addition, pre-treatment with the middle and highest dose of glabridin significantly decreased MPO activity by 28.94% and 43.42% (1.62 ± 0.25, 1.29 ± 0.24 U/mg tissue, respectively), while this effect of glabridin decreasing MPO level was not observed at the lowest dose (2.23 ± 0.42 U/mg tissue) as compared to the I/R group.

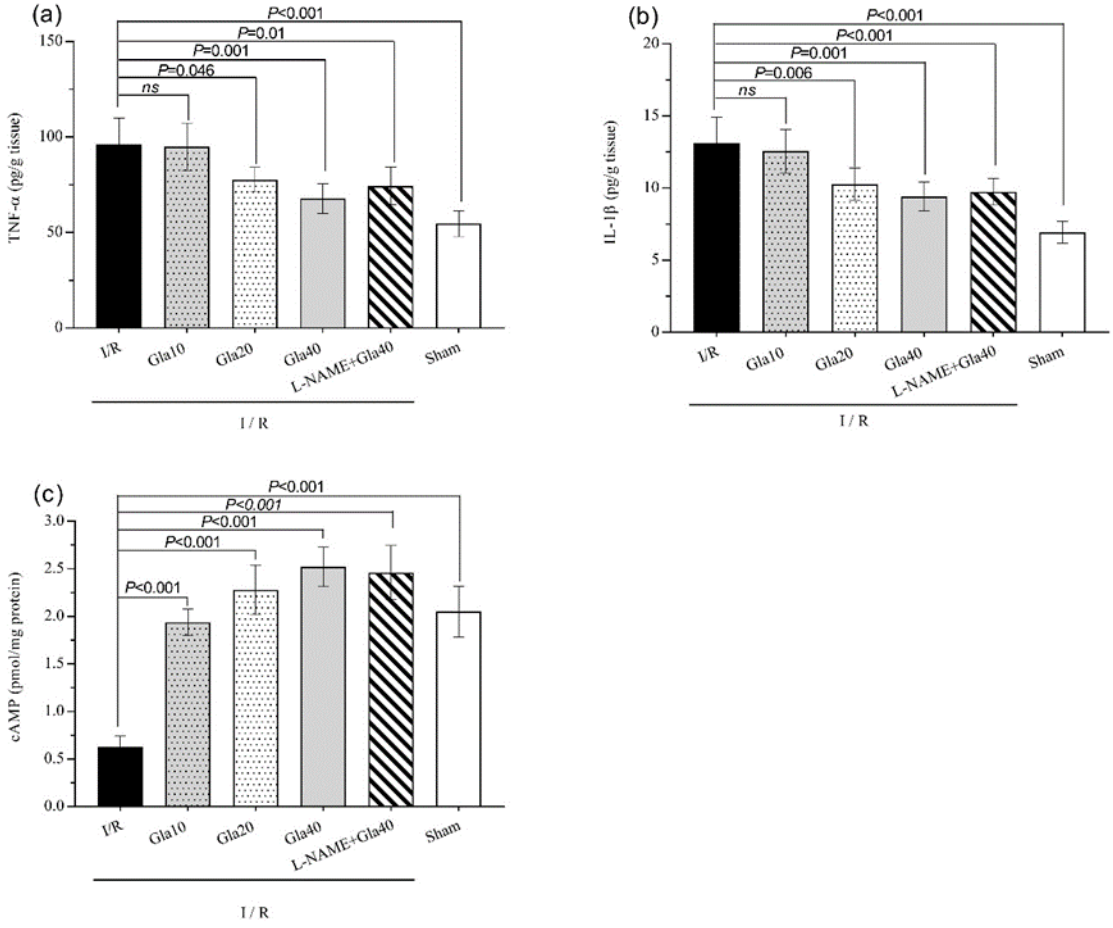

Figure No. 3

The effects of glabridin to TNF-α, IL-1β, and cAMP levels in ileum tissue. Effect of glabridin on TNF-α (a), IL-1β (b) activity, and cAMP (c) in tissue. Data are expressed as mean ± SD (n=6). One-way ANOVA followed by Bonferroni's post hoc test was used to determine statistical differences

TNF-α, IL-1β, and cAMP concentrations in ileum tissue

Figure No. 3a shows that I/R caused a significant increase in TNF-α concentration by 175% (96.13 ± 13.78 pg/g tissue) compared to the sham group (54.79 ± 6.80 pg/g tissue, $p<0.001$). However, pre-treatment with the middle and highest dose of glabridin significantly decreased TNF-α concentration (77.72 ± 6.42, 67.87 ± 7.86 pg/g tissue, respectively), while this effect of glabridin decreasing TNF-α level was not observed at the lowest dose (95.07 ± 12.37 pg/g tissue) as compared to the I/R

group.

As illustrated in Figure No. 3b, I/R caused a significant increase in IL-1β concentration (13.12 ± 1.79 pg/g tissue) compared to the sham group by 189% (6.94 ± 0.77 pg/g tissue, $p<0.001$). However, the co-administration of L-NAME with glabridin caused a decrease in IL-1β concentration by 25.68% (9.75 ± 0.95 pg/g tissue) as compared to the I/R group ($p<0.001$). In addition, pre-treatment with the middle and highest dose of glabridin significantly decreased IL-1β concentration by 21.64% and 28.27% (10.28 ± 1.12, 9.41 ± 1.01 pg/g tissue, respectively), while this effect of glabridin decreasing IL-1βlevel was not observed at the lowest dose (12.57 ± 1.56 pg/g tissue) as compared to the I/R group.

Figure No. 3c shows that I/R caused a significant decrease in cAMP concentration by 325% (0.63 ± 0.12 pmol/mg protein) compared to the sham group (2.05 ± 0.27 pmol/mg protein, $p<0.001$). However, both co-administration of L-NAME with glabridin and all of the glabridin (10, 20, and 40 mg/kg) doses caused a significantly increase in cAMP concentration by 390%, 307%, 361%, and 400%, respectively (2.46 ± 0.28, 1.94 ± 0.14, 2.28 ± 0.26, and 2.52 ± 0.21 pmol/mg protein, respectively) as compared to the I/R group ($p<0.001$).

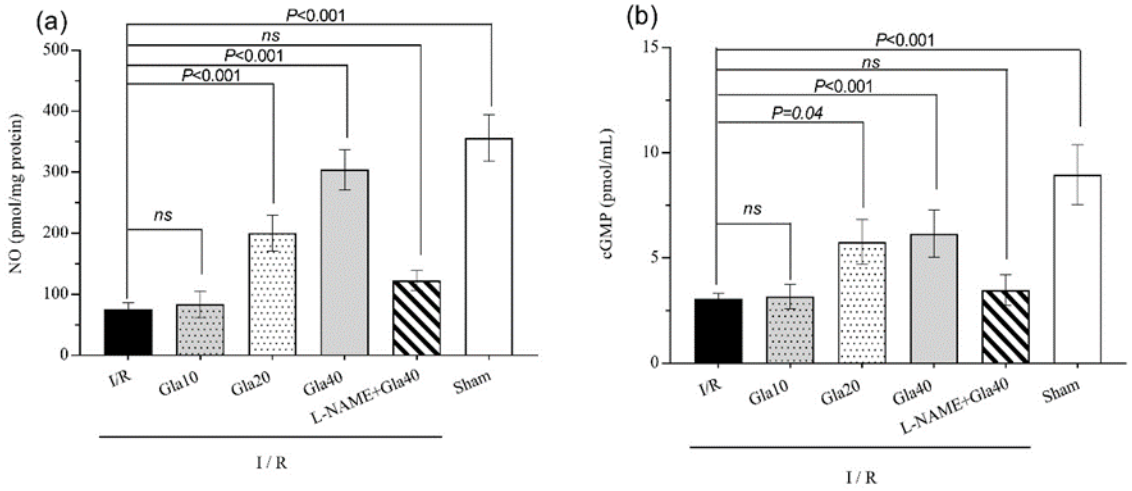

Figure No. 4
The effects of glabridin on NO, and cGMP levels in ileum tissue. Effect of glabridin on NO (a), cGMP (b) content. Data are expressed as mean ± SD (n=6). One-way ANOVA followed by Bonferroni's post hoc test was used to determine statistical differences

The level of NO in ileum tissue

As seen in Figure No. 4a, I/R caused a significant decrease in the NO level by 487% (75.14 ± 11.04 pmol/mg protein) compared to the sham (356.19 ± 38.14 pmol/mg protein, $p<0.001$). However, compared to the I/R group, two high doses of glabridin (20 and 40 mg/kg) caused a significant increase in NO concentration (by 274% and 416%, 200.61 ± 29.07, and 304.23 ± 32.98 pmol/mg protein, respectively), yet both the co-administration L-NAME with glabridin (122.59 ± 16.46 pmol/mg protein) and the lowest dose of glabridin (10 mg/kg dose, 83.51 ± 11.04 pmol/mg protein) did not change the NO level in ileum tissue.

The activities of cGMP in ileum tissue

Figure No. 4b shows that I/R caused a significant decrease in cGMP level by 293% (3.05 ± 0.26 pmol/mL) compared to the sham (8.96 ± 1.42pol/mL, $p<0.001$). However, pre-treatment with two high doses of glabridin (20, 40 mg/kg) significantly increased the cGMP level by 188% and 201% (5.76 ± 1.07, 6.15 ± 1.12pmol/mL) as compared to the I/R group ($p<0.001$). Yet, the co-administration L-NAME with glabridin and the lowest dose of glabridin did not change the cGMP level when compared to the I/R group.

DISCUSSION

Because of their many advantages, such as their relative safety and lower side effects, herbal products, which are regaining popularity among the public, and the main ingredients obtained and/or derived from their extracts continue to be the target of the pharmaceutical industry. Glabridin, derived from *Glycyrrhiza glabra* root, has biological activities such as antioxidant, anti-inflammatory, anti-atherogenic, estrogenic, neuroprotective, anti-osteoporotic, skin-whitening, and energy metabolism regulatory effects (Simmler *et al*., 2013). Because the effect of glabridin on the function of ileum smooth muscles in I/R-induced ileum injury has not been investigated, this study aimed to reveal the effect of glabridin on ileum smooth muscle functions and the contingent mechanisms on intestinal injury induced by the I/R of the mesenteric artery.

This study found that glabridin significantly reduced MDA, MPO, and cytokine levels such as TNF-α and IL-1β in ileum tissue. Moreover, it increased cAMP, cGMP, and GSH levels. In addition, in this study, L-NAME was given to test whether cGMP and NO were involved in the muscle relaxant mechanism of glabridin.

Previous studies have demonstrated a correlation between increased MPO activity and I/R injury. MPO, a heme-enzyme localized in neutrophils, macrophages, and monocytes, indicates the amount of cell migration into inflamed tissue and provides information on the extent of damage in intestinal ischemia and reperfusion injury (Koyani *et al*., 2015). In this study, glabridin was shown to inhibit MPO activity, which may be due to the inhibition of neutrophil adherence to the ileum endothelium, which suggests that prevention of neutrophil migration to ischemic tissue is the target of future treatment strategies. Previous studies have shown that L-NAME increases the MPO level further due to I/R (Arslan *et al*., 2005; Sayan *et al*., 2008). In this study, glabridin decreased the MPO level, and pre-administration of L-NAME reversed the effect of glabridin in lowering MPO activity. Thus, this study demonstrated that glabridin down-regulated the increase of MPO caused from both intestinal I/R and L-NAME.

By pre-administering glabridin, the MDA amount of the ileum tissue decreased to the level seen in the sham group. Consistent with previous findings (El-Ashmawy *et al*., 2018), results of the present investigation also showed that intestinal I/R caused an increase in the MDA level due to excessive production of ROS, which induced lipid peroxidation in the rat ileum. The findings here suggest that prevention of ROS production is the target of the future treatment of intestinal I/R. Furthermore, Dai *et al*. (2017), demonstrated that the large conductance of Ca^{++}-activated K^+ (BK_{ca}) channel activator prevents I/R damage from an oxidant-dependent mechanism by provoking increases in heme oxygenase-1 activity. In accordance with activation studies of the BK_{ca} channels by glabridin (Chanda *et al*., 2016), it may have reduced the MDA level by activation of the BK_{ca} channels.

Glutathione s-transferases, which are responsible for the detoxification of I/R-induced oxidative stress-induced endogenous substrates, lipid peroxides, hydroxy peroxides, and many other xenobiotics, are known for their ability to catalyze conjugation of the reduced glutathione (GSH) with xenobiotic substrates for detoxification (Halliwell, 1994). GSH plays an important role in reducing or removing of the second substrate as a conjugate and nucleophile. In this study, intestinal I/R caused a decrease in glutathione s-transferase activity, while glabridin reversed I/R-induced glutathione s-transferase depression. Consistent with previous findings, glabridine provides the antioxidant effect by removing intracellular radicals and inhibiting low density lipoprotein oxidation (Kang *et al*., 2015) and by activation of the BK_{ca} channels (Chanda *et al*., 2016).

TNF-α and IL-1β produced by immune system cells are pro-inflammatory agents that play an important role in the pathogenesis of I/R (Kahkhaie *et al*., 2019). In this study, a significant increase was observed in both TNF-α and IL-1β levels in I/R-induced rat ileum tissue. In additional, glabridin reversed the increase of I/R-induced pro-inflammatory cytokines. Kwon *et al*. (2008), reported that glabridin reduced TNF-α levels in dextran sulfate sodium-induced colitis in mice. Additionally, Zhang *et al*. (2017), noted that glabridin significantly reduced elevated levels of IL-1β in lipopolysaccharide-induced acute lung injury, which is consistent with the results of this study. Moreover, it has been demonstrated that glabridin mediates inhibition of the inhibitory NF-kB degradation, leading to the induction of TNF-α and IL-1β (Kang *et al*., 2015). In this study, because glabridin reduced TNF-α and IL-1β concentrations in ileum tissue, the role of glabridin in downregulating TNF-α and IL-1β

levels may be due to the inhibition of NF-kB.

The experimental intestinal I/R model mimics a pathophysiological condition that develops as a result of abdominal aortic aneurysm, trauma, hemorrhagic shock, and small bowel transplantation and continues with the disruption of cell integrity (Deitch, 1992). In this model, cell integrity is impaired, polymorphonuclear leukocytes migrate to the injured site, cell membrane lipid peroxidation levels are increased, antioxidant defense**s** are exhausted, and over-expression of pro-inflammatory cytokines occur (Gordeeva *et al*., 2017). As a result, intestinal contractility and motility are reduced (Spanos *et al*., 2007; Bayram *et al*., 2019).

In this study, the success of intestinal I/R modeling was confirmed by evaluating biochemical measurements and the contractile responses of hanged ileum in the isolated tissue bath. The I/R-induced ileum smooth muscles were contracted by neither Ach nor KCl, consistent with previous studies (Soydan *et al*., 2009). Moreover, intestinal I/R injury inhibited the spontaneous activity of the ileum, which may be due to ischemia-induced disruption of cell integrity, increased cytokine content, and the oxidation and depletion of antioxidant defense stores during the reperfusion period when the actual damage occurs (Lindeström & Ekblad, 2004). In this study, it was found that in the intestinal I/R group, MPO, MDA, TNF-α, and IL-1β levels increased, while GSH and cAMP levels decreased. The observations show its consistency with the results of previous stud**ies** (Soydan *et al*., 2009; Bayram *et al*., 2019). As a result, the mechanism underlying the abolishing effect of I/R-induced cytokine increase in ileum is that glabridin may be due to improved intracellular disrupted Ca^{++} homeostasis (Wrzosek, 2014; Dai *et al*., 2017) by activating BK_{ca} channels.

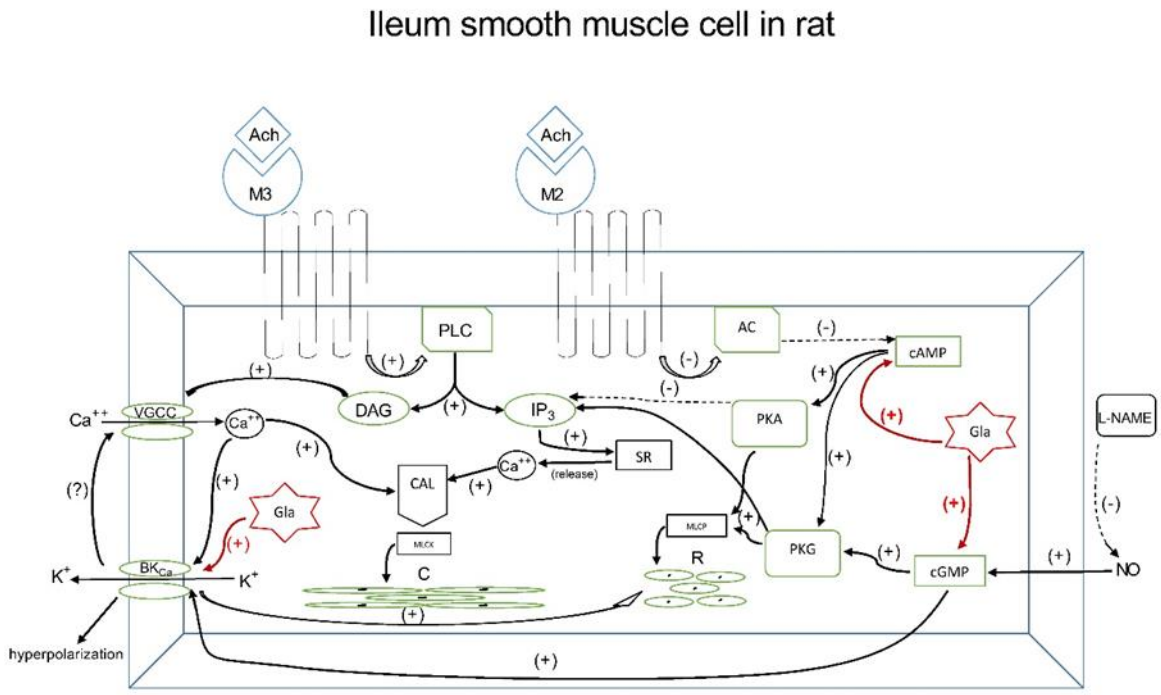

Figure No. 5

The mechanism schema of glabridin on ileum smooth muscle. Ach; acetylcholine, M; muscarinic receptor, Gla; glabridin, PLC; phospholipase C, DAG; diacylglycerol, IP_3; inositol triphosphate, BK_{ca}; large conductance Ca^{++}-activated K^+ channel, VGCC: voltage gate Ca^{++} channels, CAL; calmoduline, MLCK; myosin-light chain kinase, MLCP; myosin-light chain phospholipase, SR; Sarcoplasmic reticulum, cAMP; cyclic adenosine monophosphate, NO; nitric oxide, cGMP; cyclic guanosine monophosphate PKA; cAMP-dependent protein kinase, PKG; cGMP-dependent protein kinase, AC; adenylyl cyclase, L-NAME; NO inhibitor, R; relaxation, C; contraction

As illustrated schematically in Figure No. 5, contraction of the ileum smooth muscle with acetylcholine occurs directly via muscarinic M2 and M3 receptors in the ileum smooth muscle cell. Since adenylate cyclase is inhibited when the M2 receptor is stimulated by acetylcholine, the intracellular cAMP level is decreased (Peralta *et al*., 1988). cAMP and cGMP are the second precursor for direct relaxation of intracellular smooth muscles. cAMP is produced by adenylate cyclase, which is activated by the stimulation of beta-adrenergic receptors and is inhibited by M2 receptor activation, while cGMP is activated by mediators such as NO and natriuretic peptides (Uchiyama & Chess-Williams, 2004; Heghes *et al*., 2019).

The M3 receptor induces phospholipase C to produce both diacylglycerol, which causes Ca^{++} influx of extracellular Ca^{++} into the cell, and inositol triphosphate (IP_3), which causes the release of Ca^{++} from intracellular stores such as sarcoplasmic reticulum (Caulfield & Birdsall, 1998). cAMP induces the relaxation of smooth muscles by activating protein kinase A, which inhibits IP_3 and increases myosin-light chain phospholipase (MLCP) activity, decreasing myosin-light chain phosphorylation without changing intracellular Ca^{++} (Carvajal et al., 2000). In accordance with previous studies (El-Ashmawy *et al*., 2018), glabridin increased intracellular cAMP levels in this study.

NO-induced guanylate cyclase increases the level of intracellular cGMP which activates cGMP-dependent protein kinase (PKG). PKG mediates relaxation of smooth muscles by activating MLCP (Heghes *et al*., 2019) by inhibiting the sarcoplasmic reticulum release of Ca^{++} via IP_3 (Komalavilas & Lincoln, 1996) and by activating BK_{ca} channels (Bolotina *et al*., 1994; Dai *et al*., 2017). According to El-Ashmawy *et al*. (2018), the increased NO level in a rat model of ulcerative colitis induced by dextran sulfate sodium was down-regulated by the administration of glabridin. In this study, it was confirmed that glabridin prevented/reversed the decrease in intestinal I/R-induced NO level; however, the NO level was lower than the control group. Therefore, L-NAME was given to the group that received glabridin to test whether the NO-cGMP pathway mediates the smooth muscle relaxant effect of glabridin and whether glabridin has an effect on cGMP and NO levels. The data showed that the effect of glabridin to upregulate NO synthesis was inhibited by L-NAME. Alternatively, glabridin caused relaxation of the ileum smooth muscle via the NO-cGMP pathway. The result of the test is supported by a previous study, which concluded that the NO donor, L-Arginine, enhances the cell damage healing effect (Arslan *et al*., 2005).

In addition to the mechanisms described above, glabridin has a minor role in relaxing the ileum smooth muscle in the NO-cGMP-PKG pathway, despite the reduction of NO by L-NAME, as well as the activation of PKG (not PKA) by cAMP. White *et al*. (2000) demonstrated that cAMP-dependent vasodilators open BK_{ca} channels by cross-activation of PKG. Moreover, Jiang *et al*. (1992) noted that not only cGMP but also cAMP plays a role in PKG activation. This study's data showed that the relaxant effect of glabridin on the ileum smooth muscles is not only by the NO-cGMP-PKG pathway. Mechanisms mediating the muscle relaxant effect of glabridin may increase the level of cAMP and activate the more involved BK_{ca} channels, supported by the study of Chanda et al. According to their study, while glabridin caused relaxation of smooth muscles, it is abolished by a guanylate cyclase inhibitor and BK_{ca} channel blocker (Chanda *et al*., 2016). When the intracellular Ca^{++} level increases, the BK_{ca} channels are activated and the cell**s** are hyperpolarized (Heghes *et al*., 2019), resulting in the relaxation of smooth muscle. Chanda et al. also reported that in addition to activation of the BK_{ca} channels, the voltage-gated (KV), inwardly rectifying (KIR), and ATP-sensitive (KATP) K^+channels were involved in the role of glabridin as a relaxant of smooth vascular muscles (Chanda *et al*., 2016).

Along with the information collected above and with previous studies in an isolated tissue bath with anti-inflammatory and antioxidant agents (Arslan *et al*., 2005; Arab *et al*., 2014; Bayram *et al*., 2019), in this study, because glabridin enhanced the spontaneous activity of ileum and showed anti-inflammatory and antioxidant effects, it was expected that the ileum smooth muscles would contract with Ach. On the contrary, glabridin caused relaxation of the ileum smooth muscles. As explained by the aforementioned mechanisms, there may be two reasons for this. First, glabridin may cause the hyperpolarization of the cell by activating the BK_{ca} channel, resulting in relaxation of the smooth muscles. Second, although acetylcholine causes a decrease in cAMP level via the M2 receptor, the effects of the glabridin may be due to a significant increase in the direct intracellular cAMP level

independent of receptors, which is supported by previous studies *(Chanda et al*., 2016; El-Ashmawy *et a*l., 2018).

CONCLUSION

In this study, pre-administration of glabridin in I/R-induced ileum injury showed antioxidant, anti-inflammatory, and antispasmodic effects in ileum tissue, which is mediated by decreasing levels of MDA, MPO, and pro-inflammatory cytokines such as TNF-α, IL1β, and by increasing GSH and cAMP levels and by upregulating NO level. Moreover, glabridin improved the deterioration of spontaneous contraction of the ileum smooth muscles caused by intestinal I/R-injury. However, glabridin inhibited the contracting effect of acetylcholine on intestinal smooth muscles in vitro and even caused relaxation of the intestinal smooth muscles, which was due to increased intracellular cAMP content. Glabridin may be a potential anti-inflammatory, antioxidant, and spasmolytic agent. However, further studies are needed to test for potential therapeutic efficacy and clinical applicability. The limitation of this study is that the role of BK_{ca} channels was not considered in detail. Therefore, we plan to investigate the effects of glabridin on BK_{ca} channels.

REFERENCES

Ahn J, Lee H, Jang J, Kim S, Ha T. 2013. Anti-obesity effects of glabridin-rich supercritical carbon dioxide extract of licorice in high-fat-fed obese mice. **Food Chem Toxicol** 51: 439 - 445. **http://doi.org/10.1016/j.fct.2012.08.048**

Aoki F, Nakagawa K, Tanaka A, Matsuzaki K, Arai N, Mae T. 2005. Determination of glabridin in human plasma by solid-phase extraction and LC-MS/MS. **J Chromatograph B: Anal Technol Biomed Life Sci** 828: 70 - 74. **https://doi.org/10.1016/j.jchromb.2005.09.012**

Arab HH, Al-Shorbagy MY, Abdallah DM, Nassar NN. 2014. Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. **Plos One** 9: **https://doi.org/10.1371/journal.pone.0097193**

Arslan SO, Gelir E, Sayan H, Ozacmak VH. 2005. L-Arginine and melatonin interaction in rat intestinal ischemia--reperfusion. **Fund Clin Pharmacol** 19: 533 - 535. **https://doi.org/10.1111/j.1472-8206.2005.00365.x**

Bayram S, Parlar A, Arslan SO. 2019. The curative effect of cannabinoid 2 receptor agonist on functional failure and disruptive inflammation caused by intestinal ischemia and reperfusion. **Fund Clin Pharmacol** 84: 80 - 90. **https://doi.org/10.1111/fcp.12502**

Bolotina VM, Najibi S, Palacino JJ, Pagano PJ, Cohen RA. 1994. Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. **Nature** 368: 850 - 853. **https://doi.org/10.1038/368850a0**

Bradley PP, Priebat DA, Christensen RD, Rothstein G. 1982. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. **J Invest Dermatol** 78: 206 - 209. **https://doi.org/10.1111/1523-1747.ep12506462**

Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP. 2000. Molecular mechanism of cGMP-mediated smooth muscle relaxation. **J Cel Physiol** 184: 409 - 420. **https://doi.org/10.1002/1097-4652(200009)184:3<409::AID-JCP16>3.0.CO;2-K**

Casini AF, Ferrali M, Pompella A, Maellaro E, Comporti M. 1986. Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. **Am J Pathol** 123: 520 - 531.

Caulfield MP, Birdsall NJM. 1998. International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. **Pharmacol Rev** 50: 279 - 290.

Chanda D, Prieto-Lloret J, Singh A, Iqbal H, Yadav P, Snetkov V, Aaronson PI. 2016. Glabridin-induced vasorelaxation: evidence for a role of BKCa channels and cyclic GMP. **Life Sci** 165: 26 - 34. **https://doi.org/10.1016/j.lfs.2016.09.018**

Dai H, Wang M, Patel PN, Kalogeris T, Liu Y, Durante W, Korthuis RJ. 2017. Preconditioning with the BKCa channel activator NS-1619 prevents ischemia-reperfusion-induced inflammation and mucosal barrier dysfunction: roles for ROS and heme oxygenase-1. **Am J Physiol Heart Circ Physiol** 313: H988 - H999. **https://doi.org/10.1152/ajpheart.00620.2016**

Deitch EA. 1992. Multiple organ failure. Pathophysiology and potential future therapy. **Ann Surgery** 216: 117 - 134.

El-Ashmawy NE, Khedr NF, El-Bahrawy HA, El-Adawy SA. 2018. Downregulation of iNOS and elevation of

cAMP mediate the anti-inflammatory effect of glabridin in rats with ulcerative colitis. **Inflammopharmacology** 26: 551 - 559. **https://doi.org/10.1007/s10787-017-0373-9**

Gordeeva AE, Sharapov MG, Tikhonova IV, Chemeris NK, Fesenko EE, Novoselov VI, Temnov AA. 2017. Vascular pathology of ischemia/reperfusion injury of rat small intestine. **Cells Tissues Organs** 203: 353 - 364. **https://doi.org/10.1159/000455830**

Halliwell B. 1994. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? **The Lancet** 344: 721 - 724. **https://doi.org/10.1016/S0140-6736(94)92211-X**

Hasanein P. 2011. Glabridin as a major active isoflavan from *Glycyrrhiza glabra* (licorice) reverses learning and memory deficits in diabetic rats. **Acta Physiol Hungarica** 98: 221 - 230. **https://doi.org/10.1556/APhysiol.98.2011.2.14**

Heghes SC, Vostinaru O, Rus LM, Mogosan C, Iuga CA, Filip L. 2019. Antispasmodic effect of essential oils and their constituents: A review. **Molecules** 24: 1675. **https://doi.org/10.3390/molecules24091675**

Hoffmann KM, Beltrán L, Ziemba PM, Hatt H, Gisselmann G. 2016. Potentiating effect of glabridin from *Glycyrrhiza glabra* on GABAA receptors. **Biochem Biophys Rep** 6: 197 - 202. **https://doi.org/10.1016/j.bbrep.2016.04.007**

Jiang H, Colbran JL, Francis SH, Corbin JD. 1992. Direct evidence for cross-activation of cGMP-dependent protein kinase by cAMP in pig coronary arteries. **J Biol Chem** 267: 1015 - 1019.

Kahkhaie KR, Mirhosseini A, Aliabadi A, Mohammadi A, mosavi MJ, Haftcheshmeh SM, Sathyapalan T, Sahebkar A. 2019. Curcumin: a modulator of inflammatory signaling pathways in the immune system. **Inflammopharmacology** 27: 885 - 900. **https://doi.org/10.1007/s10787-019-00607-3**

Kang MR, Park KH, Oh SJ, Yun J, Lee CW, Lee MY, Han SB, Kang JS. 2015. Cardiovascular protective effect of glabridin: Implications in LDL oxidation and inflammation. **Int Immunopharmacol** 29: 914 - 918. **https://doi.org/10.1016/j.intimp.2015.10.020**

Komalavilas P, Lincoln TM. 1996. Phosphorylation of the inositol 1,4,5-trisphosphate receptor cyclic GMP-dependent protein kinase mediates cAMP and cGMP dependent phosphorylation in the intact rat aorta. **J Biol Chem** 271: 21933 - 21938.

Koyani CN, Flemmig J, Malle E, Arnhold J. 2015. Myeloperoxidase scavenges peroxynitrite: A novel anti-inflammatory action of the heme enzyme. **Arch Biochem Biophys** 571: 1 - 9. **https://doi.org/10.1016/j.abb.2015.02.028**

Kwon HS, Oh SM, Kim JK. 2008. Glabridin, a functional compound of liquorice, attenuates colonic inflammation in mice with dextran sulphate sodium-induced colitis. **Clin Exp Immunol** 151: 165 - 173. **https://doi.org/10.1111/j.1365-2249.2007.03539.x**

Leri O, Tubili S, De Rosa FG, Addessi MA, Scopelliti G, Lucenti W, De Luca D. 1997. Management of diarrhoeic type of irritable bowel syndrome with exclusion diet and disodium cromoglycate. **Inflammopharmacology** 5: 153 - 158. **https://doi.org/10.1007/s10787-997-0024-7**

Lindeström LM, Ekblad E. 2004. Structural and neuronal changes in rat ileum after ischemia with reperfusion. **Digest Dis Sci** 49: 1212 - 1222. **https://doi.org/10.1023/B:DDAS.0000037815.63547.08**

Maurice DH, Ke H, Ahmad F, Wang Y, Chung J, Manganiello VC. 2014. Advances in targeting cyclic nucleotide phosphodiesterases. **Nature Rev Drug Discov** 13: 290 - 314. **https://doi.org/10.1038/nrd4228**

Pal R, Chaudhary MJ, Tiwari PC, Nath R, Pant KK. 2019. Pharmacological and biochemical studies on protective effects of mangiferin and its interaction with nitric oxide (NO) modulators in adjuvant-induced changes in arthritic parameters, inflammatory, and oxidative biomarkers in rats. **Inflammopharmacology** 27: 291 - 299. **https://doi.org/10.1007/s10787-018-0507-8**

Parlar A, Arslan SO. 2019. Resveratrol normalizes the deterioration of smooth muscle contractility following intestinal ischemia and reperfusion in rats associated with an anti-oxidative effect and modulating TNF-α activity. **Ann Vasc Surg** 61: 416 - 426. **https://doi-org/10.1016/j.avsg.2019.06.027**

Parlar A, Arslan SO, Çam SA. 2020. Glabridin alleviates inflammation and nociception in rodents by activating BKCa channels and reducing NO levels. **Biol Pharmaceut Bull https://doi.org/10.1248/bpb.b20-00038**

Peralta EG, Ashkenazi A, Winslow JW, Ramachandran J, Capon DJ. 1988. Differential regulation of PI hydrolysis and adenylyl cyclase by muscarinic receptor subtypes. **Nature** 334: 434 - 437. **https://doi.org/10.1038/334434a0**

Sayan H, Ozacmak VH, Altaner S, Aktas RG, Arslan SO. 2008. Protective effects of L-arginine on rat terminal ileum subjected to ischemia/reperfusion. **J Pediat Gastroenterol Nutr** 46: 29 - 35. **https://doi.org/10.1097/01.mpg.0000304450.54057.96**

Simmler C, Pauli GF, Chen SN. 2013. Phytochemistry and biological properties of glabridin. **Fitoterapia** 90: 160 - 184. **https://doi.org/10.1016/j.fitote.2013.07.003**

Soydan G, Sökmensüer C, Kilinç K, Tuncer M. 2009. The effects of sildenafil on the functional and structural changes of ileum induced by intestinal ischemia-reperfusion in rats. **Eur J Pharmacol** 610: 87 - 92. **https://doi.org/10.1016/j.ejphar.2009.03.038**

Spanos CP, Papaconstantinou P, Spanos P, Karamouzis M, Lekkas G, Papaconstantinou C. 2007. The effect of L-arginine and aprotinin on intestinal ischemia-reperfusion injury. **J Gastrointestinal Surg** 11: 247 - 255. **https://doi.org/10.1007/s11605-007-0102-6**

Tian M, Yan H, Row KH. 2008. Simultaneous extraction and separation of liquiritin, glycyrrhizic acid, and glabridin from licorice root with analytical and preparative chromatography. **Biotechnol Bioproc Eng** 13: 671 - 676. **https://doi.org/10.1007/s12257-008-0019-2**

Uchiyama T, Chess-Williams R. 2004. Muscarinic receptor subtypes of the bladder and gastrointestinal tract. **J Smooth Muscle Res** 40: 237 - 247. **https://doi.org/10.1540/jsmr.40.237**

White RE, Kryman JP, El-Mowafy AM, Han G, Carrier GO. 2000. cAMP-dependent vasodilators cross-activate the cGMP-dependent protein kinase to stimulate BK(Ca) channel activity in coronary artery smooth muscle cells. **Circ Res** 86: 897 - 905. **https://doi.org/10.1161/01.res.86.8.897**

Wiegman CH, Michaeloudesm C, Haji G, Narang P, Clarke CJ, Russell KE, Bao W, Pavlidis S, Barnes PJ, Kanerva J, Bittner A, Rao N, Murphy MP, Kirkham PA, Chung KF, Adcock IM, COPDMAP. 2015. Oxidative stress-induced mitochondrial dysfunction drives inflammation and airway smooth muscle remodeling in patients with chronic obstructive pulmo nary disease. **J Allergy Clin Immunol** 136: 769 - 780. **https://doi.org/10.1016/j.jaci.2015.01.046**

Wrzosek A. 2014. The potassium channel opener NS1619 modulates calcium homeostasis in muscle cells by inhibiting SERCA. **Cell Calcium** 56: 14 - 24. **https://doi.org/10.1016/j.ceca.2014.03.005**

Wu F, Jin Z, Jin J. 2013. Hypoglycemic effects of glabridin, a polyphenolic flavonoid from licorice, in an animal model of diabetes mellitus. **Mol Med Rep** 7: 1278 - 1282. **https://doi.org/10.3892/mmr.2013.1330**

Zhang LEI, Chen H, Wang M, Song X. 2018. Effects of glabridin combined with 5-fluorouracil on the proliferation and apoptosis of gastric cancer cells. **Oncol Lett** 15: 7037 - 7045. **https://doi.org/10.3892/ol.2018.8260**

Zhang LP, Zhao Y, Liu GJ, Yang DG, Dong YH, Zhou LH. 2017. Glabridin attenuates lipopolysaccharide-induced acute lung injury by inhibiting p38MAPK/ERK signaling pathway. **Oncotarget** 8: 18935 - 18942. **https://doi.org/10.18632/oncotarget.14277**

Zheng XY, Wei RB, Shi SZ, Yin Z, Chen XM. 2012. Effects of Fufang Shenhua Tablet on the expression of toll-like receptors during acute kidney injury induced by ischemia-reperfusion in rats. **Chin J Integrat Med** 18: 918 - 924. **https://doi.org/10.1007/s11655-012-1295-1**