Amelioration of Visual Display Terminal-induced Ocular Fatigue by Aqueous Extracts of *Perilla frutescens* var. acuta

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Abstract In this study, we investigated the effects of *Perilla frutescens* var. acuta aqueous extract (PFA) on eyestrain using *in vitro*, *ex vivo*, and clinical tests. We confirmed the antioxidant activity of compounds present in the extract by using high-performance liquid chromatography. PFA contains antioxidant compounds such as rosmarinic acid, luteolin-7-*O*-glucuronide, and apigenin-7-*O*-glucuronide, and has been shown to inhibit reactive oxygen species generation in C2C12 muscle cells. In an *ex vivo* study, PFA inhibited the carbachol (100 μM)-induced contraction of ciliary muscle from rabbit eyeball. We then investigated the mechanism by which PFA caused muscle relaxation and found that it effectively increased cyclic GMP production and inhibited PDE5A activity, but did not affect the cyclic AMP pathway, in cultured human aortic smooth muscle cells. In addition, PFA (50, 100, and 200 μg/mL) dose-dependently decreased basal intracellular calcium in smooth muscle cells and attenuated the endothelin-1-stimulated increase in intracellular calcium. Finally, we performed a clinical study to evaluate the effects of PFA on the near point of accommodation (NPA) after visual display terminal (VDT) work. Thirty participants were randomized to either PFA (500 mg/day) or placebo, and intake for one consecutive week. NPA was evaluated before and after 2 h of VDT work, and was found to be improved in the PFA-treated group. Thus, our findings show that PFA may ameliorate visual fatigue.

Keywords: *perilla frutescens var. acuta, visual display terminal (VDT), visual fatigue, near point of accommodation, ciliary muscle*

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1. Introduction

Eye fatigue has been steadily increasing in recent years, with the growing use of visual display terminals (VDT) such as computers and smartphones [1,2,3]. The symptoms of eye fatigue include ocular pain, dry eye sensation, headache, dizziness, ghost images, eye pain, dryness, heavy eyelids, and blurred vision [4,5]. Recently, research conducted at the National Institute for Occupational Safety and Health (NIOSH) showed that over 50% of frequently VDTs users reported visual fatigue and other ocular problems associated with VDT use [6].

Some researchers reported oxidative stress as the cause of eye fatigue and various eye conditions [7]. Reactive oxygen species (ROS) produced in the crystalline lens induced eye strain [8]. Some studies have reported that certain natural plant-derived extracts, including bilberry, *Haematococcus pluvialis*, blackcurrant, and black soybean hull extracts, can ameliorate visual fatigue and exert antioxidant effects [2,7,9]. Antioxidant compounds have

Been shown to be effective in improving visual function [7,10].

Visual fatigue is related to the accommodative ability of the eye to everyday activities [9]. Clinical studies have showed visual fatigue is closely associated with ophthalmic factors, which represents the accommodative ability of the eye such as the near point of accommodation (NPA) [9,11,12]. Accommodation provides the ability to change in focusing power controlled by the crystalline lens that during ciliary muscle contraction, thus allowing the eye to focus on near objects [13]. Eye fatigue is caused by the excessive use and resultant exhaustion of the ciliary muscle [14]. A decrease in the accommodative ability of the eye is caused by the loss of ciliary muscle function, which regulates the thickness of the lens [15,16,17]. The mechanism underlying ciliary muscle relaxation has been extensively studied in the search for drug therapies for the treatment of eye diseases [18]. Relaxation of ciliary smooth muscle is mediated by two mechanisms: cyclic AMP (cAMP)-dependent pathways, including prostaglandin receptor-mediated responses, and cyclic GMP (cGMP)-dependent pathways [19]. In general, increased cAMP and cGMP induces smooth muscle

relaxation [20]. In addition, intracellular Ca^{2+} concentration $[Ca^{2+}]i$ in smooth muscles is a major factor affecting the contraction of ciliary smooth muscle [20].

Perilla frutescens var. acuta is a traditional medicinal plant used in Asian countries, including Korea, China, and Japan, for treating symptoms such as depression, anxiety, allergies, bacterial and fungal infections, common cold, shivering fits, fever, chest pains, and cough [21,22,23]. The leaves of this plant are widely consumed in sushi, soups, and salads, as a spice and food colorant, and for garnishing [24]. Previous pharmacological studies have shown that *P. frutescens* var. acuta exerts antioxidant, anti-inflammatory, anti-allergic, anti-microbial, and anti-tumor effects [25,26,27,28]. In addition, studies have reported that flavonoids, phenolic acids, and anthocyanins were isolated from the leaves of *P. frutescens* [29]. Phenolic compounds in this plant extracts exhibit high antioxidant activity [24]. However, the effect of these compounds on visual fatigue has not yet been elucidated. To the best of our knowledge, this is the first study to report the effects of an aqueous extract of *P. frutescens v*ar. acuta (PFA) on visual fatigue. We investigated the effects of PFA on of ROS generation in smooth muscle cells to confirm the antioxidant activity of its constituents, as well as its ability to cause relaxation in rabbit ciliary body muscle through the cGMP pathway. Finally, we performed a clinical study to determine the effect of PFA on NPA.

2. Materials and Methods

2.1. Preparation of PFA Extract

Dried leaves (3 kg) were extracted in 10 volumes (w/v) of distilled water for 3 h at 100°C according to the method reported by Kim et al with slight modifications [30]. The process was repeated three times and the resulting filtrates were pooled, concentrated under reduced pressure at 40°C, and lyophilized at −50°C to obtain a solid reddish-brown residue (650 g).

2.2. High-performance Liquid Chromatography (HPLC) Analysis for Identification of Phytochemical Composition

HPLC analysis of PFA was performed using a YL 9100 HPLC system (Young Lin Instrument Co. Ltd., Korea) equipped with a binary pump (YL9111), UV/VIS detector (9120), and auto sampler (9150), and a Triart C_{18} plus column (250 × 4.6 mm, 5 µm, YMC Co. Ltd.). The detection wavelength was set at 325 nm for water extract, luteolin-7-*O*-extract, apigenin-7-*O*-extract, and rosmarinic acid. The column thermostat was maintained at 35°C.

Methanol and water (containing 0.1% formic acid) were used as the mobile phase A and B, respectively. The elution profile was as follows: 0–10 min, 30% A; 10–30 min, 30–50% A; 30–35 min, 60% A; 35–40 min, 60–70% A; 40–45 min, 70–100% A; 45–53 min, 100% A; 53–56 min, 100–30% A; 56–60 min, 30% A. The flow rate was 1 mL/min, and the injection volume was 10 µL.

2.3. Measurement of ROS Levels In Muscle Cells

Mouse myoblast C2C12 cells (ATCC, Manassas, VA, USA) were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (GIBCO®; Life Technologies Co., Carlsbad, CA, USA), 100 units/mL of penicillin, and 100 µg/mL of streptomycin and maintained in a humidified cell incubator under an atmosphere of 5% CO_2 at 37°C. The ROS levels inhibition activity in muscle cells of PFA was measured using the method reported by Kim et al [31]. Cells (5×10^5 cells/mL of medium/well) were seeded in a 48-well plate and pretreated with PFA (50, 100, and 200 µg/mL) for 24 h prior to induction of stress by hydrogen peroxide (H_2O_2, 200 µM) and further incubation for 2 h. Cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. To measure ROS levels, cells were washed twice with phosphate-buffered saline (PBS), lysed with 1% Triton X-100 in PBS for 10 min at 37°C, and stained with 10 µM 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) for 30 min at room temperature in the dark. The cells were then washed twice with ice-cold PBS, and fluorescence intensity was examined using a fluorescence microscope (Olympus Optical, Japan) and measured using a microplate fluorometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 485 and 530 nm, respectively.

2.4. Relaxation of Rabbit Ciliary Muscle

2.4.1. Measurement of Isomeric Tension of Ciliary Muscle

Female clean rabbits (2.4-2.7 kg) were purchased from Samtako (Osan, Korea) and were anesthetized with an intramuscular injection of Zoletil-Rompun mixture (1:2). The isolation of ciliary muscle was performed according to the method reported by Matsumoto et al with slight modifications [19]. The eyes were immediately enucleated and placed in Krebs solution at 4°C ($CaCl_2$ 1.5 mM, NaCl 118 mM, KCl 4.7 mM, $MgSO_4$ 1.1 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, and glucose 10 mM; pH 7.4). The ciliary muscle was carefully ablated from the sclera after removal of the crystalline lens, cut into a 3 mm × 6 mm strip, and suspended in Krebs solution bubbled with a gas mixture of 5% CO_2 and 95% O_2 at 37°C. Muscle contractions were measured using force transducers connected to a physiograph recorder (Powerlab 4/30; AD Instruments, New South Wales, Australia) and a Power Lab data-acquisition system. Basal tension was applied at a loading weight of 1 g using a tension transducer. Following 90 min of equilibration, the ciliary muscle was stimulated by the addition of 100 µM/mL carbachol. When the resultant tension reached a plateau value, the ciliary muscle was washed with Krebs solution. After 30 min, upon establishment of baseline tension, a second stimulation was applied by a further addition of 100 µM/mL carbachol. This process was repeated multiple times, and each time, the ciliary muscle was washed with Krebs solution to re-establish the baseline. After the tenth stimulation, PFA (100 and 200 µg/mL) was added to the ciliary muscle, and the resultant tension was monitored.

This study was performed according to the guidelines of the Animal Care and use review Committee of Jeollanamdo Institute of Natural Resources Research (JINR 1603).

2.5. Mechanisms Underlying Smooth Muscle Relaxation

2.5.1. Measurement of cGMP and cAMP Production

Primary human aortic smooth muscle cells (hASMCs) were purchased from the ATCC (American Type Culture Collection, PCS-100-012, Manassas, VA, USA) and maintained in vascular cell basal medium (ATCC, PCS-100-030) supplemented with vascular smooth muscle cell growth kit (ATCC, PCS-100-042) in a humidified cell incubator under an atmosphere of 5% CO_2 at 37°C. For experiments, hASMCs were plated in complete medium on 6-well plates at a concentration of 5×10^5 cells/well. After 24 h, cells were pretreated for 10 min with PBS buffer containing 1 mM 3-isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor. PFA (50, 100, and 200 µg/mL) was then added and the cells were incubated for 15, 30, and 60 min. Media was aspirated and the cGMP content of each sample was determined using a cyclic GMP ELISA kit from Cell Biolabs, Inc. (San Diego, CA), according to the manufacturer's instructions. Intracellular cAMP levels were measured using a cAMP Parameter™ assay kit (R&D Systems Europe, Ltd., Abingdon, UK) according to the manufacturer's instructions.

2.5.2. Phosphodiesterase (PDE) Inhibitory Activity of PFA

PDE5A and PDE3A activities were assayed using kits according to the manufacturer's instructions (BPS Bioscience, San Diego, CA). Briefly, each 50 µL reaction mixture contained 10 ng/mL PDE5A or 20 ng/mL PDE3A, or both, and 200 nM FAM-Cyclic-3′,5′-GMP or 200 nM FAM-Cyclic-3′,5′-AMP, or both. PFA (50, 100, and 200 µg/mL) was diluted in PDE assay buffer, and 5 µL of the dilution was added to a 50 µL reaction volume. The reaction mixtures were incubated at room temperature for 1 h. A diluted binding agent (100 µL) was then added to each well, and the reaction mixtures were incubated at room temperature for 1 h with slow shaking. The fluorescence polarization of each sample was obtained using a 480 nm excitation filter and 528 nm emission filter. The percentage of inhibition was calculated using the following formula (1).

$$\% \text{ activity} = \left[\left(F_{drug} - F_{control} \right) / \left(F_{enzyme} - F_{control} \right) \right] \times 100. \quad (1)$$

2.5.3. Measurement of $[Ca^{2+}]i$

The $[Ca^{2+}]i$ levels inhibition in muscle cells of PFA was measured using the method reported by Lee et al [32]. The acetoxymethyl-ester form of Fura-2 (Fura-2/AM) was used as the fluorescent Ca^{2+} indicator. hASMCs were incubated for 60 min at room temperature with 5 µM Fura-2/AM and 0.001% Pluronic F-127 in HEPES-buffered solution of the following composition (in mM): 155 NaCl, 3 KCl,

2 $CaCl_2$, 10 HEPES, 10 glucose, and 1 µM glycine, adjusted to pH 7.4 with NaOH. The cells were stabilized in HEPES-buffered solution for 5 min, followed by PFA treatment (50, 100, and 200 µg/mL) for 100 s to confirm changes in $[Ca^{2+}]i$ levels. To induce a $[Ca^{2+}]$ response, the cells were stimulated with endothelin-1 (ET-1; 10 nM) for 100 s following treatment with PFA (50, 100, and 200 µg/mL) or control for 100 s. Next, the cells were illuminated using Lambda XL and excitation wavelengths of 340 and 380 nm were selected. Data were acquired every 2 s. All imaging data were collected and analyzed using MetaMorph software.

2.6. Clinical Measurement of Accommodation

2.6.1. Study Design

This study was approved by the Institutional Review Board (IRB) of Dongshin University Oriental Hospital (IRB approval number: DSGOH-033). The study design was a randomized, placebo-controlled, double-blind comparison study. Thirty participants who have no eye disease were randomly assigned to the test and placebo groups (n=30 per group). Each subject intake test sample and placebo during 1 week, and VDT loads were imposed by having the subjects play smartphone for 2 h on the final days.

2.6.2. Near Point of Accommodation (NPA)

NPA (in cm) was evaluated before and after 2 h of VDT work in the test sample and placebo group after using push-up tests. A 20/30 single letter on a fixation stick, positioned approximately 40 cm from the subject, served as the target and was moved gradually closer to the subject until the subject noticed the target starting to blur. This was considered the endpoint.

2.7. Statistical Analysis

The results of *in vitro* and *ex vivo* tests are expressed as the mean ± standard error of the mean (SEM). Data from the groups were compared by analysis of variance (ANOVA), followed by Dunnet's post hoc test. All statistical analyses were performed using GraphPad Prism 5 for Windows (GraphPad Software, San Diego, California, USA). Clinical test data were expressed as the mean ± SE. Analysis between the groups was performed via *t*-test using SPSS 21.0. P values < 0.05 were considered statistically significant.

3. Results

3.1. Effect of PFA on Antioxidant Activity

Quantitative HPLC analysis of PFA revealed three main antioxidant compounds: rosmarinic acid, luteolin-7-*O*-glucuronide, and apigenin-7-*O*-glucuronide (Figure 1). We investigated the inhibitory effect of PFA on H_2O_2-induced oxidative stress in muscle cells and observed that PFA inhibited ROS generation in H_2O_2-induced C2C12 cells in a dose-dependent manner (Figure 2B), without causing cell toxicity (Figure 2A).
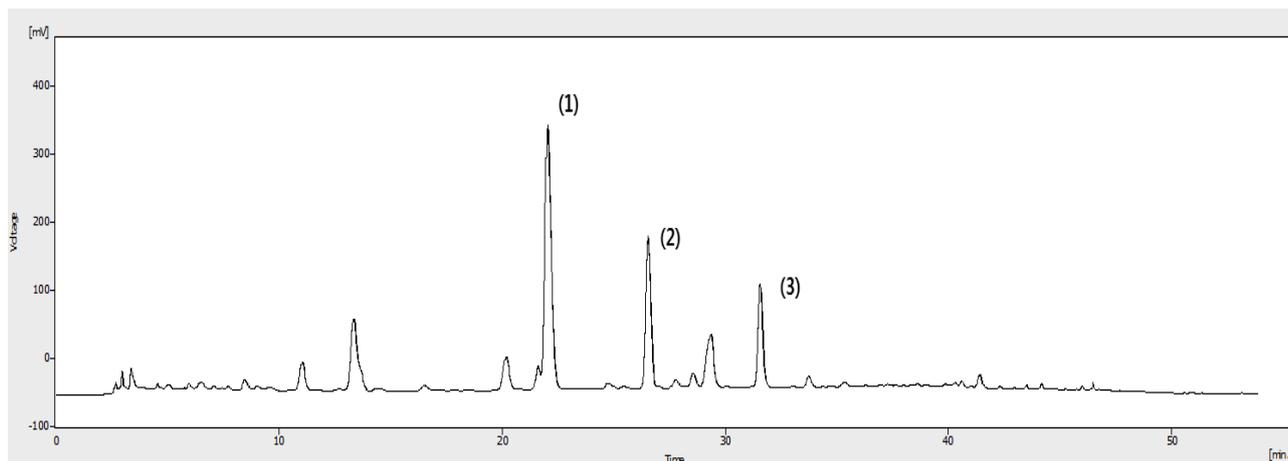
Figure 1. HPLC chromatogram of *Perilla frutescens* var. acuta aqueous extracts (PFA). (1) Luteolin-7-O-β-D-diglucuronide, (2) Apigenin -7-O-β-D-diglucuronide, (3) Rosmarinic acid
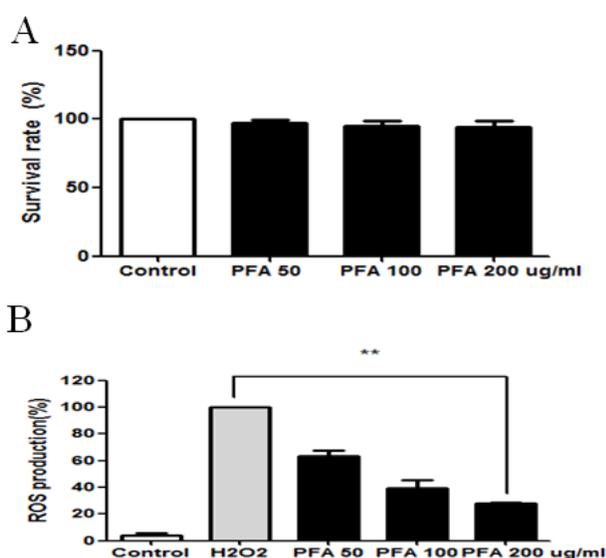

Figure 2. Effect of PFA on cell viability (A) and ROS production (B) in H_2O_2-induced C2C12 cells. Cells were pretreated with PFA (50, 100, and 200 µg/mL) for 24 h prior to treatment with H_2O_2 (200 µM) for 2 h. Cells were then stained with 10 µM DCFH-DA and fluorescence was measured at excitation and emission wavelengths of 485 and 530 nm, respectively. Data are presented as mean ± SEM of three experiments. * $P < 0.05$ and ** $P < 0.01$ compared with H_2O_2-treated cells

3.2. Effect of PFA on Ciliary Muscle Relaxation

We evaluated the ability of PFA to cause relaxation in rabbit ciliary muscle. After the resting tension of each ciliary muscle strip had stabilized, sustained contraction of approximately 100 mg was maintained by the addition of 100 µM/mL carbachol (Figure 3). After stabilization of carbachol-induced contraction, PFA was added, resulting in sustained and progressive relaxation within 40–60 min. The control (distilled water) exerted no relaxation effect on carbachol-induced contraction, while PFA (200 µg/mL) produced significant relaxation.

3.3. Effect of PFA on Cyclic GMP and Cyclic AMP Levels

We investigated whether PFA increased intracellular cGMP and cAMP production in hASMCs. PFA (50, 100, and 200 µg/mL) produced a concentration- and time-dependent increase in intracellular cGMP levels, with stimulation observed 15–60 min after the addition of the extract (Figure 4A). In addition, PFA had no apparent effect on cAMP levels at different concentrations and incubation times (Figure 4B). These results showed that PFA directly increased cGMP concentrations ($P < 0.05$ and $P < 0.01$), but no significant effect on cAMP levels was observed ($P > 0.05$).

3.4. Effect of PFA on PDE inhibitory Activity

We examined the effect of PFA on PDE5A and PDE3A inhibitory activity using the PDE assay kit. PFA significantly inhibited PDE5A activity in a dose-dependent manner, but no inhibitory effect was observed on PDE3A activity (Figure 5A, B). PFA treatment at concentrations of 50, 100, and 200 µg/mL inhibited PDE5A activity in a dose-dependent manner to 51.23 ± 0.29, 42.42 ± 0.13, and $36.58 \pm 0.37\%$, respectively (Figure 5A).
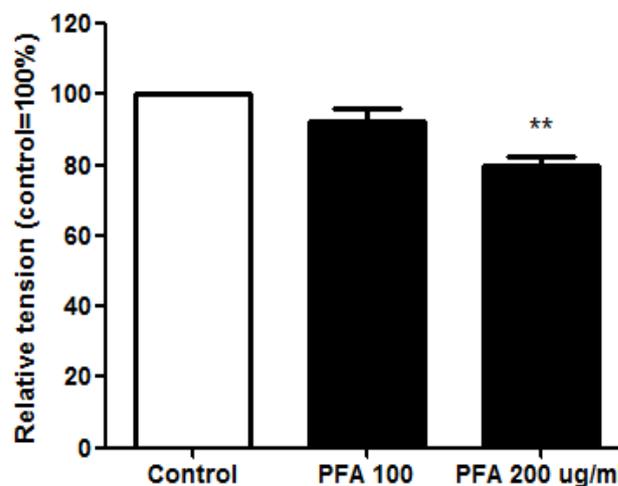

Figure 3. Relaxation effects of PFA on carbachol-induced contraction in rabbit ciliary muscles. Ciliary muscles were stimulated with carbachol (100 µM) and then treated with PFA (100 and 200 µg/mL). The relaxation rate of PFA is expressed as the percentage of the respective component control. Data are expressed as mean ± SEM of three experiments. * $P < 0.05$ and ** $P < 0.01$ compared with control (CT)
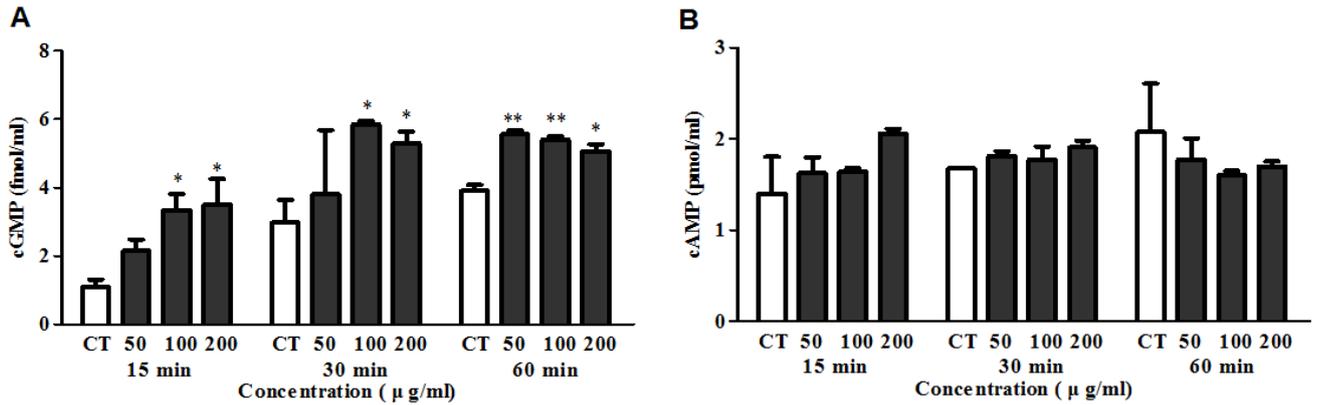
Figure 4. Effect of PFA on cGMP and cAMP levels in hASMCs. (A) Intracellular concentration of cGMP after 15, 30, and 60 min of PFA treatment at 50, 100, and 200 μg/mL. (B) Intracellular concentration of cAMP after 15, 30, and 60 min of PFA treatment at 50, 100, and 200 μg/mL. The values are expressed as mean ± SEM of three experiments. * $P < 0.05$ and ** $P < 0.01$ compared with control (CT)
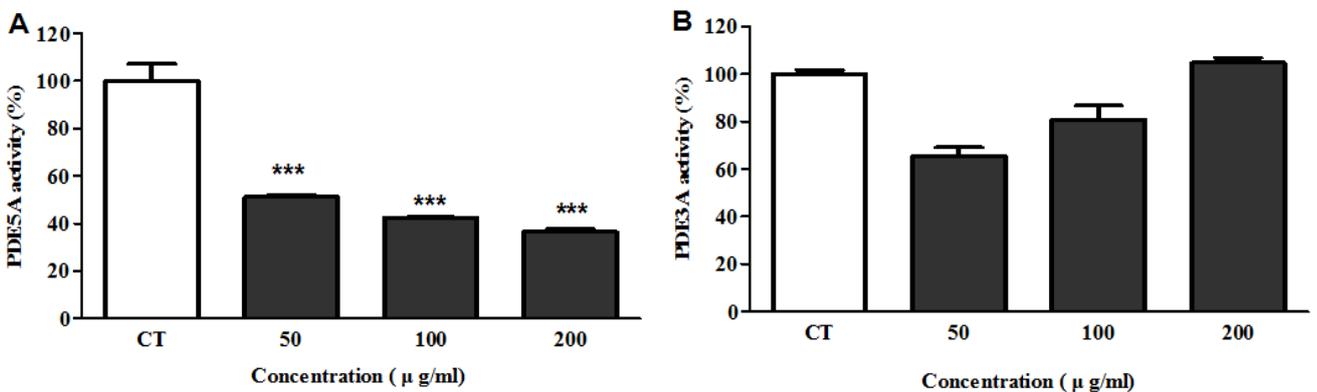

Figure 5. Effect of PFA on PDE activity. Effect of PFA on isoenzyme-specific cGMP-PDE activity for PDE5A (A) and cAMP-PDE activity for PDE3A (B) was measured. The values are expressed as the mean ± SEM of three experiments. *** $P < 0.001$ compared with control (CT)
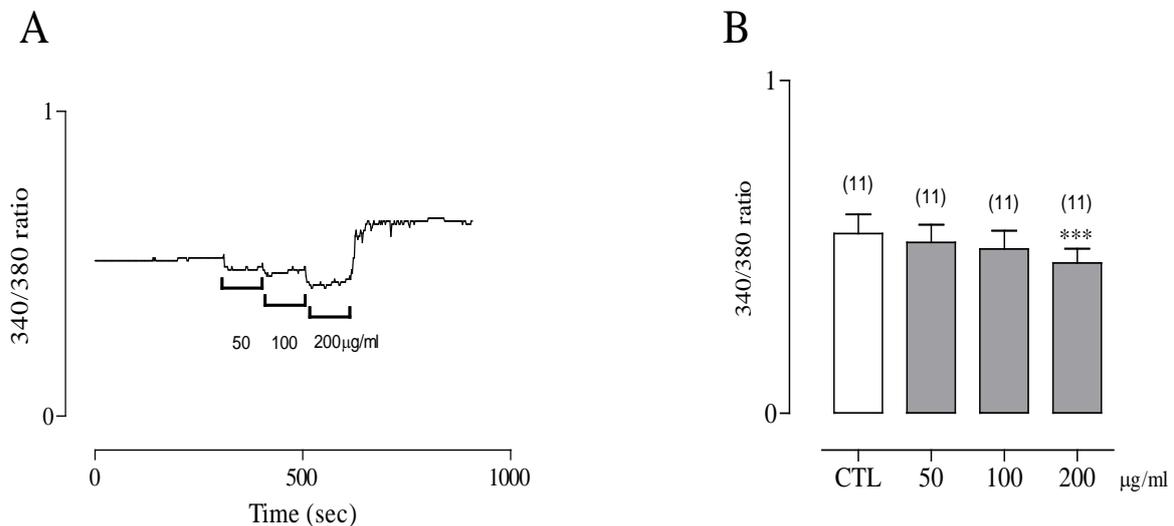

Figure 6. Effects of PFA on basal $[Ca^{2+}]_i$ of hASMCs. (a) Representative tracing of the $[Ca^{2+}]_i$ response evoked by various concentrations of PFA (50, 100, and 200 μg/mL) (b) The panel shows the summarized data of PFA-induced changes in $[Ca^{2+}]_i$ in hASMCs. The results are expressed as mean ± SEM of basal $[Ca^{2+}]_i$, in PFA-treated hASMCs. n = 11, *** $P < 0.001$ compared with control (CT)

3.5. Effect of PFA on Basal $[Ca^{2+}]_i$ in Muscle Cells

To investigate whether the inhibitory effect of PFA on muscular contraction is associated with the attenuation of $[Ca^{2+}]_i$, we examined its effects on $[Ca^{2+}]_i$ levels using Fura-2 ratiometric Ca^{2+} imaging. As shown in Figure 6, the control had no effect on $[Ca^{2+}]_i$. However, PFA significantly reduced basal $[Ca^{2+}]_i$ levels in a dose-dependent manner.

3.6. Inhibition of ET-1-induced $[Ca^{2+}]_i$ Increase by PFA in Muscle Cells

To confirm the effects of PFA on Ca^{2+} signaling in smooth muscle cells, we determined whether Ca^{2+} release

was affected by PFA. Application of 10 nM of ET-1 to hASMCs induced a statistically significant elevation $(P < 0.001)$ in $[Ca^{2+}]_i$, which was inhibited by PFA treatment (50, 100, and 200 µg/mL; Figure 7).
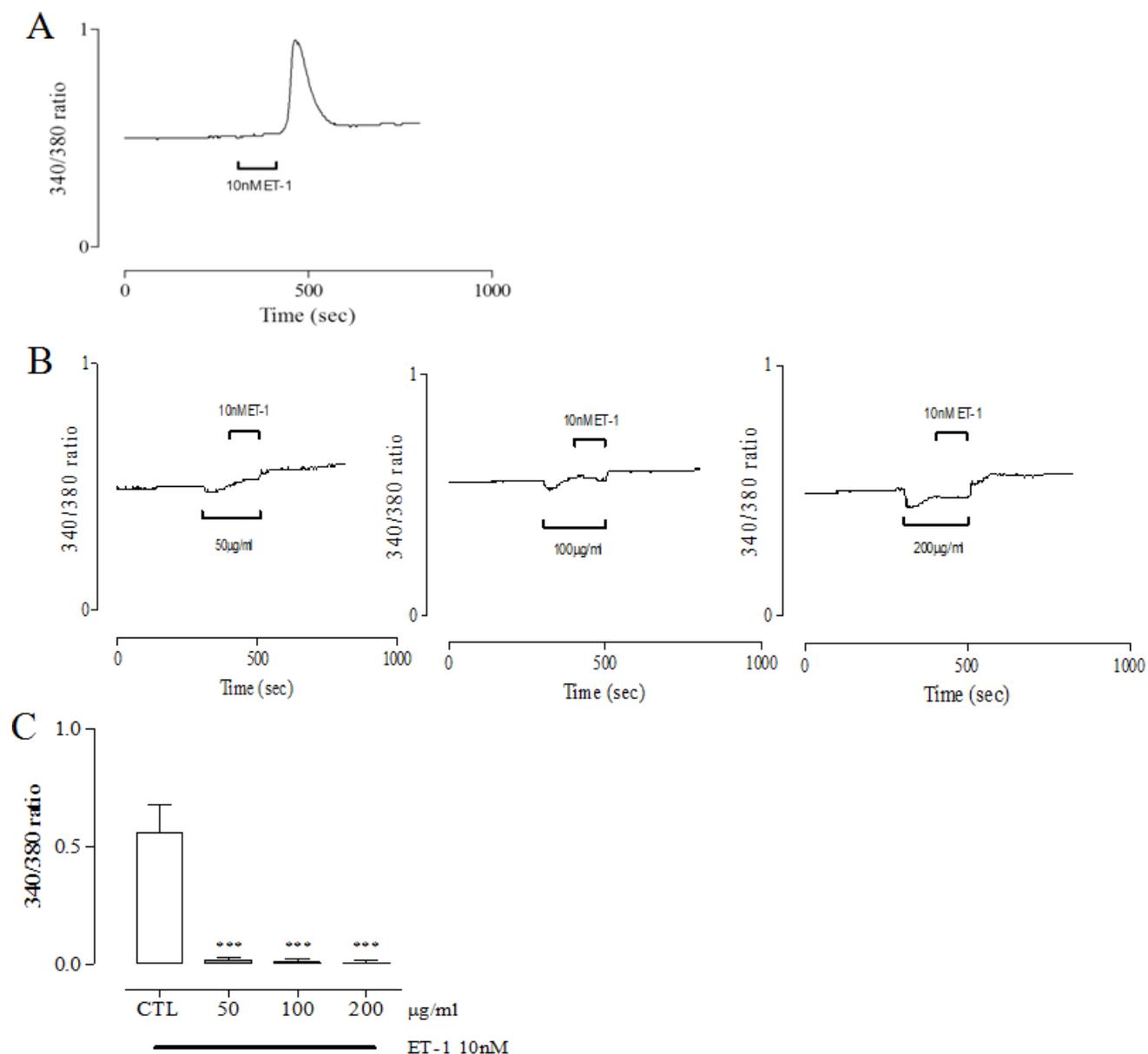

Figure 7. Inhibitory effects of PFA treatment on ET-1 (10 nM)-induced $[Ca^{2+}]_i$ response in hASMCs. (a) $[Ca^{2+}]_i$ responses induced by ET-1 (10 nM, 100 s). (b) Inhibitory effects of PFA pretreatment (50, 100, and 200 µg/mL for 100 s) on ET-1-induced $[Ca^{2+}]_l$ responses (c) The panel shows the summary of the mean ± SEM of $[Ca^{2+}]_i$. Inhibition of ET-1-induced $[Ca^{2+}]_i$ responses by PFA. n = (11), ***P<0.001 compared with control (CT), ET-1 = endothelin-1

Table 1. Change of NPA values before and after VDT work for 2 h of PFA and Placebo

NPA[1](cm)		Before	After	Change	t-test	p-value
PFA[2]	Dominant eye	8.68±2.98	7.83±3.08	-0.85±1.32	t = -3.53	***P < 0.001***
	Non-dominant eye	8.38±3.13	7.67±3.21	-0.71±1.21	t = -3.20	***P < 0.001***
	Both eye	7.96±2.97	7.27±3.25	-0.69±1.46	t = -2.60	***P < 0.001***
Placebo	Dominant eye	8.90±2.60	9.63±2.40	+0.73±0.79	t = 5.12	***P < 0.001***
	Non-dominant eye	8.60±2.49	9.43±2.42	+0.83±1.12	t = 4.09	***P < 0.001***
	Both eye	9.00±2.45	9.67±2.48	+0.67±0.84	t = 4.33	***P < 0.001***

Unit: cm, p<0.05
All values are expressed as the mean±SE.
[1]: NPA Near point accommodation, [2] PFA: *Perilla frutescens* var. acuta aqueous extracts.

3.7. Effect of PFA on VDT-induced Ocular Fatigue Assessed by NPA

NPA was measured before and after VDT work for 2 h, and was 8.68±2.98 and 7.83±3.08 cm, respectively, in the dominant eye, indicating a significant decrease ($P < 0.001$). The NPA for the non-dominant eye was 8.38±3.13 cm before VDT use and 7.67±3.21 cm after 2 h of VDT use, also indicating a significant decrease ($P < 0.001$). The NPA for both the eyes before and after 2 h of VDT work was 7.96±2.97 and 7.27±3.25 cm, respectively, representing a significant decrease ($P < 0.001$). However, NPA did not improve in the placebo group. The NPA in the placebo group before and after 2 h of VDT use was 8.90±2.60 and 9.63±2.40 cm, respectively, for the dominant eye ($P < 0.001$). For the non-dominant eye, the NPA before and after 2 h of VDT work was 8.60±2.49 and 9.43±2.42 cm, respectively ($P < 0.001$). The NPA for both eyes before and after 2 h of VDT work was 9.00±2.45 and 9.67±2.48 cm, respectively ($P < 0.001$; Table 1).

4. Discussion

Recently, the widespread use of various VDTs, such as smartphones, televisions, and computer monitors, has become a serious global health concern [33,34]. Increased VDT use has resulted in a concurrent increase in various symptoms associated with eye fatigue, including ocular pain, dry eye sensation, excess tears, and blurred vision [4,5]. The aim of the present study was to investigate whether PFA exerts ameliorating effects on visual fatigue. Several studies have demonstrated that certain food ingredients, such as docosahexaenoic acid [35], eicosapentaenoic acid [35], omega-3 fatty acids [36], lutein [37], zeaxanthin [37], astaxanthin [9], bilberry extract [2], black soybean hull extract [9], and anthocyanin [19,38] can improve visual fatigue.

Some studies have examined the relationship between oxidative stress and various ophthalmic diseases [8,39]. Food constituents with antioxidants have been reported to be effective in improving visual systems [13]. Other studies have reported that astaxanthin, bilberry extract, and lutein exert antioxidant effects [2,9]. In support of these reports, we identified the major polyphenolic components in PFA as apigenin-7-*O*-diglucuronide, luteolin-7-*O*-diglucuronide, and rosmarinic acid using HPLC analysis, and these compounds showed antioxidant activity. In addition, our experimental results showed that PFA significantly inhibited ROS generation in H_2O_2-induced muscle cells without causing cell toxicity.

The accommodative ability of the eyes, controlled by the action of ciliary muscle, may directly lead to eye fatigue [40]. In a previous study, it was reported that astaxanthin may improve accommodation owing to its relaxing effect on the ciliary muscle [41,42]. Moreover, bilberry and black soybean hull extracts improved accommodative ability and ciliary muscle relaxant effects [9]. We conducted a randomized, placebo-controlled, double-blind comparison study of 30 subjects to verify the effect of PFA on the accommodative ability of the eye. By measuring NPA, we showed that VDT work for 2 h significantly improved accommodative ability compared with placebo. Increased cGMP content in smooth muscle

cells suppresses contractions. In this study, PFA (200 µg/mL) relaxed carbachol-induced contractions of ciliary muscles. In accordance with the results of previous studies, our data indicate that PFA induced the relaxation of carbachol-induced ciliary muscle contraction via increased cGMP production. However, PFA did not affect cAMP content. PDEs selectively degrade both cAMP and cGMP through hydrolysis in smooth muscle tissues [43]. cAMP hydrolysis is mediated by PDE1, 2, 3, and 10 and cGMP hydrolysis by PDE5, 6, and 9 [43]. In this study, we demonstrated that PFA significantly inhibited PDE5A activity in a dose-dependent manner, but exhibited no inhibitory effect on PDE3A activity. In a previous study, delphinidin-3-rutinoside relaxed bovine ciliary smooth muscle by activating the ET-B receptor and the NO/cGMP pathway [20]. In addition, it has been reported that bovine ciliary smooth muscle relaxation is mediated by the cGMP pathway [44]. Furthermore, cGMP is known to decrease Ca^{2+} levels in smooth muscle cells, thus leading to smooth muscle relaxation [20]. Our results indicate that PFA treatment (50, 100, and 200 µg/mL) significantly reduced basal $[Ca^{2+}]_i$ and inhibited ET-1-induced $[Ca^{2+}]_i$ in a dose-dependent manner.

5. Conclusion

In conclusion, this is the first study to demonstrate that PFA ameliorated visual fatigue. PFA also mediated the relaxation of ciliary smooth muscle via the cGMP pathway, and significantly improved accommodative ability compared with the placebo group in a clinical study. Our results indicate the beneficial effects of PFA in improving visual fatigue.

Acknowledgements

Conflicts of Interest

The authors have no conflicts of interest to declare.

List of Abbreviations

NPA, near point of accommodation; PFA, *Perilla frutescens* var. acuta aqueous extract; ROS, reactive oxygen species; PDE, phosphodiesterase.

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