Research Results in Pharmacology

Research Results in Pharmacology 10(4): 113–124
UDC: 616.348–002.44-085.243
DOI 10.18413/rrpharmacology.10.507

Research Article

Pharmacological effects of purple basil extract on isolated mouse bladder functions and underlying molecular mechanisms

Beyza N. Arslan[1], Naciye Y. Dondas[1]

1 University of Çukurova, Faculty of Medicine, 01330, Adana, Türkiye

Corresponding author: Naciye Y. Dondas (yakdas25@cu.edu.tr)

Academic editor: Oleg Gudyrev ♦ **Received** 01 June 2024 ♦ **Accepted** 21 October 2024 ♦ **Published** 30 December 2024

Citation: Beyza NA, Naciye YD (2024) Pharmacological effects of purple basil extract on ısolated mouse bladder functions and underlying molecular mechanisms. Research Results in Pharmacology 10(4): 113–124. https://doi.org/10.18413/rrpharmacology.10.507

Abstract

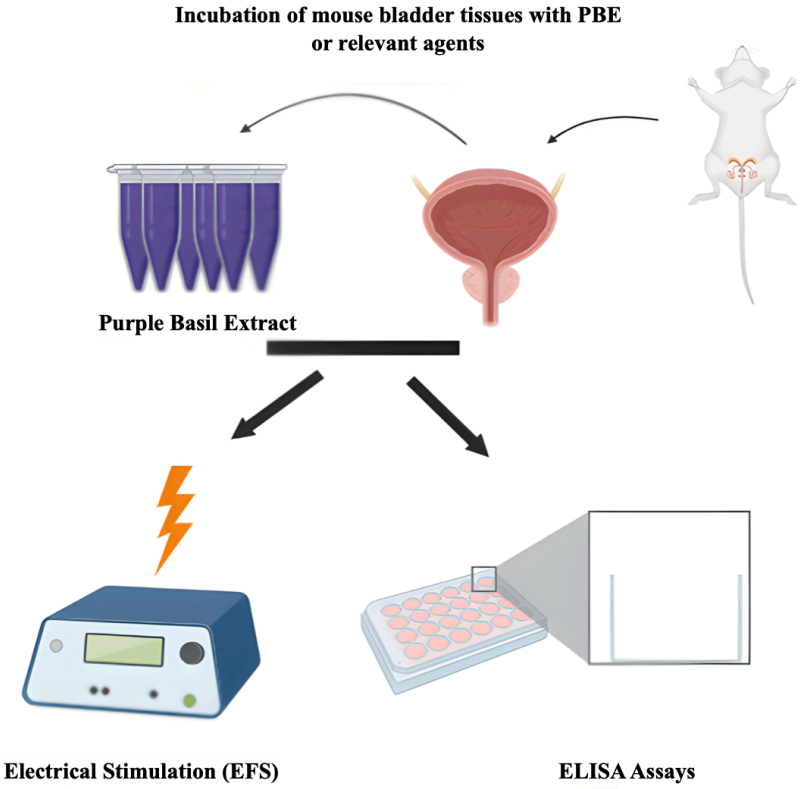
Introduction: In this study, the pharmacological effects of purple basil (*Ocimum basilicum var. purpurascens*) extract (PBE) in isolated mouse bladder strips were investigated. The effects of PBE on contractile responses induced by electrical field stimulation (EFS) in bladder strips and the molecular mechanisms involved in these effects were investigated. Anti-inflammatory and antioxidant effects of PBE in isolated tissues were investigated.

Materials and Methods: PBE was prepared. Mice were sacrificed under ketamine-xylazine anesthesia, bladder tissues were isolated. The isolated bladder tissues were cut into strips and suspended in an organ bath. The bladder strips were then incubated with the relevant agents, the contractile responses were examined by applying EFS. Then, COX and SOD enzyme levels in bladder strips incubated with the relevant agents were determined by ELISA method.

Results: EFS-induced contractions were statistically significantly inhibited by PBE. Atropine, Nω-nitro-L-arginine, Tetraethylammonium similarly caused a significant increase in EFS-induced contraction responses compared to control. PBE reversed these effects of the respective agents in a statistically significant manner. In contrast, verapamil significantly decreased the contractile responses of the respective tissues compared to the control, whereas PBE potentiated this inhibitory effect of verapamil. PBE significantly increased COX and SOD enzyme levels compared to the control group.

Conclusion: The experimental findings suggest that PBE has an inhibitory effect on contractile responses and that cholinergic and nitrergic pathways, potassium channel activation and calcium channel inhibition play a role in this inhibitory effect. It also suggests that PBE has antioxidant but not anti-inflammatory effects at the administered concentrations.

Graphical abstract



Keywords

antioxidant, bladder, EFS, purple basil extract, relaxation

Introduction

The use of plants as healing agents predates human history and is the origin of most forms of treatment in modern medicine. Many traditional medicines are plant-based; for example aspirin (willow bark), digoxin (foxglove), quinine (Cinchona bark) and morphine (poppy) (Vickers and Zollman 1999). Furthermore, although the use of synthetic drugs in treatment is widespread, there is an increasing interest in herbal treatment and the underlying mechanisms of their pharmacological effects.

Medicinal plants used for treatment have many therapeutic effects. For example; basil has anti-inflammatory, antioxidant, antimicrobial, immunomodulatory and anti-inflammatory effects due to its important pharmacophore groups such as flavonoids, anthocyanins. For this reason, it is recognized as an important medicinal plant in herbal therapy (Güez et al. 2017).

Basil (*Ocimum basilicum* L.), is an important essential oil crop, medicinal plant and culinary herb, belongs to the Lamiaceae family (Saha et al. 2016; Aldarkazali et al. 2019). Basil species have been used for many years in the food industry and also for therapeutic purposes (Gurav et al. 2022). For example, sweet basil is mostly used in the food sector (Simon et al. 1990), while purple basil is mostly used for medical treatment (Le Claire et al. 2005; Park et al. 2008; Petersen et al. 2009).

Purple basil (*Ocimum basilicum* L. *var. purpurascens*) is a good source for phenylpropanoid metabolites, secondary metabolites, mainly including anthocyanins, caffeic acid, chichoric acid and rosmarinic acid (Gang et al. 2001; Allan et al. 2008; Kiferle et al. 2011; Bertoli et al. 2013; Flanigan and Niemeyer 2014). Since secondary metabolites play a key role against oxidative damage and various environmental stress factors (La Camera et al. 2004), plants rich in secondary metabolites are considered to have various pharmacological effects such as antioxidant, antimicrobial and anti-inflammatory activities (Zheng and Wang 2001; Javanmardi et al. 2002; Shan et al. 2005; Lee and Scagel 2009).

Although some pharmacological effects of purple basil have been investigated, scientific studies investigating its effects on muscle functions are not

sufficient. And also, there is no study investigating the effects of purple basil on bladder smooth muscle functions. So in the present study, we aimed to investigate the effects of purple basil on mouse bladder functions and the underlying molecular mechanisms.

Materials and Methods

Animals

Swiss albino mice (Male, 10-12 weeks, weighing 25-30 gr) were used in all experiments. Mice were kept at room temperature, on a 12-hour light-dark cycle, with ad libitum access to water and food. Mice were bred at Çukurova University Health Sciences Experimental Application and Research Center.

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by Çukurova University Animal Experiments Local Ethics Committee (20.01.2022/Meeting number: 1/Decision number: 4).

Mice were anesthetized by intraperitoneal injection of ketamine (200 mg/kg) and xylazine (10 mg/kg) and sacrificed by cervical dislocation.

Experimental groups

Swiss albino mice were used for studies with the MP35 data acquisition system. Mice were divided into 6 groups, the number of mice in each group was between 5-11 (n=5-11). 10 ml Krebs-Henseleit solution was added to the organ baths. Isolated bladder tissues were then suspended in the organ baths. In each experimental group, 400 µl PBE and 60% ethanol (the final concentration of 60% ethanol and PBE in the bath medium is 40µl/ml for all the experimental groups) was added to the organ bath and incubated for 40 min. The experimental groups were as follows:

Group 1: Extract Control Group (Half of the bladder was used for control work and the other half for Purple Basil work).

Group 2: Negative Control (NC) Group (400 µl of 60% ethanol was applied to one half of the bladder and 400 µl of PBE to the other half and incubated for 40 min).

Group 3: Tetraethylammonium (TEA) Group (One half of the bladder was treated with 400 µl PBE only, the other half with 400 µl PBE+50µM TEA and incubated for 40 min).

Group 4: Verapamil Group (Only 400 µl PBE was applied to one half of the bladder and 400 µl PBE+50 µM verapamil was applied to the other half and incubated for 40 min).

Group 5: Nω-Nitro-L-arginine (L-NOARG) Group (One half of the bladder was treated with 400µl PBE only, the other half with 400 µl PBE+50 µM L-NOARG and incubated for 40 min).

Group 6: Atropine Group (Only 400 µl PBE was applied to one half of the bladder and 400 µl PBE+50 µM atropine was applied to the other half and incubated for 40 min).

Reagents and chemicals

Ketasol 10% (Richter pharmaag); Xylazinbio 2% (Bioveta); Tetraethylammonium chloride (TEA), (±)-Verapamil hydrochloride, Atropine sulfate salt, Nω-Nitro-L-arginine (L-NOARG), RIPA buffer, Phenylmethylsulfonyl fluoride (PMSF), Sodium orthovanadate (Sigma-Aldrich); Protease inhibitor cocktail (Thermo Scientific) were purchased from commercial sources. The dried purple basil plant used in our experiments was obtained from a reliable herbalist where medicinal and aromatic plants are sold and where specialists trained in this field work.

Preparation of purple basil extract

The method of obtaining purple basil (*Ocimum basilicum*) extract (PBE) was prepared with the support of the literature (Bunwijit et al. 2017; Baković et al. 2023; Mansour et al. 2023). To prepare PBE, dried purple basil leaves were crushed into fine powder. Weighed 5g of this powder mixture. 100ml of 60% ethanol was added and mixed well. It was left to macerate in a cool, dry and sun-free place for a total of 3 days, stirring once a day. At the end of 72 hours, the solution was boiled in boiled water in a glass flask over low heat for 20 min. After boiling, 1ml of extract was added to 1.5ml eppendorfs. The eppendorfs were tightly sealed with parafilm and stored at -20ºC until use.

Content analysis of purple basil extract by headspace solid phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS)

Volatile component analysis was determined by Agilent Brand 7890B GC, 7010B MS system. By Solid Phase Microextraction (SPME) method, 3mL of sample was placed in a 20mL vial and kept at 60°C for 15 minutes, then the volatile components were adsorbed for 30 minutes with a 50/30µm Divinylbenzene/Carboxene/Carboxene/Polydimethylsiloxane (DVB/CAR/PDMS, 1cm) coated fiber in a Solid Phase Microextraction (SPME) apparatus. Then DB-Wax (60m x 0.25mm i.dx 0.25µm, Jamp; WScientific-Folsom, USA) was desorbed and injected into the capillary column for 5 min. The injection temperature was 250°C, the column temperature was increased by 3°C per minute to 90°C after 4 min at 40°C, then by 4°C per minute to 130°C, and after 4 min at this temperature, the temperature was increased by 5°C per minute to 240°C and kept at this temperature for 8 min. He was used as the carrier gas. The electron energy is 70 eV and the mass range is 30-600 m/z. The split ratio is 1:10.

Qualitative studies: evaluation of smooth muscle contractions in ısolated mouse bladder strips

Smooth muscle function of the bladder was evaluated using the MP35 data acquisition system. After the mice were euthanized by cervical dislocation under anesthesia, bladder tissues were isolated by abdominal incision. The isolated tissues were then striped and suspended longitidunally under 0.5g tonus in organ baths containing fresh Krebs-Henseleit solution (NaCl 118 mM; KCl 4.7 mM; $MgSO_4$ 1.2 mM; $NaHCO_3$ 25 mM; KH_2PO_4 1.2 mM; $CaCl_2$ 2.5 mM; and $C_6H_{12}O_6.H_2O$ 12.7 mM; pH 7.4) gassed with 95% O_2+5% CO_2. The experimental findings were recorded using a computerized recording system with a computerized isometric force transducer (Force Displacement Transducer, May, FDT 0.5). For EFS application, bladder tissues were placed between two platinum electrodes and connected to the stimulator (Grass S88 Stimulator). Stimulator parameters; Delay: 1 ms, Duration: 1 ms, Voltage: 40 V, Rate: 5, 10, 20 Hz. The function of the stimulator was set to "Repeat". Frequencies (5, 10, 20 Hz) were applied for 1 min at

10 min intervals. Then, after a 40 min incubation period, the first application was repeated (Control group). In the test 9 groups, after washing after the first application, the experiment was repeated as in the control group in the presence of purple basil extract (PBE, 40 µl/ml; control group) or PBE (40 µl/ml)+chemical agent (50 µM atropine, L-NOARG, TEA or verapamil).

Quantitative studies: ELISA assays

Isolated mouse bladder tissues were incubated with 40µl/ml PBE for 40 min and stored at -20ºC until used in Enzyme-Linked ImmunoSorbent Assay (ELISA) experiments. ELISA method was used to determine enzyme levels in mouse tissues using SOD and COX kits. ELISA experiments were performed accordingto kit protocols.

Statistical analysis

To evaluate the experimental results we used GraphPad Prism (5.1) statistical programs for analysis of experimental data [Analysis of Variance (ANOVA; Post hoc: Bonferroni) or Student's t test] was used. $P<0.05$ values were considered statistically significant.

Results

HS-SPME-GC-MS analysis of PBE revealed the ratios and retention times of the constituents. Among the components, the highest percentage belonged to linalool (54.154%), while tau-cadinol (11.667%) and alpha-terpineol (4.708%) were the other high percentage components (Table 1 and Figure 1).

Table 1. HS-SPME-GC-MS analysis of the composition of PBE

No	Compound Names	RT (min)	Score (%)	Area (%)
1	Eucalyptol	19.99745	97.3	3.196
2	Fenchyl acetate	31.56745	94.6	1.168
3	Benzaldehyde	33.09617	89.49	1.969
4	(+)-2-Bornanone	33.46147	94.49	2.243
5	Linalool	34.47755	96.77	54.154
6	Bornyl acetate	36.5496	93.22	1.629
7	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	37.24028	79.82	1.662
8	Bicyclo[4.2.0]octa-1,3,5-trien-7-ol	38.7076	71.41	0.911
9	Cyclohexanemethanol-alpha-alpha-dimethyl-4-methylene	39.88328	89.57	0.588
10	alpha-Terpineol	40.80607	94.88	4.708
11	(+)-Sativen	42.0413	80.24	0.617
12	Cadina-1(10)-4-diene	43.52088	91.12	1.045
13	gamma-Muurolene	43.68972	93.82	0.719
14	2,7-dimethyl-2,6-Octadien-1-ol	45.45876	88.89	0.864
15	Butylated Hydroxytoluene	47.6005	93.61	0.979
16	2-Propenoic acid-3-phenyl-methyl ester	48.49992	86.06	0.624
17	Caryophyllene oxide	50.11679	92.14	0.779
18	Epicubenol	51.72613	82.53	3.490
19	(+)-Spathulenol	53.0921	96.22	1.760
20	Eugenol	53.50035	96.99	3.558
21	tau-Cadinol	54.1419	97.25	11.667
22	Carvacrol	54.3716	92.35	1.119
23	alpha-Methyl-beta-[2-thiosulfatoethyl]aminopropiophenone	60.60739	69.66	0.553

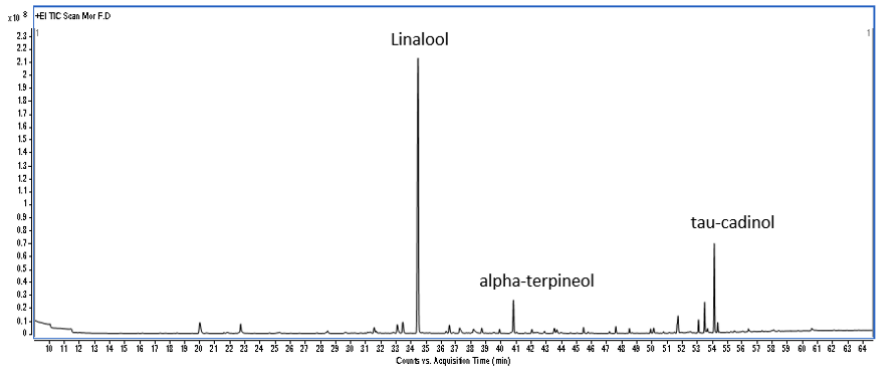


Figure 1. HS-SPME-GC-MS chromatograms showing peaks and retention time.

Discussion

In our study, PBE inhibited EFS-induced contractions. We investigated the molecular mechanisms involved in this inhibition and the anti-inflammatory and antioxidant effects of the related herbal extract in isolated mouse bladder.

Atropine, a nonselective antagonist of cholinergic muscarinic receptors, significantly increased EFS-induced isolated mouse bladder smooth muscle contractile responses at all frequency ranges of EFS. PBE significantly decreased the contractile responses of EFS at all frequency ranges. In the atropine+PBE combination, PBE reversed the potentializing effects of atropine and produced a statistically significant decrease in contractile responses compared to the atropine group (Table 2, Figure 2A-a,b,c,d). These experimental results suggest that cholinergic muscarinic receptors are involved in the inhibitory effect of PBE on isolated mouse bladder smooth muscle contractions.

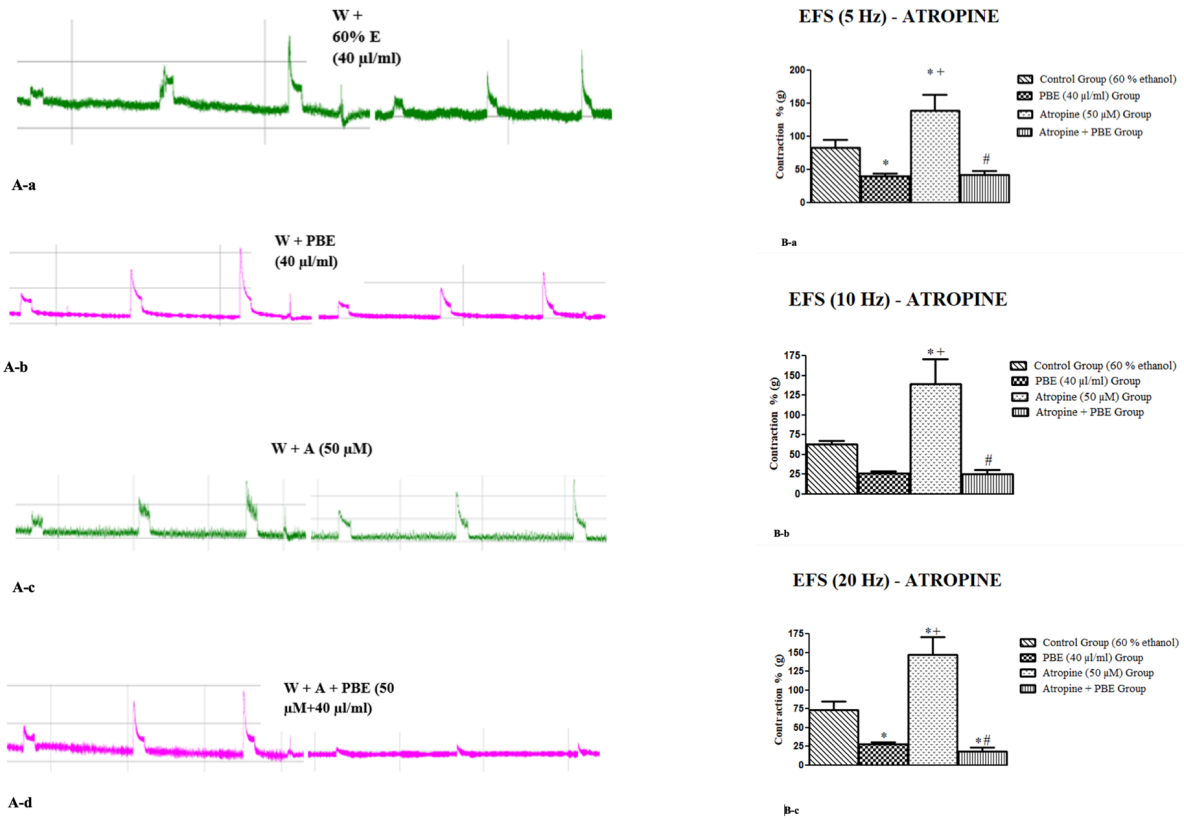


Figure 2A-2B. Effects of 60% ethanol, PBE, atropine and atropine+PBE on contraction responses induced by EFS in isolated mouse bladder strips. **Figure 2A.)** Traces of contraction responses induced by EFS in isolated mouse bladder strips, **Figure 2A-a)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with 60% ethanol incubation, **Figure 2A-b)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with PBE incubation, **Figure 2A-c)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with atropine incubation, **Figure 2A-d)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with atropine+PBE incubation. **Figure 2B.)** Effect of atropine incubation on smooth muscle contractions induced by EFS in isolated mouse bladder (n=5-11), **Figure 2B-a)** Effect of atropine incubation on smooth muscle contractions induced by 5 Hz EFS in isolated mouse bladder, **Figure 2B-b)** Effect of atropine incubation on smooth muscle contractions induced by 10Hz EFS in isolated mouse bladder, **Figure 2B-c)** Effect of atropine incubation on smooth muscle contractions induced by 20Hz EFS in isolated mouse bladder. W:Washing, E:ethanol, PBE:Purple Basil Extract, A:atropine.

In the present study, atropine potentiated the contractile responses induced by EFS in the relevant tissues in the frequency dependent manner. We also observed that PBE reversed the atropine responses and inhibited the EFS-induced contractions in a statistically significant manner. The inhibitory effect of PBE was more pronounced at higher frequencies of EFS (Table 2, Figure 2B-a,b,c). These experimental results suggest that a greater involvement of the cholinergic pathway in EFS-induced contractile responses at higher EFS frequencies. The findings of a study by Tong et al. (1997) support our hypothesis. In the related study, the authors reported that the cholinergic pathway plays a role in EFS-induced contractile responses in isolated rat bladder and that the role of the cholinergic pathway increased at high frequencies (Tong et al. 1997). Similarly, the another study made by Iguchi et al. (2021) also support our hypothesis.

Table 2. Statistical analysis data of experimental findings between groups (*Significance of other groups compared to the control group, +Significance of other groups compared to the group given PBE, #Significance of other groups compared to the group given atropine/L-NOARG/TEA/verapamil)

Groups	Control Group (60% ethanol) *	PBE (40 µl/ml) +	Atropine (50 µM) #	L-NOARG (50 µM) #	TEA (50 µM) #	Verapamil (50 µM) #
PBE (40 µl/ml)	P<0.05; 5 Hz P<0.05; 20 Hz					
Atropine (50 µM)	P<0.05; 5 Hz P<0.001; 10 Hz P<0.05; 20 Hz	P<0.001; 5 Hz P<0.001; 10 Hz P<0.001; 20 Hz				
Atropine+PBE	P<0.05; 20 Hz		P<0.001; 5 Hz P<0.001; 10 Hz P<0.001; 20 Hz			
PBE (40 µl/ml)	P<0.05; 5 Hz P<0.01; 10 Hz P<0.01; 20 Hz					
L-NOARG (50 µM)	P<0.01; 10 Hz	P<0.001; 5 Hz P<0.001; 10 Hz P<0.001; 20 Hz				
L-NOARG+PBE	P<0.05; 5 Hz P<0.01; 10 Hz P<0.01; 20 Hz			P<0.01; 5 Hz P<0.001; 10 Hz P<0.01; 20 Hz		
PBE (40 µl/ml)	P<0.05; 5 Hz P<0.001; 10 Hz P<0.05; 20 Hz					
TEA (50 µM)	P<0.05; 5 Hz P<0.001; 10 Hz	P<0.001; 5 Hz P<0.001; 10 Hz P<0.001; 20 Hz				
TEA+PBE	P<0.05; 5 Hz P<0.001; 10 Hz P<0.05; 20 Hz				P<0.001; 5 Hz P<0.001; 10Hz P<0.001; 20Hz	
PBE (40 µl/ml)	P<0.01; 5 Hz P<0.001; 10 Hz P<0.01; 20 Hz					
Verapamil (50 µM)	P<0.01; 5 Hz P<0.001; 10 Hz P<0.01; 20 Hz					
Verapamil+PBE	P<0.01; 5 Hz P<0.001; 10 Hz P<0.01; 20 Hz	P<0.001; 10 Hz				
PBE (400 µl) COX-2	P=0.0353; 400 µl					
PBE (800 µl) COX-2	P<0.0001; 800 µl					
PBE (400 µl) SOD1	P=0.0144; 400 µl					
PBE (800 µl) SOD1	P=0.0016; 800 µl					

All of the cholinergic muscarinic receptor subtypes (M1, M2, M3, M4 and M5) are present in the bladder. Although M2 receptors are more abundant, M3 receptors are more important in the related tissuefor contractile function (Levin et al. 1988; Andersson and Wein 2004; Canda et al. 2006). M2, M4 receptors inhibit adenylacyclase via Gi (inhibitory G protein) protein (Caulfield and Birdsall 1998). M1, M3 and M5 receptors activate phospholipase C by interacting with Gq/11 proteins. Thus, they increase intracellular calcium level by inducing inositol phosphate cycle and mediate bladder contraction (Canda et al. 2006).

In our study, the reason why atropine potentiated EFS contractile responses in the relevant tissues may have been due to the blockade of inhibitory M2, M4 receptors by atropine. Here, since M2 receptors are muscarinic type receptors that are abundant in the bladder and mediate relaxation responses, potentialization of atropine-induced contraction responses is expected by blocking these receptors with atropine. In our study, the reason for the reversal of atropine responses by PBE in the related tissue, may be its greater affinity for muscarinic receptors and its competition with atropine to activate Gi-mediated muscarinic receptors (M2, M4) or inhibit Gq/11-mediated muscarinic receptors (M1, M3, M5). Furthermore, PBE may have inhibited the effect of atropine through another mechanism. In our literature search, we did not find any other study investigating this effect of PBE. Further studies are needed to elucidate the molecular mechanism(s) underlying the interaction of PBE with atropine in the relevant tissue.

In our study, PBE significantly inhibited isolated mouse bladder smooth muscle contractions induced by EFS. In contrast, L-NOARG (50µM), an inhibitor of NO synthase, potentiated EFS contractions. Moreover, PBE inhibited the effect of L-NOARG in a statistically significant manner (Table 2, Figure 3A-a,b,c,d). These experimental findings suggest that the nitrergic pathway is involved in the inhibitory effect of PBE on the EFS contractions. In a study conducted by Blanco-Rivero et al. (2021), N(gamma)-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, potentiated EFS induced contractile responses in isolated rat superior mesenteric artery rings. The finding that inhibition of the nitrergic system by L-NAME in the related tissue enhances the contractile responses of EFS (Blanco-Rivero et al. 2021). This study supports our experimental data that L-NOARG potentiated the EFS-induced contractions. Here, the reversal of the L-NOARG effect by PBE suggests that PBE increases nitric oxide synthesis by inhibiting the L-NOARG effect (Figure 3B-a,b,c).

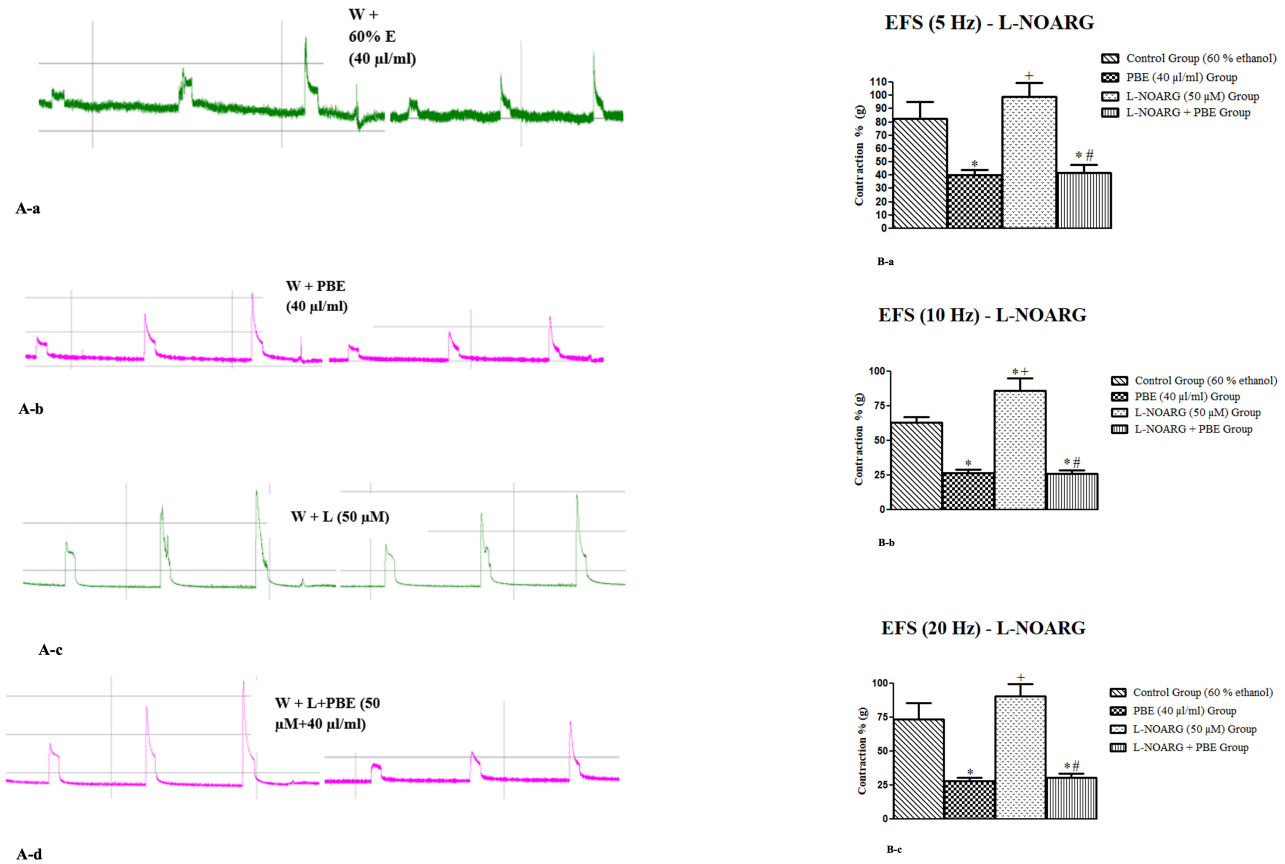


Figure 3A-3B. Effects of 60% ethanol, PBE, L-NOARG and L-NOARG+PBE on contraction responses induced by EFS in isolated mouse bladder strips. **Figure 3A.)** Traces of contraction responses induced by EFS in isolated mouse bladder strips, **Figure 3A-a)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with 60% ethanol incubation, **Figure 3A-b)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with PBE incubation, **Figure 3A-c)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with L-NOARG incubation, **Figure 3A-d)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with L-NOARG+PBE incubation. **Figure 3B.)** Effect of L-NOARG incubation on smooth muscle contractions induced by EFS in isolated mouse bladder (n=5-11), **Figure 3B-a)** Effect of L-NOARG incubation on smooth muscle contractions induced by 5Hz EFS in isolated mouse bladder, **Figure 3B-b)** Effect of L-NOARG incubation on smooth muscle contractions induced by 10Hz EFS in isolated mouse bladder, **Figure 3B-c)** Effect of L-NOARG incubation on smooth muscle contractions induced by 20Hz EFS in isolated mouse bladder. W:Washing, E:ethanol, PBE:Purple Basil Extract, L:L-NOARG.

Tetraethylammonium (TEA), a nonselective blocker of potassium channels (Weatherall et al. 2010; Zhang et al. 2012), was used to investigate the role of potassium channels. TEA significantly increased EFS contractions compared to the control. In the combination of TEA+PBE, PBE inhibited the effect of TEA in a statistically significant manner (Table 2, Figure 4A-a,b,c,d and Figure 4B-a,b,c). These results suggest that potassium channel activation plays a role in the inhibitory effect of PBE on EFS-induced contractile responses in the relevant tissues. Holt et al. (1985) applied electrical stimulation (10 Hz, 2 ms and 8 V) to isolated mouse bladder strips in a study. The researchers reported that isolated bladder voltage responses increased in a TEA concentration dependent manner (Holt et al. 1985). The results of this study support our findings.

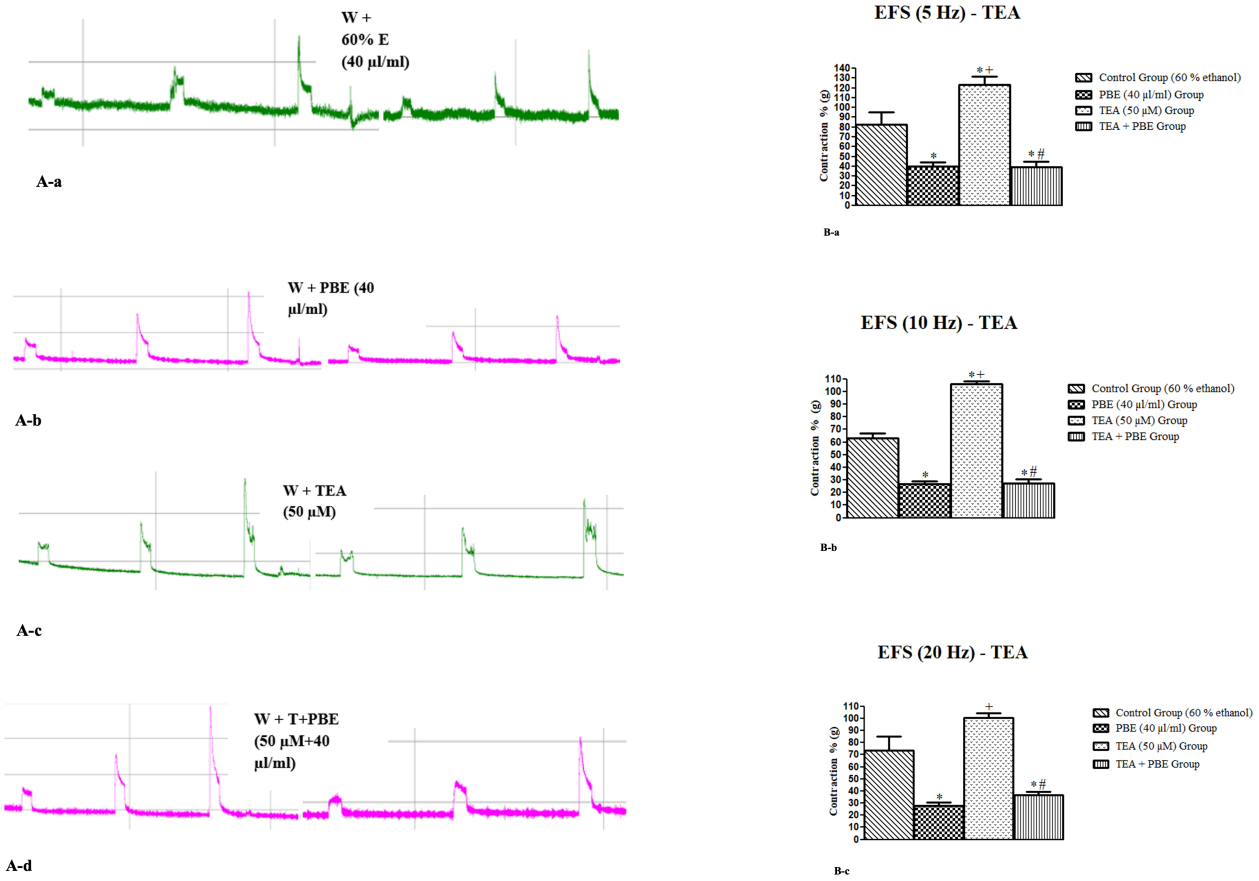


Figure 4A-4B. Effects of 60% ethanol, PBE, TEA and TEA+PBE on contraction responses induced by EFS in isolated mouse bladder strips. **Figure 4A.)** Traces of contraction responses induced by EFS in isolated mouse bladder strips, **Figure 4A-a)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with 60% ethanol incubation, **Figure 4A-b)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with PBE incubation, **Figure 4A-c)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with TEA incubation, **Figure 4A-d)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with TEA+PBE incubation. **Figure 4B.)** Effect of TEA incubation on smooth muscle contractions induced by EFS in isolated mouse bladder (n=5-11), **Figure 4B-a)** Effect of TEA incubation on smooth muscle contractions induced by 5Hz EFS in isolated mouse bladder, **Figure 4B-b)** Effect of TEA incubation on smooth muscle contractions induced by 10Hz EFS in isolated mouse bladder, **Figure 4B-c)** Effect of TEA incubation on smooth muscle contractions induced by 20Hz EFS in isolated mouse bladder. W:Washing, E:ethanol, PBE:Purple Basil Extract, TEA:Tetraethylammonium.

To investigate the role of L-type calcium channels, the L-type calcium channel blocker verapamil (50 µM) was used. Verapamil caused a statistically significant decrease in EFS-induced contractile responses in isolated mouse bladder strips compared to the control. In the combination of verapamil+PBE, PBE was observed to potentiate the inhibitory effect of verapamil (Table 2, Figure 5A-a,b,c,d and Figure 5B-a,b,c). This experimental result suggests a role for calcium channel in the inhibitory effect of PBE on EFS-induced contractile responses in the relevant tissues. In a study by Han et al. (2018) support our hypothesis. In that study the authors examined the contractile responses by applying EFS to bladder strips after inducing a diabetes model in rats. Verapamil incubation reduced the contractile responses to EFS application in both normal and diabetic rat bladder smooth muscle (Han et al. 2018).

In our experimental studies to investigate the anti-inflammatory effects of PBE, we observed that PBE increased COX enzyme levels at the concentrations used (40 and 80 µl/ml) compared to the control group and this increment was statistically significant (Table 2 and Figure 6A-a,b).

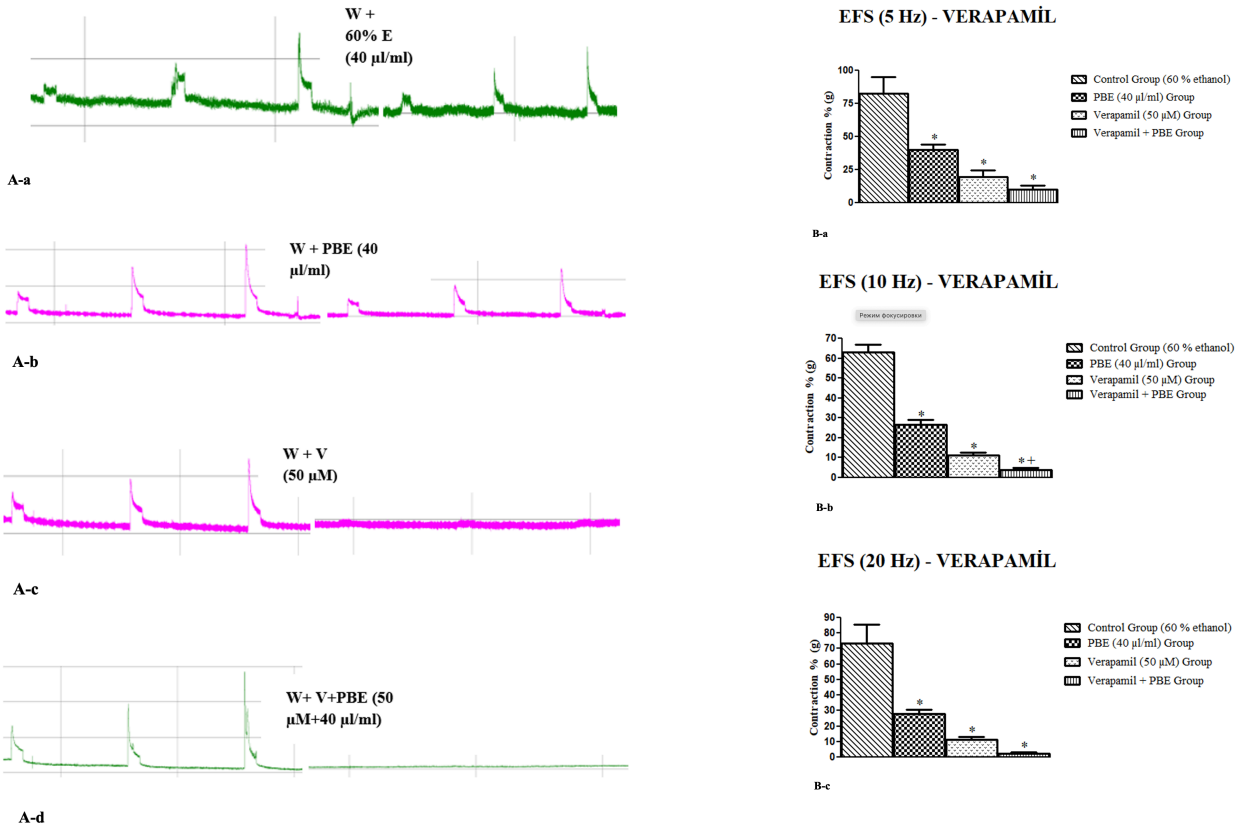


Figure 5A-5B. Effects of 60% ethanol, PBE, verapamil and verapamil+PBE on contraction responses induced by EFS in isolated mouse bladder strips. **Figure 5A.)** Traces of contraction responses induced by EFS in isolated mouse bladder strips, **Figure 5A-a)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with 60% ethanol incubation, **Figure 5A-b)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with PBE incubation, **Figure 5A-c)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with verapamil incubation, **Figure 5A-d)** Contractile responses of isolated mouse bladder strips to EFS at frequencies of 5-10-20Hz with verapamil+PBE incubation. **Figure 5B.)** Effect of verapamil incubation on smooth muscle contractions induced by EFS in isolated mouse bladder (n=5-11), **Figure 5B-a)** Effect of verapamil incubation on smooth muscle contractions induced by 5Hz EFS in isolated mouse bladder, **Figure 5B-b)** Effect of verapamil incubation on smooth muscle contractions induced by 10Hz EFS in isolated mouse bladder, **Figure 5B-c)** Effect of verapamil incubation on smooth muscle contractions induced by 20Hz EFS in isolated mouse bladder. W:Washing, E:ethanol, PBE:Purple Basil Extract, V:verapamil.

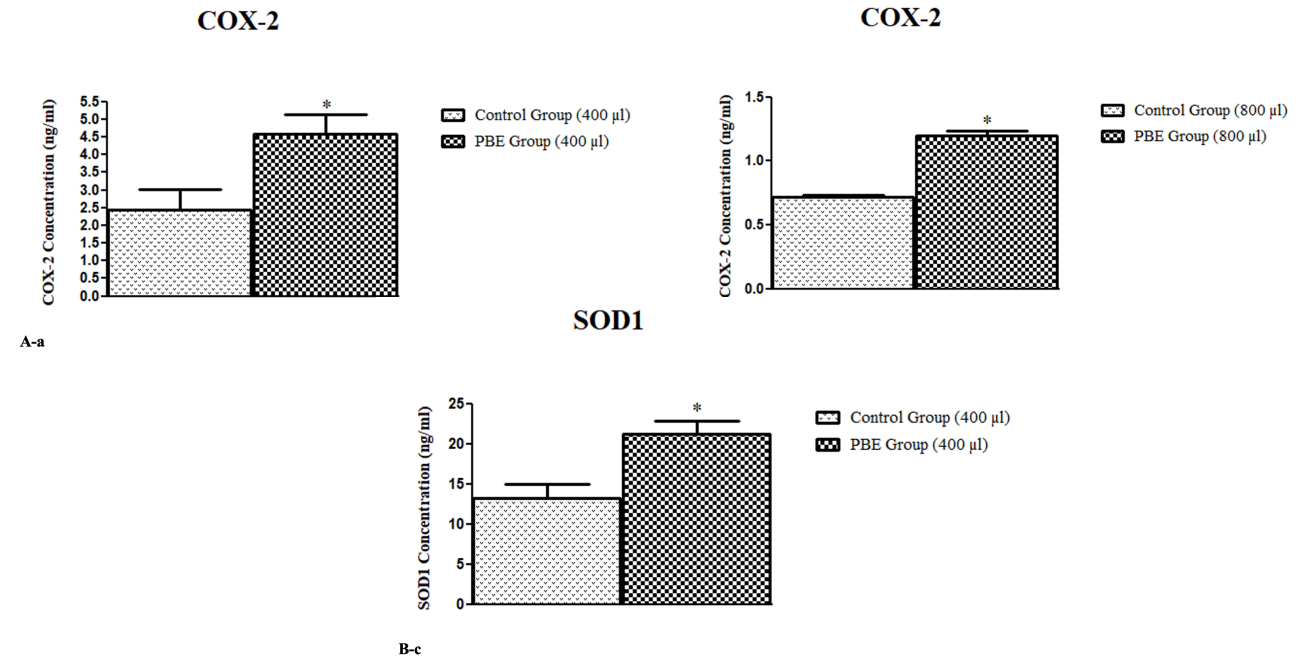


Figure 6A-6B. Effect of PBE on COX-2 enzyme and SOD1 enzyme levels in isolated mouse bladder tissues (n=4-5). **Figure 6A.)** Effect of PBE on COX-2 enzyme levels in isolated mouse bladder tissues, **Figure 6A-a)** Effect of 400 µl PBE on COX-2 enzyme levels in isolated mouse bladder tissues, **Figure 6A-b)** Effect of 800 µl PBE on COX-2 enzyme levels in isolated mouse bladder tissues. **Figure 6B.)** Effect of PBE on SOD1 enzyme levels in isolated mouse bladder tissues, **Figure 6B-a)** Effect of 400 µl PBE on SOD1 enzyme levels in isolated mouse bladder tissues, **Figure 6B-b)** Effect of 800 µl PBE on SOD1 enzyme levels in isolated mouse bladder tissues

As known, COX enzymes are important regulators of angiogenesis, inflammation and carcinogenesis (Clària 2003). There are three isoforms of cyclooxygenase; COX-1, COX-2 and COX-3 (Pang et al. 2016). COX-1 is constitutively expressed in most tissues (Soh and Weinstein 2003; Pang et al. 2016). COX-3 is a variant of COX-1 (Kis et al. 2003; Sarkar et al. 2007). COX-2 is found in normal tissues such as kidney, reproductive organs and stomach (Su et al. 2016; Obermoser et al. 2018) and is an inducible isoform (Gurram et al. 2018). In carcinogenesis-adenocarcinoma, cholangiocarcinoma, hepatocellular carcinoma, etc. (Dannenberg et al. 2005; Mortezaee 2018)-increased levels of COX-2 have been reported (Gurram et al. 2018; Raj et al. 2019). There are other studies examining the effect of PBE on COX. In their study, Szymanowska et al. (2015) examined the antioxidant and anti-inflammatory activities of purple basil leaves. The researchers reported that jasmonic acid, one of the anthocyanins contained in purple basil, inhibited COX enzyme (Szymanowska et al. 2015). Güez et al. (2017) investigated the oxidative, antigenotoxic and anti-inflammatory effects of basil extract in human leukocyte cell culture. The researchers reported that basil extract inhibited COX-2 enzyme. However, in our experimental findings, we observed that PBE significantly increased COX enzyme levels at the concentrations used. The underlying mechanisms of PBE increasing COX in our study need to be investigated.

In our experimental studies to investigate the antioxidant effects of PBE, we observed that PBE caused a significant increase in SOD enzyme levels at the concentrations used (40 and 80 µl/ml) and this increment was statistically significant (Table 2 and Figure 6B-a,b). SOD enzymes are metalloenzymes with antioxidant effect in the body and constitute the front line in defence against reactive oxygen species-mediated damage in the body (Kangralkar et al. 2010). SOD enzyme catalyses the reduction of superoxide anion radical to hydrogen peroxide (H_2O_2) (Yasui and Baba 2006). There are 3 different isoforms of SOD enzyme. These are Cu-Zn-SOD (SOD1), Mn-SOD (SOD2) and Cu-SOD (SOD3) (Sanyal et al. 2009). SOD1 is found in the cytoplasm, nuclear compartments and lysosomes. SOD2 is found in the mitochondria of aerobic cells (Takada et al. 2002; Zelko et al. 2002). SOD3 is found in plasma, lymph and synovial fluid. It has been shown that vascular smooth muscle cells synthesise SOD3 to a great extent (Fattman et al. 2003). In a study conducted by Gulcin et al. (2007), the antioxidant effects of basil were investigated. The study revealed that water and ethanol extracts of basil had concentration-dependent antioxidant activities (Gulcin et al. 2007). The results of this study support our results.

Conclusions

In conclusion, the experimental findings of our study suggest that PBE has an inhibitory effect on EFS-induced contractile responses in isolated mouse bladder and that cholinergic, nitrergic pathways, potassium channel activation and calcium channel inhibition may play a role in this inhibitory effect. In addition, in the concentrations we used in our study, PBE was found to have antioxidant effect but not antiinflammatory effect. In fact, since PBE increased COX-2 expression in a concentration-dependent manner at the concentrations we used in this study, further studies are needed to elucidate the molecular mechanism of this phenomenon.

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

The authors have no funding to report.

Acknowledgments

We gratefully acknowledge financial support from Çukurova University Scientific Research Projects Unit (TYL-2022-14595, TSA-2023-16116).

Data availability

All of the data that support the findings of this study are available in the main text.

References

- Aldarkazali M, Rihan HZ, Carne D, Fuller MP (2019) The growth and development of sweet basil (*Ocimum basilicum*) and bush basil (*Ocimum minimum*) grown under three light regimes in a controlled environment. Agronomy 9(11): 743. https://doi.org/10.3390/agronomy9110743
- Allan AC, Hellens RP, Laing WA (2008) MYB transcription factors that colour our fruit. Trends in plant science 13(3): 99–102. https://doi.org/10.1016/j.tplants.2007.11.012 [PubMed]
- Andersson KE & Wein AJ (2004) Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. Pharmacological Reviews 56(4): 581–631. https://doi.org/10.1124/pr.56.4.4 [PubMed]
- Baković M, Perković L, Matijević G, Martić A, Vujović T, Ekić S, Fumić M, Jurić S, Čož-Rakovac R, Roje M, Jokić S & Jerković I (2023) Bioprospecting of five *Ocimum* sp. Cultivars from Croatia: New potential for dietary and dermatological application with embryotoxicity tests. Pharmaceuticals 16(7): 981. https://doi.org/10.3390/ph16070981 [PubMed] [PMC]
- Bertoli A, Lucchesini M, Mensuali-Sodi A, Leonardi M, Doveri S, Magnabosco A, Pistelli L (2013) Aroma characterisation and UV elicitation of purple basil from diferent plant tissue cultures. Food Chemistry 141(2): 776–787. https://doi.org/10.1016/j.foodchem.2013.02.081 [PubMed]
- Blanco-Rivero J, Couto GK, Paula SM, Fontes MT, Rossoni LV (2021) Enhanced sympathetic neurotransduction in the superior mesenteric artery in a rat model of heart failure: role of noradrenaline and ATP. American Journal of Physiology Heart and Circulatory Physiology 320(2): H563–H574. https://doi.org/10.1152/ajpheart.00444.2020 [PubMed]
- Bunwijit J, Sripanidkulchai B, Pannangrong W, Junlatat J, Sripanidkulchai K (2017) Gastroprotective effect of hydroalcoholic extract of *Ocimum africanum* leaves. Songklanakarin Journal of Science and Technology 39(4): 539–547.
- Canda AE, Cross CR, Chapple CR (2006) Pharmacology of the lower urinary tract and management of overactive bladder. Journal of the Turkish-German Gynecological Association 7(2): 146–157.
- Caulfield MP and Birdsall NJM (1998) International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacological Reviews 50(2): 279–290. [PubMed]
- Clària J (2003) Cyclooxygenase-2 biology. Current pharmaceutical design 9(27): 2177–2190. https://doi.org/10.2174/1381612033454054
- Dannenberg AJ, Lippman SM, Mann JR, Subbaramaiah K & DuBois RN (2005) Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention. Journal of Clinical Oncology 23(2): 254–266. https://doi.org/10.1200/JCO.2005.09.112 [PubMed]

- Fattman CL, Schaefer LM & Oury TD (2003) Extracellular superoxide dismutase in biology and medicine. Free Radical Biology & Medicine 35(3): 236–256. https://doi.org/10.1016/s0891-5849(03)00275-2 [PubMed]
- Flanigan PM and Niemeyer ED (2014) Effect of cultivar on phenolic levels, anthocyanin composition and antioxidant properties in purple basil (*Ocimum basilicum* L.). Food Chemistry 164: 518-526. https://doi.org/10.1016/j.foodchem.2014.05.061 [PubMed]
- Gang DR, Wang J, Dudareva N, Nam KH, Simon JE, Lewinsohn E & Pichersky E (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. Plant Physiology 125(2): 539–555. https://doi.org/10.1104/pp.125.2.539 [PubMed] [PMC]
- Gulcin I, Elmastas M & Aboul-Enein HY (2007) Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family *Lamiaceae*) assayed by different methodologies. Phytotherapy Research 21(4): 354–361. https://doi.org/10.1002/ptr.2069 [PubMed]
- Gurav TP, Dholakia BB & Giri AP (2022) A glance at the chemodiversity of *Ocimum* species: Trends, implications, and strategies for the quality and yield improvement of essential oil. Phytochemistry Reviews 21(3): 879–913. https://doi.org/10.1007/s11101-021-09767-z [PubMed] [PMC]
- Gurram B, Zhang S, Li M, Li H, Xie Y, Cui H, Du J, Fan J, Wang J, Peng X (2018) Celecoxib conjugated fluorescent probe for ıdentification and discrimination of cyclooxygenase-2 enzyme in cancer cells. Analytical Chemistry 90(8): 5187–5193. https://doi.org/10.1021/acs.analchem.7b05337 [PubMed]
- Güez CM, de Souza RO, Fischer P, de Moura Leão MF, Duarte JA, Boligon AA, Athayde ML, Zuravski L, de Oliveira LFS, Machado MM (2017) Evaluation of basil extract (*Ocimum basilicum* L.) on oxidative, anti- genotoxic and anti-inflammatory effects in human leukocytes cell cultures exposed to challenging agents. Brazilian Journal of Pharmaceutical Sciences 53(1): e15098. https://doi.org/10.1590/s2175-97902017000115098
- Han JS, Min YS, Kim GH, Chae SH, Nam Y, Lee J, Lee SY, Sohn UD (2018) The change of signaling pathway on the electrical stimulated contraction in streptozotocin-induced bladder dysfunction of rats. The Korean Journal of Physiology & Pharmacology 22(5): 577–584. https://doi.org/10.4196/kjpp.2018.22.5.577 [PubMed] [PMC]
- Holt SE, Cooper M, Wyllie JH (1985) Evidence for purinergic transmission in mouse bladder and for modulation of responses to electrical stimulation by 5-hydroxytryptamine. European Journal of Pharmacology 116(1-2): 105–111. https://doi.org/10.1016/0014-2999(85)90190-6 [PubMed]
- Iguchi N, Carrasco A, Jr Xie AX, Pineda RH, Malykhina AP & Wilcox DT (2021) Functional constipation induces bladder overactivity associated with upregulations of Htr2 and Trpv2 pathways. Scientific Reports 11(1): 1149. https://doi.org/10.1038/s41598-020-80794-0 [PubMed] [PMC]
- Javanmardi J, Khalighi A, Kashi A, Bais HP, Vivanco JM (2002) Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. Journal of Agricultural and Food Chemistry 50(21): 5878–5883. https://doi.org/10.1021/jf020487q [PubMed]
- Kangralkar VA, Patil SD, Bandivadekar RM (2010) Oxidative stress and diabetes: A review. International Journal of Applied Pharmaceutics 1: 38–45.
- Kiferle C, Lucchesini M, Mensuali-Sodi A, Maggini R, Rafaelli A, Pardossi A (2011) Rosmarinic acid content in basil plants grown *in vitro* and in hydroponics. Central European Journal of Biology 6(6): 946–957. https://doi.org/10.2478/s11535-011-0057-1
- Kis B, Snipes JA, Isse T, Nagy K, Busija DW (2003) Putative cyclooxygenase-3 expression in rat brain cells. Journal of Cerebral Blood Flow and Metabolism 23(11): 1287–1292. https://doi.org/10.1097/01.WCB.0000090681.07515.81 [PubMed]
- La Camera S, Gouzerh G, Dhondt S, Hofmann L, Fritig B, Legrand M, Heitz T (2004) Metabolic reprogramming in plant innate immunity: the contributions of phenylpropanoid and oxylipin pathways. Immunological Reviews 198(1): 267–284. https://doi.org/10.1111/j.0105-2896.2004.0129.x [PubMed]
- Le Claire E, Schwaiger S, Banaigs B, Stuppner H, Gafner F (2005) Distribution of a new rosmarinic acid derivative in *Eryngium alpinum* L. and other *Apiaceae*. Journal of Agricultural and Food Chemistry 53(11): 4367–4372. https://doi.org/10.1021/jf050024v [PubMed]
- Lee J, Scagel CF (2009) Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. Food Chemistry 115(2): 650–656. https://doi.org/10.1016/j.foodchem.2008.12.075
- Levin RM, Ruggieri MR, Wein AJ (1988) Identification of receptor subtypes in the rabbit and human urinary bladder by selective radioligand binding. The Journal of Urology 139(4): 844–848. https://doi.org/10.1016/s0022-5347(17)42659-0 [PubMed]
- Mansour AT, Diab AM, Khalil RH, Eldessouki EA, El-Sabbagh N, Elsamannoudy SI and Younis NA (2023) Physiological and immunological responses of Nile tilapia fed dietary supplementation of sweet basil ethanolic and aqueous extracts. Frontiers in Marine Science 9: 1064455. https://doi.org/10.3389/fmars.2022.1064455
- Mortezaee K (2018) Human hepatocellular carcinoma: Protection by melatonin. Journal of cellular physiology 233(10): 6486–6508. https://doi.org/10.1002/jcp.26586 [PubMed]
- Obermoser V, Baecker D, Schuster C, Braun V, Kircher B, Gust R (2018) Chlorinated cobalt alkyne complexes derived from acetylsalicylic acid as new specific antitumor agents. Dalton Trans 47(12): 4341–4351. https://doi.org/10.1039/C7DT04790H [PubMed]
- Pang LY, Hurst EA, Argyle DJ (2016) Cyclooxygenase-2: A role in cancer stem cell survival and repopulation of cancer cells during therapy. Stem Cells International 2016: 2048731. https://doi.org/10.1155/2016/2048731 [PubMed] [PMC]
- Park SU, Uddin R, Xu H, Kim YK, Lee SY (2008) Biotechnological applications for rosmarinic acid production in plant. African Journal of Biotechnology 7(25): 4959–4965.
- Petersen M, Abdullah Y, Benner J, Eberle D, Gehlen K, Hücherig S, Janiak V, Kim KH, Sander M, Weitzel C, Wolters S (2009) Evolution of rosmarinic acid biosynthesis. Phytochemistry 70(15-16): 1663–1679. https://doi.org/10.1016/j.phytochem.2009.05.010 [PubMed]
- Raj V, Bhadauria AS, Singh AK, Kumar U, Rai A, Keshari AK, Kumar P, Kumar D, Maity B, Nath S, Prakash A, Ansari KM, Jat JL & Saha S (2019) Novel 1,3,4-thiadiazoles inhibit colorectal cancer via blockade of IL-6/COX-2 mediated JAK2/STAT3 signals as evidenced through data-based mathematical modeling. Cytokine 118: 144–159. https://doi.org/10.1016/j.cyto.2018.03.026 [PubMed]
- Saha S, Monroe A, Day MR (2016) Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum* L.) under soilless agricultural systems. Annals of Agricultural Sciences 61(2): 181–186. https://doi.org/10.1016/j.aoas.2016.10.001 [PubMed] [PMC]
- Sanyal J, Bandyopadhyay SK, Banerjee TK, Mukherjee SC, Chakraborty DP, Ray BC & Rao VR (2009) Plasma levels of lipid peroxides in patients with Parkinson's disease. European Review for Medical and Pharmacological Sciences 13(2): 129–132. [PubMed]
- Sarkar FH, Adsule S, Li Y, Padhye S (2007) Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy. Mini Reviews in Medicinal Chemistry 7(6): 599–608. https://doi.org/10.2174/138955707780859431 [PubMed]
- Shan B, Cai YZ, Sun M, Corke H (2005) Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of Agricultural and Food Chemistry 53(20): 7749–7759. https://doi.org/10.1021/jf051513y [PubMed]
- Simon JE, Quinn J, Murray RG (1990) Basil: A source of essential oils. In: J. Janick and J. E. Simon, Eds., Advances in New Crops, Timber, Portland, pp 484-989.
- Soh JW, Weinstein IB (2003) Role of COX-independent targets of NSAIDs and related compounds in cancer prevention and treatment. Progress in Experimental Tumor Research 37: 261–285. https://doi.org/10.1159/000071377 [PubMed]
- Su CW, Zhang Y, Zhu YT (2016) Stromal COX-2 signaling are correlated with colorectal cancer: A review. Critical Reviews in Oncology/Hematology 107: 33–38. https://doi.org/10.1016/j.critrevonc.2016.08.010 [PubMed]
- Szymanowska U, Złotek U, Karaś M, Baraniak B (2015) Anti-inflammatory and antioxidative activity of anthocyanins from purple basil leaves induced by selected abiotic elicitors. Food Chemistry 172: 71–77. https://doi.org/10.1016/j.foodchem.2014.09.043 [PubMed]
- Takada Y, Hachiya M, Park SH, Osawa Y, Ozawa T, Akashi M (2002) Role of reactive oxygen species in cells overexpressing manganese superoxide dismutase: Mechanism for induction of radioresistance. Molecular Cancer Research 1(2): 137–146. [PubMed]
- Tong YC, Hung YC, Shinozuka K, Kunitomo M, Cheng JT (1997)

Evidence of adenosine 5'-triphosphate release from nerve and P2x-purinoceptor mediated contraction during electrical stimulation of rat urinary bladder smooth muscle. The Journal of Urology 158(5): 1973–1977. https://doi.org/10.1016/s0022-5347(01)64196-x [PubMed]

- Vickers A, Zollman C (1999) ABC of complementary medicine Herbal medicine. The BMJ 319(7216): 1050–1053. https://doi.org/10.1136%2Fbmj.319.7216.1050 [PubMed] [PMC]
- Weatherall KL, Goodchild SJ, Jane DE, Marrion NV (2010) Small conductance calcium-activated potassium channels: from structure to function. Progress in Neurobiology 91(3): 242–255. https://doi.org/10.1016/j.pneurobio.2010.03.002 [PubMed]
- Yasui K, Baba A (2006) Therapeutic potential of superoxide dismutase (SOD) for resolution of inflammation. Inflammation Research 55(9): 359–363. https://doi.org/10.1007/s00011-006-5195-y [PubMed]
- Zelko IN, Mariani TJ, Folz RJ (2002) Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radical Biology & Medicine 33(3): 337–349. https://doi.org/10.1016/s0891-5849(02)00905-x [PubMed]
- Zhang J, Halm ST, Halm DR (2012) Role of the BK channel (KCa1.1) during activation of electrogenic K+ secretion in guinea pig distal colon. American Journal of Physiology Gastrointestinal and Liver Physiology 303(12): G1322–G1334. https://doi.org/10.1152/ajpgi.00325.2012 [PubMed] [PMC]
- Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. Journal of Agricultural and Food Chemistry 49(11): 5165–5170. https://doi.org/10.1021/jf010697n [PubMed]

Author contributions

- **Naciye Y. Dondas,** Professor Doctor, Department of Medical Pharmacology – Faculty of Medicine, Department of Biotechnology - Institute of Natural and Applied Sciences, Department of Translational Medicine - Institute of Health Sciences, 01330, Adana, Türkiye; e-mail: yakdas25@cu.edu.tr; **ORCID ID:** https://orcid.org/0000-0002-9386-091X. Individual contributions: She played an important role in the design of the experimental procedure, the proper conduct of the experiments and the publication of this manuscript.

- **Beyza N. Arslan,** hD student, Institute of Health Sciences, Department of Medical Pharmacology, Faculty of Medicine, Çukurova University, 01330, Adana, Türkiye; e-mail: beyza94nur@hotmail.com; **ORCID ID:** https://orcid.org/0000-0002-4770-4182. Individual contributions: She was instrumental in the performance of the experimental procedure and the publication of this manuscript.