Original Article

Luteolin-Rich Fraction from *Dracocephalum lindbergii*: Promising Agent for Hypertension Treatment

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Abstract

Background: High blood pressure is one of the most critical issues in maintaining health; it requires prevention and management methods. In traditional medicine, the combination of medicinal plants was usually used to control high blood pressure. One of these plants is *Dracocephalum lindbergii*. Therefore, this article examines the vasodilating effects of the flavonoid fractions of this plant and deals with this extract as a new suggestion for the prevention and control of high blood pressure. **Methods:** This research evaluates the hemodynamic properties of flavonoid-rich fractions extracted from this plant in a rat model under conditions of blood pressure induction. First, the phytochemistry laboratory prepared flavonoid fractions by using a chromatography column. Then, after surgical preparation, the arterial pressure of the rats was controlled until stabilization, and it was possible to record basal systolic pressures (SYS), diastolic pressures (DBP), and mean arterial pressures (MAP). Hypertension was maintained by continuous phenylephrine infusion at 0.1 mg/kg per minute, while the vascular responses were monitored during the infusion. After the animal tests, polyamide and Sephadex columns were used to analyze the most bioactive fractions, which led to the isolation of several flavones identified by regular one- and two-dimensional NMR spectra. **Results:** In this model, administration of nifedipine led to an 8% decrease in SYS and a 9% decrease in DBP. Meanwhile, treatment with flavonoid-rich fractions 3, 4, 5, and 6 reduced SYS from 15% to 42% and DBP blood pressure from 6% to 30%. Among these samples, fraction number 6, followed by fraction number 4, showed more effects. Phytochemical studies of these fractions led to the identification of their major components probably responsible for observer effects, including apigenin (1) and apigenin-7-O-β-D-glucopyranoside (2), isolated from fraction number 4, as well as luteolin-4′-O-β-D-glucopyranoside (3) and luteolin-7-O-β-D-glucopyranoside (4) isolated from fraction 6. However, other minor components in fraction 6 are still possible with blood-pressure-lowering effects. **Conclusions:** Flavonoid fractions, especially fraction number 6, rich in luteolin derivatives, can provide promising results in reducing blood pressure based on traditional medicine and complementary intervention in a model of acute phenylephrine-induced blood pressure. This study highlights the importance and potency of luteolin-rich fractions of *D. lindbergii* to serve as a complementary intervention in essential blood pressure control.

Keywords: *Dracocephalum lindbergii, Diastolic blood pressure, Systolic blood pressure, flavonoids, Public health*

Introduction

The Lamiaceae family is one of the largest plant families, showing significant botanical diversity, particularly in the Mediterranean region. Among its many genera, Dracocephalum comprises over 60 species, including Dracocephalum lindbergii (*D. lindbergii*), prevalent in the temperate zones of Europe and Asia. Hypertension is a major public health issue, recognized as a leading modifiable risk factor for serious disorders such as coronary heart diseases and strokes, which significantly contribute to global morbidity and premature mortality.[1]

Despite the variety and abundance of antihypertensive drugs, many patients suffer from inadequate control and treatment of hypertension. This issue leads to their vulnerability to many cardiovascular complications in the long term. Therefore, in parallel with the development of new drugs, there are other studies to control and reduce high blood pressure along with standard treatments. They include changing and improving lifestyle, dietary changes, consumption of fruits and vegetables, as well as traditional and complementary medicines.[2,3]

Among the traditional treatments for managing blood pressure, *Dracocephalum*

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moldavica has been used in traditional Chinese medicine to treat high blood pressure and coronary artery disease.[4] Recent research has shown the antioxidant and heart-protecting effects of the flavonoid compounds found in this plant. Evidence obtained from another study on ischemia-reperfusion has shown *that D. moldavica extract improves cardiac rhythm and coronary blood flow and lowers* lactate dehydrogenase and creatine kinase enzyme levels. These cases indicate the protective effects of this plant against myocardial ischemia with the mechanism of reducing tissue oxidative stress.[5]

In traditional Tibetan and Uyghur medicine, another species of this plant with the scientific name *Dracocephalum heterophyllum* Benth is used to control and reduce blood pressure. In a recent study, it was found that it increases the renal hypertension model's left ventricular function and overall heart contraction. Phytochemical research on this plant has identified various flavonoids, including luteolin and kaempferol.[2]

More research has been done on another plant of this genus, *Dracocephalum kotschyi*. Various flavonoid compounds and polyphenolic compounds have been reported in this plant. Studies have shown that some flavonoids can reduce vasoconstriction caused by norepinephrine in smooth muscles and increase vasodilation by inhibiting specific enzymes related to protein kinase C activity.[6] Therefore, according to the reported effects of flavonoids, the present study was conducted on the vasodilator effects of flavonoid-rich fractions of D. *lindbergii* to investigate their potential as a complementary treatment against high blood pressure. To the best of our knowledge, it is the first antihypertensive report on this species. In the present study, high blood pressure was done in rats by continuous injection of phenylephrine to induce and simulate high blood pressure conditions. However, while the above model effectively creates a hypertensive state, it primarily reflects the acute (rather than chronic) hypertension seen in humans.

Materials and Methods

Plant material

The aerial part of *Dracocephalum lindbergii* was collected from Khorasan during the flowering season in late May 1400. It was confirmed and identified by Dr. Mohammad Reza Joharchi, a senior expert in plant sciences at Ferdowsi University of Mashhad. A herbarium specimen of this plant, number SAM-4050, is available in the Samsam-Shariat herbarium, Faculty of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences.

Extraction and initial purification process

First, 6 kg of plant powder was extracted using 25 L of 70% ethanol by percolation, set to 20 drops per minute. Concentration was conducted using a rotary evaporator at 40°C. Next, sodium bicarbonate was added to alkalize the extract, enabling the ionization of phenolic compounds and flavonoids and facilitating their transfer to the aqueous phase. Chloroform was utilized to extract and eliminate non-polar, non-phenolic compounds. The aqueous phase containing the ionized compounds was collected for further processing. The aqueous phase was neutralized using 0.2 N hydrochloric acid. The obtained extract was concentrated with a rotary device connected to a vacuum pump and submitted to a polyamide column for fractionation, using chloroform: methanol as the solvent with a gradient increase in the concentration of methanol as follows: Fr. 1: 100:0; Fr. 2: 95:5; Fr. 3: 90:10; Fr. 4: 85:15; Fr. 5: 80:20. For determination of flavonoid-rich fractions, fractions were plated on a TLC and analyzed using flavonoid natural product reagent.

Animals

The experiments were performed in 29 male (200–250 g) Wistar rats. Animals were purchased from the Isfahan Faculty of Pharmacy and kept in the animal care unit at the Isfahan Faculty of Medicine. At least 1 week before the experiment, animals were acclimated in the animal room in standard conditions (light/dark cycle of 12/12 h with the room temperature of 23 ± 2°C) with complete access to water and food in standard rat chows. The ethics committee of the university approved the study procedure by IR.MUI. AEC.1401.011 number. Rats were randomly divided into 11 experimental groups, and each contained three rats: Group 1 (control group) received 0.2 mL of saline as a vehicle; group 2 (nifedipine group) received a bolus dose of nifedipine 1 mg/kg; group 3 (fraction 3 group) received a bolus dose of fraction 3, 50 mg/kg; group 4 (fraction 4 group) received a bolus dose of fraction 4, 50 mg/kg; group 5 (fraction 5 group) received a bolus dose of fraction 5, 50 mg/kg; and group 6 (fraction 6 group) received a bolus dose of fraction 6, 50 mg/kg. A dose-response study was also done for the most bioactive fraction at doses of 10, 25, 50, 75, and 100 mg/kg in five divided groups. All the experimental groups were carefully monitored to evaluate the effects of the studied fractions in the different phases.[3,7]

Surgical procedures and measurements

Each rat was anesthetized with urethane 1.7 g/kg i.p. (Merck, Germany). The animals were subjected to tracheostomized to help with ventilation. Then, the left carotid artery was isolated, and the polyethylene catheter was implanted into the artery to measure systolic (SYS) and diastolic blood pressure (DBP). The arterial pressures were recorded by pressure transducers connected to the PowerLab system (AD Instruments, Australia). The left jugular vein was selected for drug infusion. During the experiment, a microsyringe infusion pump (New Era Pump System Inc. Farmingdale, NY, USA) was used for continuous infusions of phenylephrine.[7,8]

Experimental protocol

Following the surgical intervention, subjects were allowed to recover briefly before initiating the monitoring phase. The arterial pressure was closely monitored for 30 minutes. This monitoring aimed to determine when the arterial pressure reached a steady state, indicating stability. Once a steady state was established, baseline measurements for the following parameters were recorded: SYS, DBP, and mean arterial pressure (MAP). After establishing baseline values, phenylephrine was administered continuously at a rate of 0.1 mg/kg per minute to induce controlled hypertension. Vascular responses were evaluated immediately following the commencement of the phenylephrine infusion. Following the induction of hypertension with phenylephrine, a bolus dose was administered to each subject. The treatments varied as follows: group 1 received a vehicle (in mL/kg), group 2 received nifedipine at a dosage of 1 mg/kg, and groups 3–6 each received one of the prepared fractions 3, 4, 5, and 6, with a dosage of 50 mg/kg each. Physiological parameters were measured at three distinct time intervals after administering the treatments: 5 minutes, 10 minutes, and 15 minutes. These measurements aimed to evaluate and quantify the effects of the vehicle, nifedipine, or the prepared fractions 3–6 on the subjects' hemodynamic status in their respective groups.[9]

Phytochemical analysis post *in vivo* studies

Following the *in vivo* studies, fractions with significant effects in animal testing were prioritized for further purification. Specifically, fractions 3 and 6 were selected for further processing. Fraction 3 was dissolved in a small amount of an appropriate solvent and then purified using a Sephadex column. Methanol was used as the solvent for this column chromatography technique. Fraction 6 underwent purification using a polyamide column, employing water: methanol gradient solvent system in sex step gradient: Fr. 6.1: 70:30; Fr. 6.2: 60:40; Fr. 6.3: 50:50; Fr. 6.4: 40:60; Fr. 6.5: 30:70; Fr. 6.6: 20:80; Fr. 6.7: 10:90; Fr. 6.8: 0:100. This method allows for the gradual separation of compounds based on their polarity. Additionally, fractions 6.1 and 6.2 were subjected to further purification using a Sephadex column with methanol as the solvent to enhance the separation of desired compounds. Fraction 6.6 underwent separation but was ultimately set aside due to the detection of minor impurities during the purification process, which led to its exclusion from further analysis.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). SPSS software (version 20) was applied for data analysis and interpretation. The baseline data (before) and the phenylephrine infusion phase in each group were compared using paired-sample *t*-tests. The vascular responses to vehicle/nifedipine/fractions 3–6 are reported as a percentage (%) of change from the average values after phenylephrine infusion and subjected to ANOVA for repeated measures.

Results

Extraction and initial purification process

The extraction and initial purification process yielded 900 g of extract from 6 kg of plant powder. The resulting extract contained ionized phenolic compounds and flavonoids, successfully transferred to the aqueous phase after alkalization. The resulting solution was transferred to a polyamide column for initial fractionation. Six distinct fractions were obtained from the fractionation process, identified as Fr. 1–Fr. 6. Upon analysis, fractions 3–6 were determined to contain significant flavonoid content and were selected for subsequent *in vivo* studies.

Verification of phenylephrine-induced hypertension

Phenylephrine is an agonist for alpha-1 adrenergic receptors, leading to vasoconstriction in both arteries and veins. This action renders it effective for elevating MAP. The affirmation of phenylephrine-induced hypertension was assessed 3 minutes post-infusion, at which point the arterial pressure had stabilized. Table 1 reveals a significant rise in SYS and DBP across all experimental groups following phenylephrine administration.

Hemodynamics responses

The study results demonstrate a reduction in the percentage change of SYS and DBP in rats treated with nifedipine (8% reduction in SYS and 9% reduction in DBP) and treatments involving fractions 3–6 (~15%–42% reduction in SYS and 6%–30% in DBP) compared to the phenylephrine-only phase in each group, assessed 15 minutes after the administration of nifedipine or the respective fractions. A statistically significant decrease in SYS and DBP was observed in the groups receiving nifedipine or fractions 3–6 compared to the vehicle-treated group. Notably, a 42% reduction in SYS was recorded in the group treated with fraction 6, highlighting a significant difference in this parameter between the nifedipine and fraction six groups, thus indicating that fraction 6 exhibits a more potent effect than other tested groups [Figures 1a and b and 2].

The study also assessed changes in MAP after administering the vehicle, nifedipine, or selected fractions during continuous phenylephrine infusion. The vehicle group showed no significant change in MAP, while nifedipine and all four fractions significantly reduced MAP compared to the vehicle. Additionally, fraction 6 resulted in a notable decrease in MAP compared to the nifedipine group, suggesting its potential effectiveness in lowering blood pressure [Figures 1c and 2].

Table 1: Induction of blood pressure across all experimental groups following phenylephrine administration

A:

Factors	Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)		
Groups	Base	Phenylephrine	*P*	Base	Phenylephrine	*P*
Control	108±9.7	203±6.6	0.002	87±11.7	125±2.9	0.019
Nifedipine	119±3.9	176±9.9	0.012	103±2.9	145±1.3	0.003
Fraction 3	123±3.2	191±4.5	0.014	83±3.6	122±1.7	0.032
Fraction 4	113±4.7	209±6.7	0.001	82±1.8	137±7.4	0.014
Fraction 5	128±0.9	195±8.7	0.005	89±6.5	117±1.5	0.018
Fraction 6	114±1.3	186±8	0.012	84±1.5	129±3.2	0.005

B:

Factors	Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)		
Fraction 6	Base	Phenylephrine	*P*	Base	Phenylephrine	*P*
10 mg	118±1.3	196±1.9	<0.0001	88±4.7	142±1.9	0.003
25 mg	108±2.1	165±3.7	0.003	91±2.2	143±2.2	0.001
50 mg	114±1.3	186±8	0.012	84±1.5	129±3.2	0.005
75 mg	113±3.1	174±2.3	0.002	94±2.7	143±4.2	0.01
100 mg	105±6.4	181±12.7	0.007	90±2.9	143±4.9	0.007

Mean arterial blood pressure (mmHg) measured at baseline and three minutes following the initiation of phenylephrine infusion in both experimental and control groups (vehicle and Nifedipine). A) Fractions 3, 4, 5, and 6 were administered at a dose of 50 mg/kg; B) Fraction 6 was administered at various doses. Data are presented as mean±SEM. Statistical analyses were performed using paired-sample *t*-tests

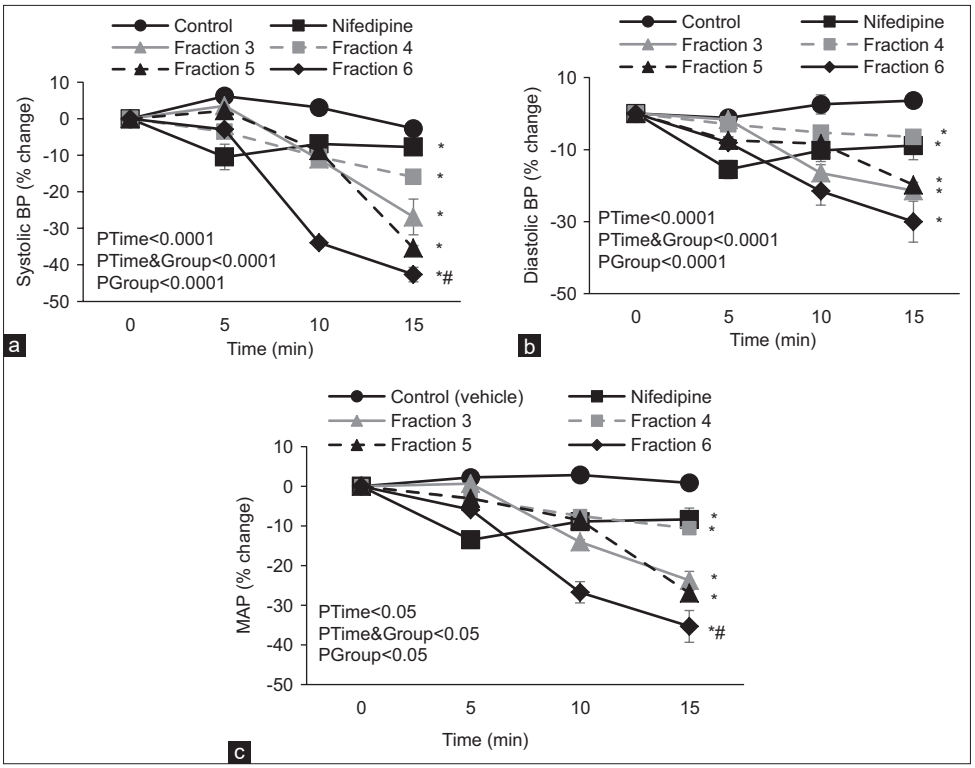

Figure 1: Effect of *Dracocephalum lindbergii* flavonoid-rich fractions in phenylephrine-induced hypertension in rats. (a) The changes in systolic blood pressure (BP); (b) The changes in diastolic blood pressure (DBP); (c) The changes in mean arterial pressure (MAP). Data are presented as mean ± SEM and analyzed using repeated-measures ANOVA. * Significant difference from the control group; # Significant difference from the nifedipine group

Phytochemical analysis post *in vivo* studies

Fractions 3 and 6 were identified as having significant effects in animal testing. Purification of fraction 3 yielded compounds 1 and 2. Fraction 6 was separated into eight distinct fractions (Fr. 6.1–Fr. 6.8). Purification of fractions 6.1 and 6.2 successfully yielded compounds 3 and 4. Fraction 6.6 was excluded from further analysis due to the detection of minor impurities [Figure 3].

Apigenin (compound 1): ^{1}H-NMR in DMSO-d6, δ_H: 12.97 (1H, s, OH), 10.55 (1H, bs, OH), 7.93 (2H, d, J = 8.8 Hz, H-6',2'), 6.93 (1H, d, J = 8.8 Hz, H-5',3'), 6.79 (1H, s, H-3), 6.48 (1H, d, J = 2.0 Hz, H-8) and

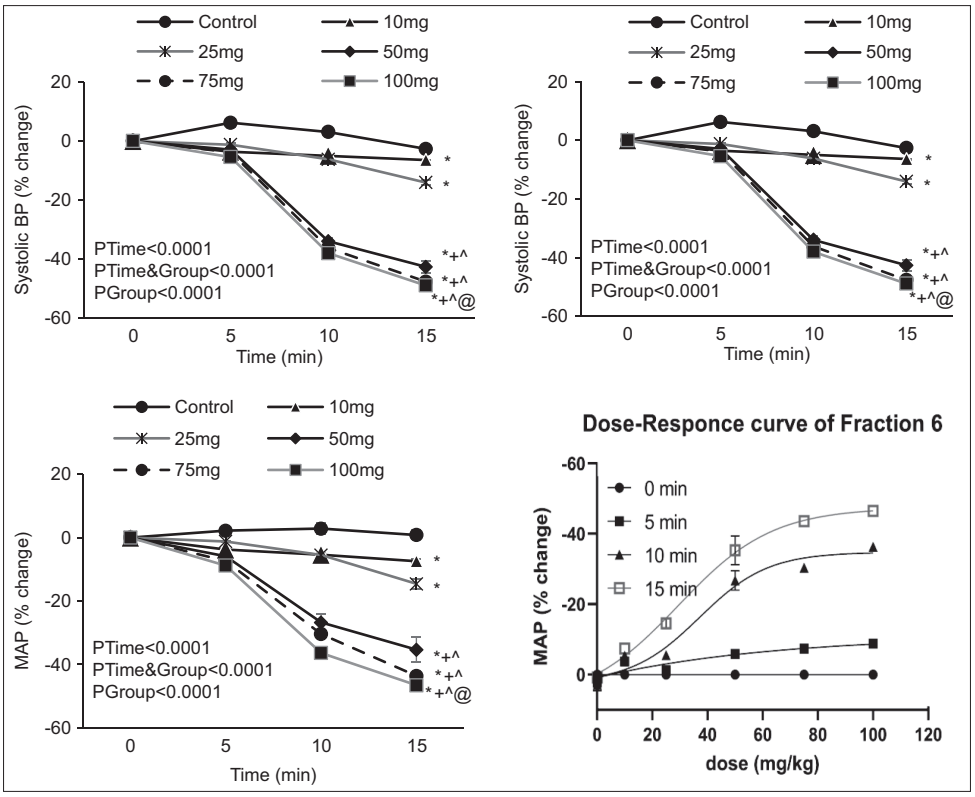

Figure 2: The effect of fraction 6 on systolic blood pressure (BP) and diastolic blood pressure (DBP) at various doses. Data are expressed as mean ± SEM and were analyzed using repeated-measures ANOVA. * Significant difference from the control group; @ Significant difference from the 50-mg group; ^ Significant difference from the 25-mg group; + Significant difference from the 10-mg group

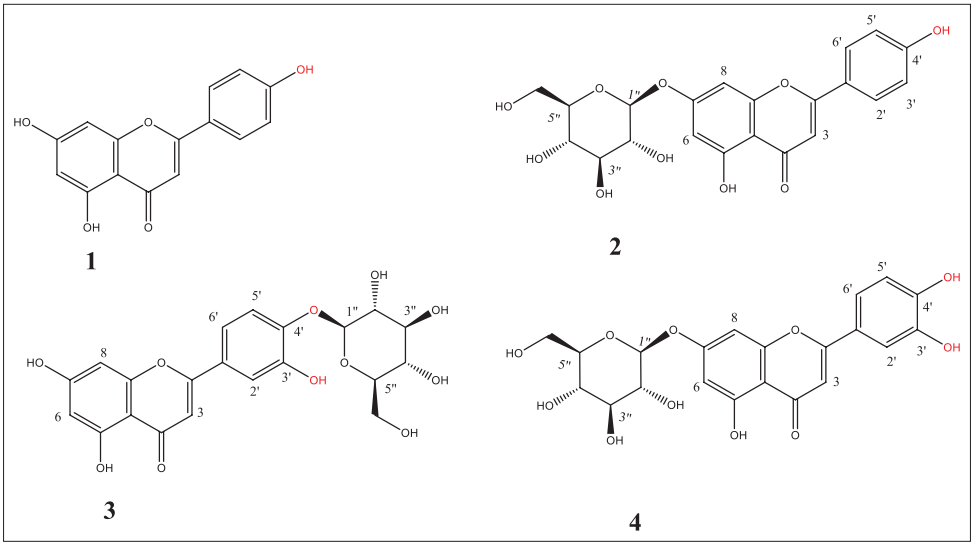

Figure 3: Identification of major phytochemicals from *D. lindbergii* bioactive fractions in a rat model of epinephrin-induced hypertension

6.19 (1H, d, *J* = 2.0 Hz, H-6); δ_C: 183.2 (C4), 166.4 (C2), 163.8 (C7), 163.3 (C5), 163.2 (C4'), 159.0 (C9), 129.4 (C5', C3'), 117.3 (C6', C2'), 122.8 (C1'), 106.5 (C10), 104.4 (C3), 100.5 (C6), 95.3 (C8), Negative ESI mass: 269 *m*/*z*.

Apigenin-7-O-β-D-glucopyranoside (compound 2): ^{1}H-NMR in DMSO-d6, δ_H 12.9 (1H, s, 5-OH), 7.96 (2H, d, J = 8.4 Hz, H-2',6'), 6.91 (2H, d, *J* = 8.4 Hz, H-3',5'), 6.85 (1H, s, H-3), 6.77 (1H, d, *J* = 2.0 Hz, H-8), 6.46 (1H, d, *J* = 2.0 Hz, H-6), 5.12 (1H, d, *J* = 7.1 Hz, H-1''), 4.5 to 3.0 (6H, overlapped, H-1'' to H-6''). δ_C: 182.02 (C4), 164.28 (C2), 162.97 (C5), 161.41 (C7), 161.13 (C9), 156.97 (C4'), 128.65 (C6', C2'), 121.02 (C1'), 116.02 (C5', C3'), 105.35 (C10), 103.12 (C3), 99.89 (C1''), 99.53, (C6), 94.87 (C8), 77.18 (C5''), 76.41 (C3''), 73.10 (C2''), 69.53 (C4''), 60.59 (C6''). Negative ESI mass: 431 *m*/*z*.

Luteolin-4′-O-β-D-glucopyranoside (compound 3): H-NMR in DMSO-d6 (400 MHz) ppm: δ 3.2 to 3.8 (6H, overlapped, H-2'' to H-6''), 5.04 (1H, d, J = 7.4 Hz, H-1''), 6.38 (1H, d, J = 1.9 Hz, H-8), 6.55 (1H, s, H-3), 6.59 (1H, d, J = 8.5 Hz, H-5'), 6.75 (1H, d, J = 1.9 Hz, H-6), 7.26 (1H, d, J = 1.9 Hz, H-2'), 7.37 (1H, dd, J = 8.6, 1.9 Hz, H-6'). C-NMR in DMSO-d6 (100 MHz): C NMR (101 MHz, DMSO) δ: 181.69 (C4), 166.05 (C2), 163.00 (C7), 161.57 (C5), 157.27 (C9), 148.06 (C4', C3'), 121.48 (C6, C1'), 115.40 (C5'), 110.66 (C2'), 105.55 (C10), 100.87 (C3), 100.31 (C1''), 99.67 (C6), 94.96 (C8), 77.55 (C5''), 76.83 (C3''), 73.57 (C2''), 70.03 (C4''), 61.07 (C6'').

Luteolin-7-O-β-D-glucopyranoside (compound 4): H NMR (400 MHz, DMSO-d6) δ 3.22 to 3.8 (6H, overlapped, H-2'' to H-6''), 5.08 (1H, d, J = 7.3 Hz, H-1''), 6.44 (1H, d, J = 2.2 Hz, H-8), 6.73 (1H, s, H-3), 6.78 (1H, d, J = 2.2 Hz, H-6), 6.88 (1H, d, J = 8.3 Hz, H-5'), 7.41 (1H, d, J = 2.3 Hz, H-2', 7.44 (1H, dd, J = 8.3, 2.3 Hz, H-6'). Spectrum of C-NMR in DMSO-d6 (100 MHz): C NMR (101 MHz, DMSO) δ: 182.36 (C4), 165.02 (C2), 163.41 (C7), 161.62 (C5), 157.43 (C9), 150.90 (C4'), 146.39 (C3'), 121.48 (C1'), 119.72 (C6), 116.44 (C5'), 113.0 (C2'), 105.80 (C10), 103.49 (C3), 100.35 (C1''), 99.99 (C6), 95.18 (C8), 77.63 (C5''), 76.87 (C3''), 73.59 (C2''), 70.02 (C4''), 61.08 (C6'').

Discussion

Post *in vivo* phytochemical analysis

In a rat model, this study investigates the hemodynamic effects of flavonoid-rich fractions from *D. lindbergii* in epinephrine-induced hypertension. After *in vivo* studies, bioactive fractions 3 and 6, due to their best responses, were analyzed, and compounds 1–4 were identified as follows:

Compound 1 was isolated as a pale-yellow powder with a positive reaction to the natural product TLC reagent for flavonoids. ^{1}H-NMR spectrum, two doublets coupled in the meta position in the region of 6.49 (d, J = 2.1 Hz, 1H) and 6.20 (d, J = 2.1 Hz, 1H) corresponding to H-8 and H-6, one singlet proton at 6.79 corresponds to H-3 and two pairs of protons in the ortho position in the region 7.93 (d, J = 8.8 Hz, 2H) and 6.93 (d, J = 8.8 Hz, 2H) corresponding to (H- 6',2') and (H- 5', 3') which was characteristic for apigenin.[10]

Compound 2: NMR data suggested a flavone glycoside. Resonances of the carbon spectrum of its sugar part include 99.89 (C1''), 77.18 (C5''), 76.41 (C3''), 73.10 (C2''), 69.53 (C4''), and 60.59 (C6''), which are related to pyranoglucoside. Its aglycon part, like compound 1, contains two doublets paired in the meta position in the region of 6.77 (1H, d, J = 2.0 Hz) and 6.77 (1H, d, J = 2.0 Hz), which correspond to H-8 and H-6. A singlet proton at 85/6 corresponds to H-3, and two pairs of protons in the ortho position in the region of 7.96 (2H, s, J = 8.4) and 6.91 (2H, d, J = 8.4 Hz) correspond to (H- 6',2') and (H- 5', 3'). The ^{1}H-NMR spectrum had absorption in the 12.9 (s, 1H) region corresponding to 5-hydroxy, but there was no absorption related to 7-hydroxy. In the ultraviolet spectrum, band 1 showed absorption at the wavelength of 344 nm and band 2 at the wavelength of 269 nm. After adding ALCl3/HCl, band 1 showed a Schiff shift at 386 nm equal to 44 nm, suggesting that 5-hydroxy is not substituted. NaOAc powder was added, and the UV spectrum was taken after shaking. Sodium acetate ionized only the most acidic phenolic group, that is, 7-hydroxy, which resulted in a 5-nm redshift. In the case of compound 2, no significant change was seen in band II at 268 nm, confirming that C-7 does not have free hydroxy and is substituted. Therefore, compound 3 was identified as apige nin-7-O-β-D-glucopyranoside.[10]

The H-NMR of compound 3 showed six overlapping protons at δc = 3.2–3.8 from H-2″ to H-6″, with an anomeric proton at 5.04 ppm with a coupling constant of 7.4 Hz. Based on the C-NMR data (100.31 (C1″), 99.67 (C6), 94.96 (C8), 77.55 (C5″), 76.83 (C3″), 73.57 (C2″), 70.03 (C4″), 61.07 (C6″) ppm), this compound was also identified as β-glucopyranoside. Two pairs of meta-coupled doublet peaks in the 6.38- and 6.59-ppm regions with a coupling constant of 2.0 Hz correspond to the H-8 and H-6 protons. A single proton at 6.55 ppm corresponds to H-3, and two pairs of ortho-coupled protons at 6.59 and 7.44 ppm, observed as doublets with coupling constants of 8.5 and 1.9 Hz, correspond to H-5′ and H-6′. A single proton at 7.26 ppm with a coupling constant of 1.9 Hz corresponds to H-2′. Based on the C-NMR data, this structure was identified as luteolin aglycone. The HMBC correlation between the anomeric sugar proton and C-4′ at 148.06 ppm confirmed the identification of this compound as luteolin-4′-O-β-D-gl ucopyranoside.[11,12]

The H-NMR of compound 4 showed six overlapping protons at δc = 3.2–3.8 from H-2″ to H-6″, with an anomeric proton at 5.08 ppm with a coupling constant of 7.3 Hz. The C-NMR data (103.48, 77.63, 76.89, 73.58, 70.01, 61.07 ppm) identified it as β-glucopyranoside. The two pairs of meta-coupled doublet peaks in the 6.44 and 6.78 ppm regions with a coupling constant of 2.0 Hz correspond to the H-8 and H-6 protons. A single proton at 6.73 ppm corresponds to H-3, and two pairs of ortho-coupled protons at 6.88 and 7.44 ppm, observed as doublets with coupling constants of 8.3 and 2.3 Hz, correspond to H-5′ and H-6′. A single proton at 7.41 ppm with a coupling constant of 2.0 Hz corresponds to H-2′. Based on the C-NMR data, this structure was identified as luteolin aglycone. The HMBC correlation between the anomeric sugar proton and C-7 at 163.41 ppm confirmed the identification of this compound as luteolin-7-O-β-D-gl ucopyranoside.[13]

Biological analysis

As an overview of the experiments and comparison of treatments in epinephrine-induced hypertension in a rat model, briefly, nifedipine treatment outcomes showed an 8% reduction in SYS and 9% in DBP. Treatments with fractions 3–6 are SYS reductions ranging from 15% to 42% and DBP reductions ranging from 6% to 30%. The effectiveness of fraction 6 was shown with a 42% reduction in SYS and a notable decrease in MAP compared to nifedipine.

For mechanism, based on the literature, the process of vasoconstriction initiated by phenylephrine (PE) begins when it binds to its specific receptors (α1-adrenergic receptors, α1ARs). This interaction activates phospholipase C, producing inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). These molecules subsequently mobilize intracellular Ca(^2+) stores.[14-16]

According to the data of SI *et al*., the relaxing effect of luteolin on PE-induced tension was partially reduced by L-NAME, which is a nitric oxide (NO) synthase inhibitor.[17]

This suggests that luteolin modulates vascular tension, at least in part, through the eNOS/NO-dependent pathway. In addition to enhancing NO production, luteolin may inhibit contractions through an ionotropic mechanism. The phosphorylation of eNOS was enhanced by luteolin, indicating that the vasodilatory effect of luteolin on aortic rings is predominantly mediated by eNOS activation.[17]

Given the significance of NO in preventing and treating essential hypertension, a critical strategy involves augmenting endogenous NO availability, which may help maintain normal vascular tone. In this context, natural phytochemicals, particularly flavonoids, have shown considerable promise, with more than 12 flavonoids having undergone experimental evaluation.[18] A previous study established a hierarchical classification of common flavonoids based on their efficacy in inducing vascular relaxation: flavones (apigenin and luteolin) > flavonols (kaempferol and quercetin) > isoflavones (genistein and daidzein) > flavanes (catechin and epicatechin).

Several intracellular signaling pathways involving PI3K/Akt, PKA, and AMP-activated kinase can modulate eNOS activation.[19] However, the mechanism by which luteolin rapidly activates eNOS in endothelial cells remains unclear. Previous studies have demonstrated that luteolin inhibits cAMP-specific phosphodiesterase in a concentration-dependent manner, with effective doses ranging from 10 to 300 μmol/L.[20] As a result, the accumulation of cAMP is possible. Research has shown that activating the cAMP/PKA pathway enhances eNOS phosphorylation and increases NO production in endothelial cells.[21] Consequently, it has been hypothesized that luteolin may stimulate eNOS through a mechanism involving cAMP/PKA and PKA.

In summary, exploring the effects of natural compounds such as flavonoids on vascular health offers a promising avenue for public health initiatives aimed at managing hypertension. Leveraging the mechanisms that enhance endothelial function can contribute to effective prevention strategies against hypertensive-related cardiovascular diseases, thereby improving overall community health outcomes. Further research into the specific effects of *Dracocephalum lindbergii* and similar phytochemicals can help inform dietary recommendations and therapeutic approaches within hypertension management protocols.

Limitations of the study

We selected unisex because biological differences between the sexes can influence drug action and response to blood pressure treatments. However, for valid research and the application of results to human populations, it is necessary that both sexes (male and female) be included in future studies to correctly generalize the effects of treatment to humans.

Conclusions

This study successfully isolated luteolin and apigenin derivatives from the most potent fractions of *Dracocephalum lindbergii* in a rat model of phenylephrine-induced hypertension, which involved inducing hypertension causing vasoconstriction by binding to α1-adrenergic receptors. These flavonoid fractions, particularly those containing luteolin derivatives, are promising, cost-effective alternatives for preventing and managing high blood pressure. This model mimics conditions of essential hypertension in humans. Therefore, any drug discovered using this model could be suggested for the treatment of this type of hypertension, which is characterized by chronic high blood pressure due to increased arterial resistance. To fully elucidate the anti-contractile effects of luteolin derivatives from *Dracocephalum lindbergii* fractions at different dosages, further animal studies will be essential to understand luteolin derivative therapeutic potential in managing hypertension.

Author contributions

MG, MHV, and MV conceived, designed, extracted, and identified the compounds of plant materials. ZP and MHV did the biological analysis.

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Abbreviations

D. lindbergii: *Dracocephalum lindbergii*; α1-AR: α (1) adrenergic receptor; [Ca2+]i: cytosolic Ca2 + levels; cGMP: Cyclic guanosine monophosphate; EGM2: Endothelial growth supplements; eNOS: endothelial nitric oxide

synthase; L-NAME: N-nitro-L-arginine methyl ester; NO: Nitric oxide; PE: phenylephrine; PKA: Protein kinase A

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Conflicts of interest

There are no conflicts of interest.

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References

1. Heydari P, Yavari M, Adibi P, Asghari G, Ghanadian S-M, Dida GO, *et al*. Medicinal properties and active constituents of Dracocephalum kotschyi and its significance in Iran: A systematic review. Evid Based Complement Alternat Med 2019;2019:9465309.
2. Turner JM, Spatz ES. Nutritional supplements for the treatment of hypertension: A practical guide for clinicians. Curr Cardiol Rep 2016;18:1-9.
3. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, *et al*. 2013 ESH/ESC practice guidelines for the management of arterial hypertension: ESH-ESC the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Blood Pressure 2014;23:3-16.
4. Fattahi M, Nazeri V, Torras-Claveria L, Sefidkon F, Cusido RM, Zamani Z, *et al*. Identification and quantification of leaf surface flavonoids in wild-growing populations of Dracocephalum kotschyi by LC–DAD–ESI-MS. Food Chem 2013;141:139-46.
5. Jiang J, Yuan X, Wang T, Chen H, Zhao H, Yan X, *et al*. Antioxidative and cardioprotective effects of total flavonoids extracted from Dracocephalum moldavica L. against acute ischemia/reperfusion-induced myocardial injury in isolated rat heart. Cardiovasc Toxicol 2014;14:74-82.
6. Jiang H, Zhang C, He W. The effects of dracocephalum heterophyllum benth flavonoid on hypertrophic cardiomyocytes induced by angiotensin II in rats. Med Sci Monit 2018;24:6322-30.
7. Numonov SR, Qureshi MN, Aisa HA. Development of HPLC protocol and simultaneous quantification of four free flavonoids from Dracocephalum heterophyllum Benth. Int J Anal Chem 2015;2015:503139.
8. Pezeshki Z, Nematbakhsh M. Sex differences in the renal vascular responses of AT1 and mas receptors in two-kidney-one-clip hypertension. Int J Hypertens 2021;2021:8820646.
9. Talmor D, Roytblat L, Artru AA, Yuri O, Koyfman L, Katchko L, *et al*. Phenylephrine-induced hypertension does not improve outcome after closed head trauma in rats. Anesth Analg 1998;87:574-8.
10. Ghanadian M, Khamesipour F, Hejazi SH, Razavi SM, Sadraei H, Namdar F. *In vitro* and *in vivo* anti-toxoplasma activities of Dracocephalum kotschyi extract in experimental models of acute toxoplasmosis. Acta Parasitol 2022;67:487-95.
11. Myagchilov A, Kalinin A, Gorovoy P. Flavonoids from the aerial part of picris koreana. Chem Nat Compd 2023;59:839-41.
12. Chang C-C, Ho SL, Lee S-S. Acylated glucosylflavones as α-glucosidase inhibitors from Tinospora crispa leaf. Bioorg Med Chem 2015;23:3388-96.
13. Erdenetsogt U, Nadmid S, Paulus C, Chanagsuren G, Dolgor E, Gotov C, *et al*. Bioactive flavonoids from plant extract of Pyrethrum pulchrum and its acute toxicity. Nat Prod Res 2021;35:5960-3.
14. Wu D, Lee CH, Rhee SG, Simon MI. Activation of phospholipase C by the alpha subunits of the Gq and G11 proteins in transfected Cos-7 cells. J Biol Chem 1992;267:1811-7.
15. Wang L, Rolfe M, Proud CG. Ca2+-independent protein kinase C activity is required for alpha1-adrenergic-receptor-mediated regulation of ribosomal protein S6 kinases in adult cardiomyocytes. Biochem J 2003;373:603-11.
16. Sica DA. Pharmacotherapy review: Calcium channel blockers. J Clin Hypertens 2006;8:53-6.
17. Si H, Wyeth RP, Liu D. The flavonoid luteolin induces nitric oxide production and arterial relaxation. Eur J Nutr 2014;53:269-75.
18. Xu Y, Leung S, Yeung D, Hu L, Chen G, Che C, *et al*. Structure–activity relationships of flavonoids for vascular relaxation in porcine coronary artery. Phytochemistry 2007;68:1179-88.
19. Kitamura T, Kimura K, Makondo K, Furuya D, Suzuki M, Yoshida T, *et al*. Proinsulin C-peptide increases nitric oxide production by enhancing mitogen-activated protein-kinase-dependent transcription of endothelial nitric oxide synthase in aortic endothelial cells of Wistar rats. Diabetologia 2003;46:1698-705.
20. Ko W-C, Shih C-M, Leu I-J, Chen T-T, Chang J-P. Mechanisms of relaxant action of luteolin in isolated guinea pig trachea. Planta Med 2005;71:406-11.
21. Liu D, Homan LL, Dillon JS. Genistein acutely stimulates nitric oxide synthesis in vascular endothelial cells by a cyclic adenosine 5′-monophosphate-dependent mechanism. Endocrinology 2004;145:5532-9.