



Hypotensive effect of *Cichorium intybus* extract in rats

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ABSTRACT

Introduction: Oxidative stress is involved in many diseases, including hypertension, kidney failure, and heart disease. This study aimed to evaluate the effect of hydroalcoholic *Cichorium intybus* extract on blood pressure in rats. Antioxidant activity, phenolic and flavonoid contents of the plant extract were also evaluated.

Methods: In this study, 32 male Wistar rats weighing 250-300 g were divided into four groups of eight each. Animals in the control group were administered with normal saline and in the *C. intybus* groups with extract at 25, 50, and 100 mg/kg for two weeks. Then, the homodynamic parameters were examined by the Power lab. The phenolic and flavonoid contents were also evaluated by a spectrophotometer and the rate of free radical scavenging activity was measured by the diphenyl-1-picryl-hydrazyl (DPPH) free radical method.

Results: The free radical scavenging activity of *C. intybus* extract was obtained 47.85% of DPPH, and flavonoid and phenolic contents were 8.21 and 27.19 mg/g of dry extract, respectively. Meanwhile, median (MAP), systolic (SAP) and diastolic arterial pressure (DAP) significantly decreased in the 50 mg/kg extract-treated group compared to the control and 200 mg/kg extract-treated groups.

Conclusion: Ethanol extract of *C. intybus* plays a protective role against hypertension, which, in part, might be due to antioxidant compounds of the plant against free radicals.

Implication for health policy/practice/research/medical education:

The ethanol extract of *C. intybus* had a protective effect against hypertension. Therefore, it might be a good source for preparation of hypotensive drugs.

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Introduction

Hypertension in most adult cases is a prognosis for some dangerous diseases, such as stroke, coronary heart disease, congestive heart failure, and advanced kidney damage (1). Despite various pharmacotherapies and lifestyle changed, the treatment of hypertension is still being studied and remains challenging, so that in about half of hypertensive patients, blood pressure does not remain at a normal level despite all therapeutic approaches. The reason for the failure of these approaches is the underlying pathophysiology that is resistant to drugs, and also certain limitations such as lack of patient satisfaction with

multidrug therapy throughout a lifetime (2). Free radicals also damage the vascular endothelium, stop nitric oxide (NO) synthesis, and decrease vasodilation. Decreased vasodilation leads to the development of heart disease, hypertension, and kidney disease. Therefore, the role of antioxidants in scavenging free radicals and reducing damage has drawn attention.

Medicinal plants have been used for a long time in various diseases and have shown to possess relatively low toxicity. Chicory is one of them which botanically referred to as *Cichorium intybus* L. Physicians introduced the nature and properties of this plant in their books and

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used it in the treatment of various diseases, including hypertension. *C. intybus* contains medicinal substances, including polysaccharides such as inulin, as well as sesquiterpenes, lactones, coumarins, flavonoids, cichoric acid, and vitamins, which have various medicinal uses (3). Reactive oxygen species (ROS) are reactive oxygen molecules, such as oxygen and peroxide ions, which play an important role in the development of dangerous diseases such as cancer, coronary artery disease, and hypertension. These effects are usually eliminated by antioxidants and radical scavenging systems (4). Due to the presence of antioxidants, *Cichorium intybus* prevents tissue damage induced by oxygen free radicals. Consumption of the plant results in a greater uptake of blood glucose and antimicrobial activity (5). The plant's root reduces the risk of atherosclerosis by reducing the levels of triglycerides and fatty acids in the liver and modulating the levels of the insulin and glucagon hormones (6). Due to phenolic compounds, the plant produces a special antioxidant effect (7). *C. intybus* also has anti-inflammatory effect by inhibiting the synthesis of prostaglandin and the cyclooxygenase 2 enzyme (8). The purpose of this study was to investigate the antioxidant effect and phenolic and flavonoid contents of *C. intybus*, as well as its effect on blood pressure.

Materials and Methods

In this experimental study, Sprague-Dawley rats (250-300 g) were purchased from Lorestan University of Medical Sciences, Lorestan Province, Iran. Thirty-two male Wistar rats were divided into four groups of eight each. Extraction was performed by maceration. Animals in the control group were administered normal saline and in experimental groups *C. intybus* 25, 50, or 100 mg of extracts by gavage for two weeks. The rats were kept under standard conditions (12-hour light/dark cycle and 22±2°C temperature) while they had ad libitum access to food and water.

Preparation of extract

Achillea millefolium was prepared from Hafshejan located in Chaharmahal and Bakhtiyari province and confirmed in the Herbariorum Unit of Shahrekord University of Medical Sciences. Then leaves twigs prepared from the plant were maintained in a suitable environment (dark and dry) and dried completely. After drying and grinding, they were ground to powder. Then, 50 g of the plant powder was soaked in ethanol alcohol for 72 hours and filtered by Büchner funnel. The obtained solution was put in a rotary evaporator to evaporate its solvent in 35°C. Finally, the solution was poured into a watch glass and then put in incubator. The obtained extract powder was maintained in refrigerator until it was used and 1% fresh solution was produced and used in the experiment day (9).

Preparing the animal to measure blood pressure

First, animals were anesthetized by intraperitoneal injection of thiopental sodium (60 mg/kg). The neck area was thoroughly shaved and placed on a surgical bed. A small bulb was placed on the floor of the surgical floor to maintain the temperature of the animal's body at 37°C. A thermometer was placed inside the anus to measure the temperature of the animal's body throughout the duration of the test.

Surgical procedure

After the preparation of the animal, their organs were fixed on the surgical bed. A longitudinal incision was made in the midline of the neck, about one cm from the manubrium symphysis upwards, and after pushing the muscles of the neck, the trachea was separated and intubated. By placing a small tufa (for example, an insulin syringe body) under the neck, the head of the animal was positioned upright, and after pushing the neck muscles, the right carotid artery was identified, and the vague nerve was isolated from it. Then, a thread was passed through under the artery and knotted where the carotid splits. The vessel was then closed by the bulldog clamp near the sternal manubrium, and the second knot was loosely created after the clamp was already placed on it. A small incision was made diagonally 1 cm above the clamp, and the heparinized PE-50 catheter [normal saline (100 IU/mL)] was entered into the midline of the heart through its curvature. Then, the loose knot on the vessel and catheter tightened, and the catheter pushed slightly downwards after removing the clamp so that the carotid artery was cannulated to prepare blood samples and record the blood pressure. The carotid artery was also connected to the power lab to record the arterial pressure fluctuations, and the homodynamic parameters of the left ventricle were recorded as well.

Phenolic and flavonoid contents and antioxidant compounds

The free radical scavenging was measured by the diphenyl-1-picryl-hydrazyl (DPPH) free radical method. To measure the antioxidant activity of the extract, at first, a stock solution of DPPH was prepared by adding 0.01 mg powder to 50 mL of methanol. Then, in the next stage 10 mg of the extract was dissolved in 1 mL of methanol and diluted 10 times more. Half a milliliter (0.5 mL) of the dissolved extract was poured into 4.5 mL of methanol and the desired concentrations were prepared. The colorimetric method was conducted by a spectrophotometer. First, the device was set to zero by ethanol, and then the absorption of the samples was read at a wavelength of 517 nm for 1 hour (10).

Statistical analysis

Data were expressed as mean ± SEM in all groups. The

hemodynamic parameters were analyzed using one-way ANOVA, and then Tukey's post test.

Results

The percentage of free radical scavenging by *C. intybus* extract was obtained by 47.85%. The flavonoid and phenolic contents were 8.21 and 27.19 mg/g of dry extract, respectively.

Table 1 shows the systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), and pulse pressure (PP) on day 14 after gavage. SAP, MAP, and DAP significantly decreased in the 50 mg/kg extract-treated group compared to the control and 200 mg/kg extract-treated groups.

Discussion

Blood pressure is one of the most common causes of other diseases. Some plant compounds are effective in the reduction of blood pressure. *C. intybus* is one of the plants that is traditionally used to decrease blood pressure. In this study, the hydroalcoholic extract of *C. intybus* had a strong anti-hypertensive effect. This effect was reflected by the effect of extract in reducing SAP, DAP, PP, and MAP. The percentage of free radical scavenging by *C. intybus* extract was obtained by 47.85%. The antioxidant activity of the extract was obtained 47.85% of radical scavenging and its flavonoid and phenolic contents 8.21 mg/g and 27.19 mg/g of dry extract, respectively. From over two decades ago, the role of ROS in cardiovascular diseases has been studied, and it has been shown that under normal conditions, there is a balance between the forms of oxygen radicals and the level of antioxidants. However, in some pathophysiological conditions, the balance may be disrupted. In these conditions, the free radical products are reduced in the presence of antioxidants (11). Studies have shown the protective roles of antioxidants in cardiovascular and kidney diseases and hypertension (12). The inverse relationship between the amount of flavonoids and antioxidant capacity, and their inverse relationship with blood pressure has been demonstrated (13). Besides, clinical trials have indicated that flavonoids may reduce mortality in cardiovascular disease patients. The great importance of flavonoids is related to their antioxidant activity and scavenging of free radicals (14).

This study and previous studies have shown the strong antioxidant activity of *C. intybus* extract due to phenolic compounds and flavonoids. Therefore, antioxidants may play an important role in preventing hypertension and heart disease (15).

Various studies have shown that *C. intybus* has potent antibacterial (16), immunity (17), antidiabetic (32), anti-inflammatory (18) and antioxidant (19) activities. Cinnamaldehyde is found in relatively high amount in *C. intybus* (20). Therefore, in addition to antioxidant effect, this compound may be one of the main causes of lowering blood pressure by *C. intybus*. In the present study, the MAP, SAP, DAP and PP in rats were reduced after 14 days of gavage with *C. intybus* extract in the treatment groups, especially the group treated with 50 mg/g, compared to the control group. Other studies on *C. intybus* extract have shown that the extract reduces the extracellular calcium flow, which can cause aortic vasodilation and reduction of blood pressure (21).

In other studies, the vasodilatory effect of flavonoids has been demonstrated by scavenging peroxynitrite. Hydroxyls released from oxidation or through endothelial cells cause oxidation of NADPH oxide and production of O₂ and certain forms of peroxynitrite by the endothelium (22). Therefore, coronary artery and blood flow improvement might be related to this effect of the extract.

The degree of action potential duration is determined by the degree of repolarization, which is related to the active effect on calcium flow and potassium outflow (23). It can be concluded that the effect of the extract on the degree of repolarization and the potassium outflow causes a decrease in blood pressure.

NO is rapidly degraded by the oxygen-derived free radical superoxide anion. Superoxide anion acts as a vasoconstrictor and is a major determinant of NO biosynthesis and bioavailability. Activation of reduction-oxidation (redox)-dependent signaling cascades and NADPH oxidase-driven generation of ROS are involved in the role of angiotensin-II-induced hypertension. Angiotensin II stimulates nonphagocytic NADPH oxidase, causing the accumulation of hydrogen peroxide, superoxide, and peroxynitrite. So, they are involved in high blood pressure (24). Some studies suggest that diets with high antioxidants content may reduce blood pressure

Table 1. Effects of *Cichorium intybus* (CI) extract on median (MAP), systolic (SAP), and diastolic (DAP) arterial pressure and pulse pressure (PP) on day 14 after gavage (n = 8)

Groups	MAP (mm Hg)	SAP (mm Hg)	DAP (mm Hg)	PP (mm Hg)
Control	84.12 ± 6.55	100.71 ± 5.75	80 ± 6.81	26 ± 5.09
CI-50	56.75 ± 4.30**	70.71 ± 4.99**	49.28 ± 4.64**	19.25 ± 1.65
CI-100	64.12 ± 3.17	82.57 ± 2.39	58.57 ± 3.04	17.5 ± 1.64
CI-200	85.75 ± 7.39##	97.14 ± 7.39#	78.14 ± 6.66##	25.88 ± 2.93

** P < 0.01 compared to control group.

0.01 compared to *Cichorium intybus* extract (50 mg/kg)- treated group.

and cardiovascular complications. Some relations between other antioxidants and BP have been reported. Antioxidants at high pharmacologic doses have revealed inconsistent BP findings (25). Although excessive ROS are central pathway in induction and exacerbation of hypertension, however, there are controversies about the efficacy of antioxidant consumption in hypertension therapy. Antioxidants inhibit molecules' oxidation, which in turn, produces free radicals capable of starting chain reactions. When the chain reaction starts in a cell, it could damage the cell or cause its death. By removing free radicals, antioxidants terminate these chain reactions and inhibit other oxidation reactions by being oxidized (26).

In this study, the lowest dose of the examined extract (50 mg/kg) had the most potent hypotensive effect. The reason is not clear. However, it has been shown that in some conditions, especially in high doses, antioxidants may act as pro-oxidants. Antagonistic activity of the extract component might be another reason. Some compounds in low doses act as agonist and in high doses as antagonist (27).

Conclusion

The present study showed that the ethanol extract of *C. intybus* had a protective effect against hypertension. Due to the relationship between the amount of flavonoid and antioxidant capacity, and their inverse relationship with blood pressure, this antihypertensive effect may be partly related to the strong antioxidant activity of *C. intybus* extract due to phenolic compounds and flavonoids. More investigations are needed to find out other components involved in the hypotensive activity of the extract.

Authors' contributions

MS performed the practical work and prepared the first draft, MCh, MF, MD, MRM, and BR helped in writing the manuscript, AN supervised the protocol. All authors were consulted for the project. All read the final proof and confirmed it for publication.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The study protocol was approved by Ethics Committee of Lorestan University of Medical Sciences (Ethical code: LUMS.REC.1394.79).

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