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Evaluation of the Interactive Effects of Glycyrrhiza Glabra Hydroalcoholic Extract and LNAME Drug on Blood Pressure and Heart Rate of Male Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: *Glycyrrhiza glabra* rhizome extracts have been widely used in traditional medicine for the treatment of peptic ulcers and cough; therefore, evaluation of the side-effects of these extracts seems essential. In this study, we aimed to evaluate the effect of *G. glabra* rhizome extract on blood pressure and heart rate of male rats and assess its interaction with the nitrergic system.

METHODS: In this experimental study, 10 male rats intravenously received *G. glabra* extracts and LNAME drug in three different modes: 1) administration of the physiological serum at baseline, 2) concomitant administration of LNAME (5 mg/kg) and the physiological serum in the control mode, and 3) concomitant administration of *G. glabra* extract (90 mg/kg) and LNAME in the trial mode. Heart rate and blood pressure of the animals were measured after the administration of *G. glabra* extract and LNAME drug. The measurements were performed, using organ electrodes, an arterial catheter connected to a pressure transducer, and a Power Lab A-D device.

FINDINGS: Based on the findings, a significant decline was observed in the mean arterial pressure in the trial mode (93±8.04), compared to the control mode (129±2.7) ($p \leq 0.05$). In addition, a significant reduction was observed in the mean systolic blood pressure in the trial mode (98±7.9), compared to the control mode (136±2.9) ($p<0.04$). Moreover, a significant decline was observed in the mean diastolic blood pressure in the trial mode (377±3.04) in comparison with the control mode (423±2.7) ($p<0.04$).

CONCLUSION: According to the results of the present study, concomitant administration of *G. glabra* extract and LNAME drug caused a significant decline in blood pressure.

KEY WORDS: *Glycyrrhiza glabra, Nitric oxide, Blood pressure, Heart rate, Rat*.

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Introduction

Medicinal herbs contain natural compounds with therapeutic features. The extracts of herbal medicines have been used for the treatment of various diseases since ancient times (1). Glycyrrhiza glabra, belonging to the pea family, is an ancient herbaceous plant, native to the Mediterranean regions, Russia, and Asia. This herb has been long applied in traditional medicine, with a wide range of therapeutic features in medical sciences (2).

The root of G. glabra has been used in traditional medicine, given its soothing and mucokinetic features (3). G. glabra rhizome is also used in traditional medicine for the treatment of muscle cramps, rheumatism, asthma, chest infections, excess bile secretion, hepatitis, respiratory and skin diseases, fever, cough, gout, tonsillitis, abdominal bloating, jaundice, hiccups, anemia, sore throat, and bleeding. Moreover, this herb can be used as a laxative agent, diuretic drug, or contraceptive agent (4, 5).

G. glabra has been applied in Asian and European traditional medicine for the treatment of gastritis, respiratory infections, and peptic ulcer (6). Moreover, it has been applied in traditional Chinese medicine to overcome hepatitis and prevent tumor growth and cardiac diseases (7). In traditional Iranian medicine, this herbal medicine has been used for the treatment of gastritis and cough (8). Today, the therapeutic effects of G. glabra on complex diseases such as cancer have been confirmed (9); this herb is also a constituent of cough syrup (10).

G. glabra activates the mineralocorticoid receptors and leads to false hyperaldosteronism, water and salt retention, and acute hypertension through deactivating 11-beta-hydroxysteroid dehydrogenase (11).

Nitric acid is an important endogenous substance, which plays a pivotal role in the regulation and maintenance of vascular tone, given its laxative properties (12). Nitric oxide (NO) is produced by one of the isoforms of NO synthase (NOS), which comprises of neuronal NOS, endothelial or epithelial enzymes (eNOS), and inducible nitric oxide synthase (iNOS) (13).

In several studies, the ineffectiveness of G. glabra hydroalcoholic rhizome extracts on the nitrergic system of the duodenum has been demonstrated (14). In recent years, there has been a growing interest in the use of medicinal herbs due to the adverse side-effects of pharmaceutical synthetic compounds (15). Considering the applicability of medicinal herbs in the treatment of various cardiovascular diseases (e.g., heart failure, hypertension, and atherosclerosis), further research on the effects of these herbs on the cardiovascular system seems essential (16).

G. glabra has been widely applied for the treatment of diseases such as peptic ulcer, atherosclerosis, and complex diseases such as cancer (11). In addition, this herb has been shown to decrease blood pressure in patients (17). Given the increasing importance and special status of medicinal herbs (such as G. glabra) in today's pharmaceutical industries and the tendency towards the use of medicinal herbs and their derivatives (due to their fewer side-effects and wider therapeutic effects) (18), we aimed to evaluate the effects of G. glabra extract on the blood pressure and heart rate of male rats and assess its interaction with the nitrergic system.

Methods

Extract preparation: G. glabra extract was prepared using a percolator device. The G. glabra rhizome powder was placed in a percolator. A sufficient amount of 70% ethanol (73 ml of ethanol and 27 ml of distilled water) was added to the powder in order to cover it with the solvent.

As the solvent penetrated into the *G. glabra* powder after 0.5 h, 70% ethanol was again added to the mixture. Over 24 h, the pressure from the percolator resulted in the accumulation of hydroalcoholic droplets of G. glabra. Afterwards, the diluted extract was placed in a rotary device to thicken; the duration of the procedure varied depending on the herb (17).

Preparation of laboratory animals: In this experimental study, 10 mature male Wistar rats (weight: 200 g) were selected and kept in an environment with a temperature of 22°C and 12 h light/12 h dark cycle. The animals had access to water and food ad libitum during the experiment. Ethical guidelines for animal research (e.g., anesthesia and surgery) were followed under the supervision of the Bioethics Committee at the biology department of the university (No: SU900786).

The animals were anesthetized by the intraperitoneal injection of sodium pentobarbital (50 mg/kg). Afterwards, femoral artery and vein of the samples were cannulated. Venous cannulation was applied for the injections (such as the physiological serum injection), and the arterial catheter was

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connected to a pressure transducer, which was attached to a Power Lab AD system (using the Bridge Amplifier) to record the changes in arterial, systolic, and diastolic blood pressure. In addition, the heart rate of the samples was recorded using subcutaneous electrodes, connected to a BioAmplifier device, PowerLab system, and lead II derivation. The body temperature of the samples was maintained at nearly 37°C during the experiment. After acclimatization within 60 min, the animals' blood pressure was recorded at baseline. The effective dose of G. glabra rhizome extract was considered to be 90 mg/kg (17).

In order to determine the interaction between G. glabra extract and the NO system, the LNAME drug and plant extract were injected to anesthetized samples through venous cannulation. Then, blood pressure and heart rate were recorded simultaneously over 120 min. Generally, LNAME as the inhibitor of NOS and NO production is considered as a suitable agent for evaluating the role of the nitrergic system in the management of blood pressure (19).

Therefore, this drug was used in the present study to evaluate the interaction between G. glabra and the nitrergic system. Normal heart rate in male rats was 300 bpm, while the mean arterial, systolic, and diastolic blood pressure was 95 mmHg (20), 118 mmHg, and 83 mmHg, respectively (19).

Animal classification: In total, baseline, control, and trial modes were applied for 10 mature male rats. For this purpose, each animal received an intravenous injection of LNAME after recording the baseline blood pressure and heart rate. Afterwards, the effects of this drug on blood pressure and heart rate were recorded. Then, the animals were allowed to rest until the blood pressure returned to the baseline.

Finally, G. glabra extract and LNAME were concomitantly injected to animals, and blood pressure and heart rate were recorded. Three modes were evaluated in the animals, which are as follows: 1) baseline: sole administration of the physiological serum; 2) control mode: concomitant administration of LNAME (Sigma Aldrich Co., Germany) and the physiological serum (equal to the amount of the extract); and 3) trial mode: concomitant administration of G. glabra extract (90 mg/kg) and LNAME (5 mg/kg). The mean arterial, systolic, and diastolic blood pressure (mmHg) and heart rate (bpm) were measured and compared in all the three modes.

Statistical analysis: In this study, data analysis was performed, using SPSS version 19. Kolmogorov-Smirnov test was performed to evaluate the normal distribution of the data. In addition, paired-sample t-test was used for inter-group comparisons. P≤0.05 was statistically considered significant.

Results

According to the results, G. glabra extract, independent of the nitrergic system, could significantly decrease the mean arterial pressure. Concomitant administration of LNAME and G. glabra extract significantly reduced the mean arterial pressure in the trial mode (93±8.04), compared to the control mode (129±2.7) (p≤0.05) (Diagram 1).

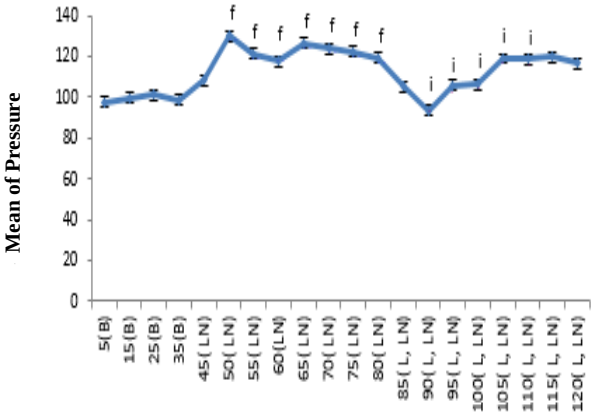


Diagram 1. Changes in the mean arterial blood pressure in the control (physiological serum+ LNAME) and trial modes (G. glabra extract+ LNAME drug) in comparison with the baseline (physiological serum)

(L, LN: trial mode, LN: control mode, B: baseline) (f: significant difference between the control mode and the baseline, p<0.03; i: significant difference between the trial and control modes, p<0.05).

G. glabra extract caused a significant decrease in systolic pressure, independent of the nitrergic system. Also, concomitant administration of LNAME and G. glabra extract led to a significant decline in systolic blood pressure in the trial mode (98±7.9), compared to the control mode (136±2.9) (P<0.04). Moreover, diastolic blood pressure significantly decreased in the trial mode (89±8.04), compared to the control mode (124±2.06) (P≤0.05) (Diagram 2).

According to Table 1, the studied extract could increase the samples' heart rate through the activation of the nitrergic system. However, concomitant administration of LNAME drug and G. glabra extract led to a significant decline in the heart rate of the

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samples in the trial mode (377±3.04), compared to the control mode (423±2.7) ($p<0.04$).

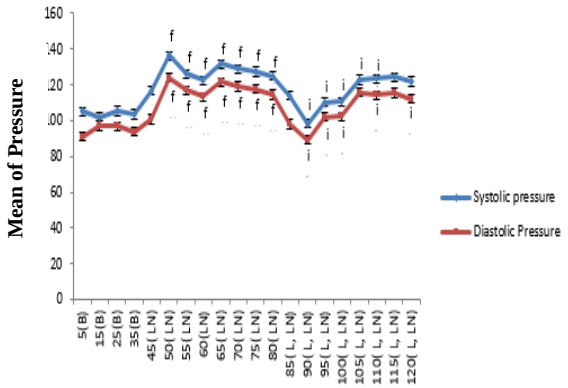


Figure 2. Changes in systolic and diastolic blood pressure in the control (physiological serum + LNAME drug) and trial modes (G. glabra extract + LNAME drug) in comparison with the baseline (physiological serum)

(L, LN: trial mode, LN: control mode, B: baseline) (f: significant difference between the control mode and the baseline, $p<0.03$; i: significant difference between the trial and control modes, $p<0.05$)

Table 1. Changes in heart rate in the control (physiological serum+LNAME drug) and trial modes (G. glabra extract + LNAME drug) in comparison with the baseline (physiological serum)

Heart rate	Baseline (bpm)	Control mode (bpm)	Trial mode (bpm)
5 min	23±349	23±452^{f}	22±436
10 min	16±363	16±435^{f}	18±392
15 min	4±397	4±387	1±395^{i}
20 min	7±397	7±377	2±379
25 min	7±390	7±380	4±370
30 min	4±373	4±399	5±368^{i}
35 min	13±349	13±423^{f}	11±377^{i}
40 min	8±363	8±383	4±369^{i}

(f: significant difference between the control mode and the baseline, $p<0.02$; i: significant difference between the trial and control modes, $p<0.04$)

Discussion

According to the results of the present study, intravenous injection of G. glabra extract, independent of the nitrergic system, caused a significant decline in the blood pressure of the rats. On the other hand, the effect of the extract on the increase in heart rate might be dependent on the nitrergic system. In this study, LNAME (NOS inhibitor) was used to evaluate the interaction between G. glabra extract and the nitrergic system. NO plays a pivotal role in the regulation and maintenance of vascular tone through its relaxant effects. Moreover, NOS in the vascular endothelium leads to the relaxation of smooth muscles and decreased blood pressure (21). Therefore, intravenous injection of LNAME as an NOS inhibitor could cause a significant increase in blood pressure. On the other hand, concomitant administration of G. glabra extract and LNAME eliminated this effect and led to a decline in blood pressure. It can be concluded that the diminishing effects of G. glabra extract on blood pressure is independent of the nitrergic system.

Consistent with our findings, Kenarkuhi et al. indicated that the hydroalcoholic extract of G. glabra could reduce tension in the isolated aortic tissues independent of the nitrergic system (22). Moreover, Ajay et al. revealed that the relaxant effects induced by some flavonoids were applied via mechanisms independent of the nitrergic system (23). In addition, the results obtained by Khoshnazar et al. were indicative of the reducing effects of hydroalcoholic G. glabra extracts on the movements of duodenal smooth muscles in rats, which were independent of the nitrergic system (14).

Gharib Naseri et al. demonstrated that G. glabra extract exerted its relaxant effects on the smooth muscle of the ileum independent of the nitrergic system (24). In a previous study by Pilijia et al., Ginkgo biloba extract caused a significant decline in spontaneous contractions of the smooth muscle of the ileum in rabbits due to the presence of flavonoids in a dose-dependent manner.

Moreover, Ginkgo biloba extract caused a reduction in the number of contractions induced by acetylcholine in ileum by antagonizing muscarinic receptors through the cholinergic pathway (25). Considering the presence of pharmacologically important compounds in G. glabra (e.g., flavonoids), the reducing effects of this plant on blood pressure could be attributed to these compounds (26, 27).

On the other hand, the results reported by Morello et al., which indicated the dilation effects of flavonoid galanin induced by NO synthesis (26), were not in line with the present findings. This discrepancy might be due to the presence of different combinations and ratios of flavonoids in G. glabra extracts in these studies. According to the literature, the hydroalcoholic rhizome extract of G. glabra could significantly reduce blood pressure through the inhibition of the adrenergic system and the effects consistent with the cholinergic

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system (17). Overall, particular attention should be paid to the role of calcium and potassium channels in the evaluation of the mechanism contributing to the effects of G. glabra extract on decreasing blood pressure. In a study by Chen et al., the antispasmodic effects were induced by flavonoids present in G. glabra extract in the rabbit jejunum and guinea pig ileum through the inhibition of potassium channels (28). Also, according to the results reported by Gharib Naseri et al., the relaxant effects of G. glabra extract on the smooth muscles of the ileum were exerted through ATP-sensitive potassium channels and their interaction with calcium channels (24).

It seems that the reducing effect of G. glabra extract on blood pressure might be due to the activation of ATP-sensitive potassium channels and inhibition of calcium channels, given the effects of this extract on the isolated aortic smooth muscle tissues (22). In the present study, injection of G. glabra extract could lead to an increase in heart rate (17). Meanwhile, concomitant injection of G. glabra extract and LNAME significantly decreased the heart rate of the samples. Therefore, the increasing effect of G. glabra extract on heart rate might be attributed to NOS and the nitrergic system; however, this effect was diminished by the injection of LNAME drug (NOS inhibitor). In line with the present study, Parisella et al. showed that glyceric acid and glycon glycerin caused an increase in heart rate and resulted in negative inotropism. In addition, the signalling pathway activated the endothelin receptor type B, NOS, and nitric oxide synthesis (29); this finding was in congruence with the present results.

Also, in a study by Bahaoddini et al., G. glabra extract exerted positive chronotropic effects on rats with normal blood pressure through baroreceptory reflex pathway and activation of endothelin receptor type B. In addition, this compound was able to induce negative inotropism effects in heart tissues through the cholinergic system (30). According to the results of the present study, it seems that the hydroalcoholic extract of G. glabra could significantly decrease blood pressure (mean arterial, systolic, and diastolic blood pressure), independent of the nitrergic system. However, the increasing effects of the extract on heart rate might be dependent on the nitrergic system; also, these effects could be induced by the activation of the baroreceptor reflex pathway.

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