ORIGINAL ARTICLE

ANTIHYPERTENSIVE EFFECTS OF ETHANOLIC EXTRACT OF *TRAPA NATANS* SEEDS IN L-NAME-INDUCED HYPERTENSIVE RATS

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Background: There is need for safer and cost-effective therapeutic intervention for hypertension. *Trapa natans* exhibits antioxidant and anti-inflammatory activity. However, its efficacy as an antihypertensicve has not yet been established. This study aimed to examine the potential antihypertensive effect of ethanolic extract of *Trapa natans* seed in L-N^{G}-Nitro Arginine Methyl Ester (L-NAME) induced hypertensive rats. **Methods:** This study used 30 rats which were divided into six groups of five rats each. The control group was given 5 mg/Kg normal saline. L-NAME and captopril (20 mg/Kg) were given to the second group. L-NAME and different doses of *Trapa natans* were given to 3rd, 4th, 5th and 6th groups. Non-invasive blood pressure was checked weekly. On the 29th day, the invasive blood pressure of the animals was checked. **Results:** The results showed that the non-invasive blood pressure altered substantially across the groups over time, especially the dose of 400 mg/Kg *Trapa natans* notably reduced the blood pressure. By the end of 28 days the invasive blood pressure also showed a noteworthy reduction in blood pressure for the dose of 400 mg/Kg *Trapa natans*. **Conclusion:** This study supports the beneficial ability of *Trapa natans* in lessening L-NAME induced hypertension. Its effects are likely to be caused by its phytochemical constituent, which demonstrates strong antioxidants and anti-inflammatory activities of *Trapa natans*.

Keywords: Hypertension, Invasive blood pressure, L-NAME, Non-invasive blood pressure, *Trapa natans*

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INTRODUCTION

Hypertension is a chronic disease of the cardiovascular system that is characterized by consistently high blood pressure in the arteries, which is determined by systolic blood pressure (SBP) 140 mmHg and/or diastolic blood pressure (DBP) 90 mmHg.[1] It is a significant global health issue and the principal risk factor to cardiovascular morbidity and mortality.

The World Health Organization (WHO) claims that hypertension causes about 9.4 million deaths every year around the globe and over 1.28 billion adults aged between 30 and 79 years are affected.[2] It is raising the rate of morbidity and mortality.[3] Nearly 64% of stroke patients have hypertension.[4] Approximately half of its patients never get a diagnosis, especially in low- and middle-income countries because their awareness of the disease and access to healthcare services are less. The current trends in lifestyles and demographic shifts have also contributed to the fact that the prevalence of hypertension has grown significantly throughout the last several decades across the globe.[2]

Prevalence of hypertension is 31.5% in low- and middle-income countries and 28.5% in high-income countries.[5] Countries with high population densities and low and moderate income are more susceptible to hypertension. Hypertension is one of the serious health burdens in Pakistan and prevalence of the conditions has been reported as 21.6% in urban population and 16.2% in rural population.[6] Almost three-quarters of people with hypertension in Pakistan do not even know that they have the disease.[7] Regional differences have also been noted as women are suffering more in Khyber Pakhtunkhwa and Baluchistan, and men are found to be more prevalent in the Punjab and Sindh.[6,8]

Risk factors of hypertension can be categorized in the broad scope as non-modifiable factors, e.g., age, sex, and inherited genetic makeup, and modifiable factors, e.g., excessive salt intake in the diet, obesity, inactivity, smoking, and alcoholism.[8] The pathophysiology of hypertension is linked to dysregulation of rennin-angiotensin-aldosterone system (RAAS), hyperactivity of the sympathetic nervous system, endothelial dysfunction, and a lack of nitric oxide bioavailability.[9]

Despite existence of various pharmacological classes like diuretics, angiotensin-converting enzyme inhibitors, calcium channel blockers, and β-adrenergic antagonists to manage hypertension, prolonged intake of these drugs is known to cause complications such as electrolyte imbalance, gastrointestinal upsets, and cardiovascular complications.[9] As a result, research on antioxidant and anti-inflammatory properties of plant-based therapies has become of interest. It is approximate that 75–80% of the world population depends, partially, on herbal medicines as primary healthcare.[10]

Aquatic polyphenol-flavonoid-rich plant, *Trapa natans*, has been shown to be useful in antioxidant and anti-inflammatory processes.

Nevertheless, its antihypertensive effect has not been properly investigated. The aim of the current work was to determine the antihypertensive properties of ethanolic extract of seeds of *Trapa natans* on L-N^G-Nitro Arginine Methyl Ester (L-NAME), (a non-selective nitric oxide synthase inhibitor widely used to induce experimental hypertension by reducing nitric oxide bioavailability and increasing vascular resistance) induced hypertensive rat model.

MATERIAL AND METHODS

This study was conducted in June 2024 in the University of Lahore after approval of Ethical Review Board of Pharmacy Department vide IREC No. IREC-2023-54. The laws of the National Institute of Health regarding animal experiments were adhered to in this study.

The experiment lasted for 28 days. Rats were randomly divided into 6 groups, i.e., control, L-NAME hypertensive control, L-NAME plus captopril (20 mg/Kg), and L-NAME plus *Trapa natans* at doses of 100 (T-100), 200 (T-200), and 400 (T-400) mg/Kg.

Both non-invasive and invasive blood pressure measurements were carried out. The non-invasive blood pressure was measured every week, with rats being acclimated to a comfortable, quiet, and dark atmosphere and restrained to reduce cases of stress as much as possible.[11] The tail was warmed and tail cuff was used to feel the pulse with a photoplethysmograph to stimulate a pressure transducer. In the invasive measurements, carotid cannulation was done following anaesthesia with diazepam and ketamine.[12,13] The neck was shaved and disinfected, and under close dissection the carotid artery and trachea were revealed. A cannula filled with heparin saline was inserted into the carotid artery, fastened with clamps and threads and attached to a pressure transducer, with the monitoring of bleeding.

The Mean±SEM was used to express the data. In order to determine the level of significance between the control and experimental groups, the data obtained were analyzed through two-way analysis of variance (ANOVA) with post hoc Bonferonni test, and $p \leq 0.05$ was considered as statistically significant.

RESULTS

Daily administration of L-NAME resulted in rise in SBP from 135±4 mmHg in week 1 to 208±10 mmHg by week 4. *Trapa natans* treatment induced an antihypertensive effect in dose dependence manner. The T-100 group recorded a reduction of 13.75 mmHg (9.34%) by week 4, T-200 had a reduction of 23.55 mmHg (19.5%) and T-400 recorded a reduction of 48.30 mmHg (42.58%), which was similar to captopril (48.4 mmHg, 43.26%) and the control group (42.0 mmHg).

Bonferonni was used to determine the group significantly different in comparison to L-NAME group. As there was a constant change in blood pressure, L-NAME group had the greatest increase. Blood pressure of the T-100 and T-200 groups revealed a constant increase although it started to decline gradually. T-400 progressively presented a decrease in blood pressure, indicating an antihypertensive effect. (Table-1).

Invasive blood pressure measurement in all groups differed. The mean difference, however, revealed that doses of 100 and 200 mg/Kg of *Trapa natans* are less effective as no differences were observed; the 400 mg/Kg dose of *Trapa natans* showed significant differences in the invasive blood pressure after 28 days. The results demonstrate that such changes were similar to those caused by captopril. (Table-2).

Table-1: Non-invasive blood pressure of animals at weekly intervals (mmHg, Mean±SE)

Group	Week 1	Week 2	Week 3	Week 4
Control	130±3	131±4	130±4	131±4
L-NAME	135±4	158±6	186±8	208±10
Captopril	128±2	129±3	121±3	118±3
T-100	132±3	148±4	166±5	189±6
T-200	129±3	140±3	160±4	167±4
T-400	127±3	128±3	123±3	115±4

Table-2: Mean invasive blood pressure (IBP) after 28 days of treatment compared to L-NAME group (mmHg, Mean±SE)

Group	Mean IBP	Change in BP	*p*
Control	125±4	N/A	N/A
L-NAME	175±7	---	N/A
Captopril	110±3	-65	<0.001*
T-100	165±5	-10	NS
T-200	145±3	-30	<0.01*
T-400	115±3	-60	<0.001*

*Significant

A dose-dependent response was seen among the test compound-treated groups; T-100 showed an increase of 14.40 mmHg (p=0.0137), T-200 showed a larger effect of 37.20 mmHg (p=0.000), and T-400 produced an increase of 66.00 mmHg (p=0.000), which was almost identical to the effect of captopril.

DISCUSSION

One of the most important physiological indicators of cardiovascular functioning is blood pressure, and its proper measurement is crucial with experimental hypertension models.[14,15] Both non-invasive and invasive techniques were used to assess the antihypertensive effect of *Trapa natans* in L-NAME induced hypertensive rats. The results of both approaches were consistent.[14,16]

With the non-invasive measurements, it was demonstrated that *Trapa natans* at the dose of T-400 generated significant and enduring effect of alleviating blood pressure during treatment. Notably, the extent of pressure drop on this dose was similar to captopril indicating clinically useful antihypertensive effect.[13] Weaker doses (T-100 and T-200) also had blood pressure-lowering activity, but the relatively small size of the effects shows that there is a dose-dependent effect as opposed to identical therapeutic effect.[17] This was further confirmed by invasive blood pressure measurements.

The invasive data proved that *Trapa natans* had a salient effect to reduce hypertension in L-NAME-treated rats with T-400 dose showing the strongest effect. The stability of the antihypertensive effect of *Trapa natans* is emphasized by the consistency between dynamic monitoring of blood pressure by non-invasive methods and terminal measurements reported by invasive method, and accuracy of tail-cuff method to determine the tendency of a trend.[15,16]

The combined analysis of invasive and non-invasive results suggests that *Trapa natans*, especially with higher levels of dosing, possesses a great potential of antihypertensive effect in L-NAME-induced hypertension. Such findings indicate its potential therapeutic value and need to explore its pharmacology and safety in the long term.

CONCLUSION

Trapa natans has an antihypertensive effect in L-NAME induced hypertension in Wistar rats. There is a definite dose-ranging effect with the T-400 dose causing a significant decrease in blood pressure similar to the conventional antihypertensive medication, captopril. Smaller dosage (T-100 and T-200) had a moderate effect, and it is another indicator of criticality of dosage.

RECOMMENDATIONS

Future research ought to use a wider dose to determine the therapeutic window and explain the dose response relationship of *Trapa natans*. An exploration of its molecular mechanisms, especially the nitric oxide regulation, oxidative stress, and vascular protection, should be investigated. It is suggested to use chronic hypertension models in long-term studies to determine safety and long-term effectiveness. Further investigation of *Trapa natans* can be conducted through separation of bioactive compounds to place *Trapa natans* in pharmacological context and use in humans.

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