Does copper enhance the antihypertensive effect of *Elaeocarpus ganitrus* in experimentally induced hypertensive rats?

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ABSTRACT

Ayurveda, one of the traditional systems of medicine of India, reports that the seeds of *Elaeocarpus ganitrus* Linn. (Tilaceae) can be used for the treatment of hypertension. The main aim is to evaluate the antihypertensive effect of *Elaeocarpus ganitrus* (Rudraksha) seeds. Powdered seeds were extracted by maceration, overnight, using water, in copper (E1) and glass vessel (E2) and analyzed for antihypertensive activity in cadmium chloride (1 mg/kg intraperitoneally, for a period of 15 days) induced hypertensive male Wistar rats at three dose levels. E1 was administered at the dose of 5, 10, and 15 mg/kg and E2 at dose of 10, 20, and 30 mg/kg. All the data were analyzed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. E1 and E2 did not show any toxicity at the dose of 5 g/kg in rats. It was found that 15 mg/kg of E1 and 30 mg/kg of E2 decreases the blood pressure by 30.20 mmHg and 28.96 mmHg, respectively, in hypertensive rats. Thus, it can be said that 15 mg/kg of E1 produced similar decrease in blood pressure as was observed with 30 mg/kg of E2. Copper ions in E1 might be additively affecting the reduction in blood pressure with the usage of *Elaeocarpus ganitrus* extracts.

Key words: Ayurveda, cadmium chloride, copper, hypertension, rudraksha

INTRODUCTION

Hypertension is the most common cardiovascular disease. The prevalence of hypertension increases with advancing age; in industrialized countries there is greater prevalence of hypertension among the elderly.[1] Hypertension is the principal cause of stroke, a major risk factor for coronary artery disease, myocardial infarction, and sudden cardiac death. It is also a major contributor to cardiac failure, renal insufficiency, and dissecting aneurysm of the aorta.[2]

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First line drugs involved in the treatment of hypertension include diuretics, beta blockers, angiotensin converting enzyme (ACE)-inhibitors, angiotensin-II receptor blockers, calcium channel blockers, alpha blockers, etc., Second line drugs include adrenergic neuron inhibitors, central α_2 inhibitors and direct vasodilators. All these antihypertensive agents have some degree of side-effects. Hypertension ensures a prolonged use of antihypertensive drugs, which may have deleterious consequences on body.[3] Hence it is of utmost importance to find a safe and potent alternative to these drugs. *Elaeocarpus ganitrus* Linn. (Tilaceae) known as Rudraksha, which is an *Ayurvedic* drug, acknowledged for its antihypertensive activity.

The drug consists of the seeds of *Elaeocarpus ganitrus* Linn. (Gaertn.) K. Schum (syn. *E sphaericus* Linn.) (Tiliaceae). The seed oil contains fatty acids, alkaloids, minerals, and vitamins.[4] According to the *Ayurvedic* pharmacopoeia, the seeds in a dose of 1-2 g taken internally are used for the treatment of uchcha raktatapa (hypertension) and hridayaroga (heart diseases) apart from being used in the treatment of mental disturbances, nervine disease,

hypertension, cardiovascular, and hepatic problems.[5,6] Similar uses are also mentioned in compendia of *Ayurvedic* texts.[7] Tamra bhasma, a metallic *Ayurvedic* preparation containing some form of copper, is also reported in the management of heart diseases.[8] A study, which has used copper complexes of synthetic drugs, has shown improved anti inflammatory effect, due to activation of copper dependant opiod receptors.[9] Thus, copper if combined with any extract can modulate the therapeutic efficacy. This concept has been used in the folklore medicine wherein decoction of Rudraksha soaked overnight in a copper vessel full of water is said to possess antihypertensive activity. The present article deals with evaluation and comparison of the effects of Rudraksha extracts on experimentally induced hypertension in rats.

MATERIALS AND METHODS

E. ganitrus sample was obtained from a supplier based in Gujarat and authenticated by at Piramal Life Sciences, Mumbai.

Nine male Swiss albino mice weighing 20-30 g were used for the acute toxicity studies. Twenty-four male Wistar albino rats, weighing 200-250 g were used for antihypertensive studies. All animals were placed in cages maintaining standard environmental conditions [23ºC ± 5, 60% ± 5 RH], fed with standard diet and allowed free access to drinking water during the period of acclimatization. All animal experiments were carried out in accordance with guidelines of Committee for the Purpose and Supervision of Experiments on Animals (CPCSEA) with the approval by the Institutional Animal Ethics committee (IAEC) bearing a protocol number CPCSEA/IAEC/SPTM/P-10/2011.

E. ganitrus seeds (250 g) were powdered using a grinder and soaked overnight in water for maceration in a copper vessel.[10] The extract was filtered, dried in the hot air oven at 60ºC for 5 h, weighed and labeled as E1. Similar extraction process was repeated using a glass vessel and the extract labeled as E2.

Both the extracts were screened for the presence of different phytoconstituents.[10] The extracts were evaluated using IR spectroscopy, UV spectroscopy, differential scanning calorimeter (DSC), X-ray diffraction (XRD), scanning electron microscope (SEM), and differential light scattering (DLS). Thin layer chromatography (TLC) system was developed for both the extracts E1 and E2 using n-butanol: acetic acid: water in the ratio of 4:1:5 as the mobile phase, silica gel GF_{254} as the stationary phase, and vanillin sulfuric acid as the spraying reagent.[4]

Acute oral toxicity

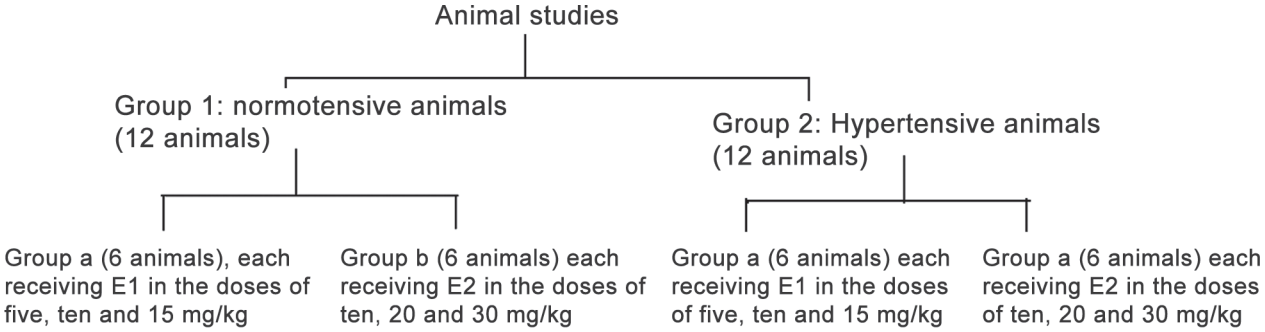
Acute oral toxicity studies for the extracts were carried out as per the organization for economic cooperation and development (OECD) guidelines 423.The animals were divided into three groups of three animals each. Two of the groups, received 2 g/kg of E1 and 2 g/kg of E2, the third group received only vehicle. All animals were observed within the period of 48 h for the sign of toxicity. Accordingly, the food intake and body weight were monitored for a period of 14 days.[11]

Antihypertensive studies

Experimental animals were divided as shown in the Graph 1 below. Hypertension was induced in the animals by intraperitoneal injection of cadmium chloride at dose of 1 mg/kg for a period of 15 days.[12] The acute effect on mean arterial blood pressure was observed for a period of 15 min at each dose level, using the invasive blood pressure monitoring system (Iworks data recorder). All the animals of both groups also received normal water and water kept in copper vessel to observe the effect of vehicle on blood pressure.

Physicochemical screening

E2 revealed the presence of tannins and carbohydrates, whereas E1 revealed the presence of steroids along with the above-mentioned constituents. The IR spectra, UV spectra, and DSC analysis of E1 and E2 were similar.



Graph 1: Division of experimental animals

Figure 1 shows the presence of crystalline copper form at 43.62 theta value in E1, whereas the same was absent for E2. DLS study revealed that E2 shows size around 26 micron with a potential of around -6.81 mV, whereas E1 shows size of around 1.34 micron with a potential of around -30 mV. SEM for E1 also showed the size of around 1 micron.

Thin layer chromatography

Both E1 and E2 showed similar spots with the given mobile phase.

Acute toxicity test

The animals receiving either of E1 or E2 showed no signs of toxicity. Thus it can be said that LD_{50} of E1 and E2 is more than 5 g/kg.[11]

Antihypertensive studies

Extracts E1 and E2 show considerable and significant effect on the blood pressure of normotensive animals [Figure 2] and hypertensive animals [Figure 3]. Further it was observed that the decrement in blood pressure is same for 10 and 15 mg/kg dose of E1 and 20 and 30 mg/kg dose of E2. Administration of vehicles did not show significant change in the blood pressure of either group.

Statistical analysis

All the data was analyzed using one way analysis of variance (ANOVA) followed by Dunnette's multiple comparison test.

DISCUSSION

The study shows that the extracts have significant anti-hypertensive activity, but E1has improved potency as compared with E2. Copper can act as a catalyst in the synthesis of alkaloids.[13] During the process of preparation of water extract, there is chance of such reactions happening, wherein copper might interact with the alkaloids present in the extract. Hence the chromatography was performed, but E1 and E2 showed the same profile, indicating absence of any new/modified compound formed in E1. Moreover the IR, UV, and DSC analysis of E1 and E2 did not show any major difference, hence it can be concluded that the chemical properties of the extracts were similar. At the same time the DLS, XRD analysis and SEM of E1 and E2 showed that there is change in the physical properties of the extracts. Another interesting study reports that Rudraksha seeds help in the formation of silver nanoparticles due to their electromagnetic and inductive properties.[14] Similarly, the present study demonstrates that E1 might have transformed into a nanoformulation, exerting greater efficacy.

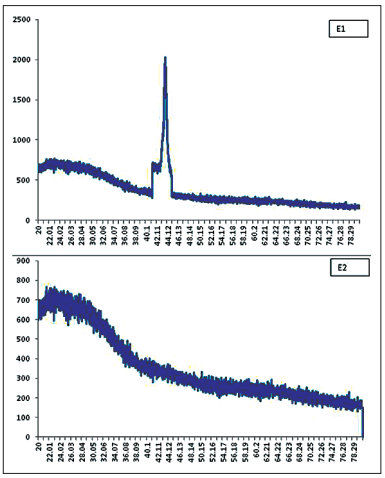


Figure 1: XRD pattern for E1 and E2

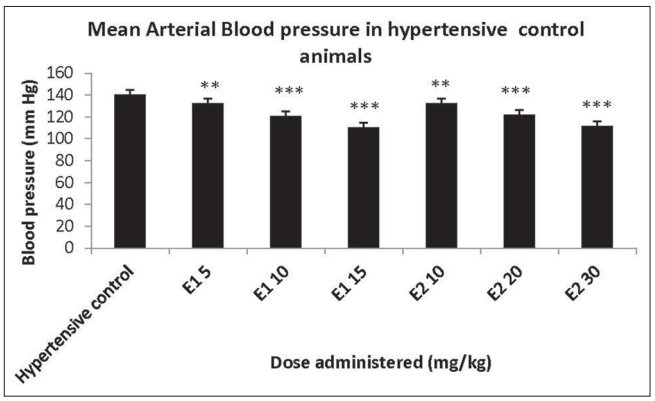


Figure 2: Effect of E1 and E2 on hypertensive animals . Each values represent Mean ± S.E.M. for the number of animals utilized during experiment. ***P<0.001. **P<0.01 when compare to hypertensive animals. Actual figures from which graph is drawn Hypertensive control: 140±3.99, E1 5: 132.46±4.14, E1 10: 121.13±3.29, E1 15: 110.3±3.60, E2 10: 132.32±3.46, E2 20: 121.99±2.39, E2 30: 111.54±2.51

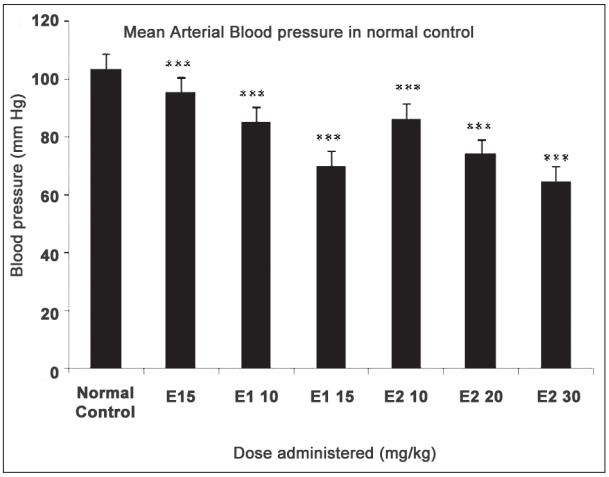


Figure 3: Effect of E1 and E2 on normotensive animals . Each values represent Mean ± S.E.M. for the number of animals utilized during experiment. ***P<0.001, when compare to normal control animals. Actual figures from which graph is drawn Normal control: 103.66±4.13, E1 5: 95.5±0.85, E1 10: 85.15±1.08, E1 15: 70.11±0.83, E2 10: 86.15±1.5, E2 20: 74.18±1.19, E2 30: 64.6±1.35

It is reported that imbalance in copper availability occurs in hypertension as suggested by decreased lysyl oxidase activity, reduction in endothelium-dependant relaxation in resistance vessels and in aorta,[15] increased sensitivity to pressor hormones, impaired oxidative defense, and altered function of locus coeruleus.[16] Lower plasma copper levels have been found in people with newly diagnosed essential hypertension.[17] Copper in contact with water ionizes to give copper ions and this might directly be responsible for lowering blood pressure. The model used in the study was cadmium-induced hypertension, which produces hypertension by one of the proposed mechanisms, an increase of Na^+ retention,[18] interaction with Ca^{2+} channels,[12] activation of sympathetic nervous system,[19] decrease in the concentration of vasodilating substances,[20] suppression of endothelium-dependent vasorelaxation,[21] and/or reduction of the kallikrein activity.[22] Thus, considering the model used and the results obtained from the acute study, the most probable mechanisms for copper directly affecting the blood pressure might be endothelium-dependant relaxation or decrease in the effect of pressor hormones. It is reported that Rudraksha water extract alone shows antihypertensive activity through an action on rennin-angiotensin system[23] and is effective to counter the action of adrenaline.[24]

The improved efficacy of E1 might be due to the combined effects of both these agents, namely, copper ions in water and Rudraksha extract counter-acting the effects of pressor hormones. But, further studies need to be carried out to confirm the nanoform of E1, which could pave way for antihypertensive mechanistic studies in future.

It can be concluded that *E. ganitrus* extracts have significant antihypertensive activity. E1 is potent than E2 in lowering blood pressure of hypertensive animals, thus providing evidence for the folklore use of *E. ganitrus* seeds in treatment of hypertension.

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