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Evaluation of anti-hypertensive activity of ancient Unani cardiovascular drug "Saad Kufi" (*Cyperus scariosus*) in adrenaline-induced hypertensive rats

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Abstract

Background: Saad Kufi (*Cyperus scariosus* R.Br) is a hardy grass-like perennial plant that belongs to the family Cyperaceae. Ibn Sina (Avicenna), the most significant thinkers and writers of the Islamic Golden Age first time systematized the individual cardiac drugs in "*Risala Advia Qalbia*," which deals with 63 cardiac drugs which are claimed to be beneficial for heart ailments as well as for psychiatric ailments. He described Saad as a root of a plant that is nodular, long, and slender and resembles the wheat plant. The roots which used medicinally are thick, elongated, slender, and color black and has an aromatic smell with a pungent taste. An antihypertensive effect of 50% ethanolic extract of Saad Kufi (*Cyperus scariosus* R.Br) [EESK] was evaluated in adrenaline-induced hypertension in Wistar albino rats. The induction of systolic blood pressure (SBP) and the percentage of inhibition was measured in EESK (15 and 25 mg/100 g, orally) with standard as Metoprolol (0.5 mg/100 g) orally, using tail-cuff apparatus with AD instrument power lab to evaluate the antihypertensive effect.

Results: EESK significantly decreased the induction in SBP as compared to disease control rats ($p < 0.05$), and there is a significant increase in the percentage of inhibition in SBP in EESK and metoprolol-treated rats as compared to the disease control group ($p < 0.05$).

Conclusion: Result data suggest that EESK possess significant antihypertensive activity in adrenaline-induced hypertensive rats.

Keywords: *Cyperus scariosus*, Saad Kufi, Metoprolol, Anti-hypertensive effect, Adrenaline, Non-invasive hypertensive test

Background

Chronically elevated arterial blood pressure (BP) is called hypertension (HTN) [1]. HTN is a major public health issue and the commonest among cardiovascular diseases in developing and developed countries. 18–54% of the total world's population is hypertensive. Direct effects of hypertension caused 12% of deaths, and about 20% of the general population should expect to have high blood pressure during their life [2].

The essential hypertension of humans is a multifactorial, complex, quantitative trait under polygenic control [3]. The etiology of hypertension in 90% of cases is unknown (essential hypertension). Although it is common and readily detectable, if it is untreated, it can lead to lethal complications. Several classes of drugs and regimens are available to control hypertension, but their concomitant risk factors remain uncontrolled [4]. There are various anti-hypertensive plants available in traditional medicine, and their screening has been performed in

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several animal models [5]. The first herbal drug used on a large scale to treat systemic hypertension was Reserpine. Several other anti-hypertensive plants to treat hypertension are also used today. For example, *Uncaria rhynchophylla*, *Stephania tetrandra*, and the root of *Ligusticum wallichii* are used to treat hypertension in Traditional Chinese Medicine [6].

In order to understand the pathogenesis and to investigate the treatment and prevention of a disease, it is essential to develop animal models of hypertension. These animal models have many similar features that are present in human hypertension [3]. Tail-cuff non-invasive method is one of those models to study blood pressure measurements which can be consistent, accurate, and reproducible in awake and anesthetized mice and rats. Furthermore, multiple animal testing is cost-effective for large scale, high throughput screening [7].

Saad Kufi (*Cyperus scariosus* R.Br) belongs to the family Cyperaceae, consisting of 600 species distributed in the tropical and warm temperate regions of the world. Cyperus is a greek word which means sedge. Medicinally, the root of *Cyperus scariosus* is used for the same purpose as those of *Cyperus rotundus*, and this has long been in use in Hindu medicine and perfumery under the Sanskrit name Nagar mustaka [8].

The root of Saad Kufi (*Cyperus scariosus* R.Br) finds a pride of place in Unani literature for their ability to produce tonic and exhilarant effect on the heart. In Unani literature, the *Afa'al* (actions) have been described as *Muqawwe Qalb* (cardiotonic), *Muffarah* (exhilarant), *Muffatah Sudad* (deobstruent) [9, 10], *Mudir Boul* (diuretic), and *Dafesumoom* (antidote) [11] and it has been to be useful in *Khafqan* (palpitation) and *Zof-e-Qalb* (weakness of the heart) [12, 13]. Phytochemical investigations have revealed that major chemical constituents of this Unani drug are polyphenol, flavonol, alkaloid, glycoside, saponins, essential oil, cardiac glycosides, and sesquiterpenes and many therapeutic phyto-chemical components (i.e., alkaloids, glycosides, flavanoids, resins, tannin) with no known adverse effects [14], and additionally, the drug is also available at low cost.

Objective

The objective of the study is to investigate the anti-hypertensive effect of 50% ethanolic extract of Saad Kufi [(*Cyperus scariosus*) EESK] in hypertensive rats induced by adrenaline.

Methods

Collection and authentication of the test drug

The roots of Saad Kufi (*Cyperus scariosus* R.Br) were procured from the local market and identified in the Pharmacognosy Section, Department of Ilmul Advia, Ajmal Khan Tibbiya College. They were also authenticated by the National Institute of Science Communication and Information Resources, New Delhi (NISCAIR/RHMD/Consult/2017/3064-13-1). The sample of the test drug was submitted to Mawalid-e-Salasa Museum of the Department of Ilmul Advia after identification, for future reference with the voucher no. SC-0220/17.

Preparation of plant extract

Saad Kufi (*Cyperus scariosus*) was cleaned from the earthy material, washed with double distilled water, and dried at 45 °C in a hot air oven and powdered by an electrical grinder. Extraction was done in 50% ethanolic solvent by Soxhlet's apparatus for 6 h. The extract was filtered and dried by evaporation on a water bath. The yield percentage was calculated with reference to a crude drug and was found to be 13.07%. A fresh suspension of the extract was prepared in distilled water (as per calculation w/v) at the time of the experiment.

Ethical statement

All experimental procedures and protocols used in the study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) on August 26, 2017, Jawaharlal Nehru Medical College, and care of laboratory animals was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The registration number is 401/GO/Re/S/2001/CPCSEA.

Study design

Group I: Normal control (20 ml/kg distilled water)
Group II: Negative control (adrenaline 0.05 mg/100 g b. w. i.p.)
Group III: Standard group (metoprolol tartrate 0.5 mg/100 g/day, p.o.)
Group IV: Test group (low dose): Saad Kufi (*C. scariosus* R.Br): 15 mg/100gm, p.o.
Group V: Test group (high dose): Saad Kufi (*C. scariosus* R.Br): 25 mg/100gm, p.o.

Experimental procedure

The in vivo anti-hypertensive (non-invasive method) effect of the test drug on systolic blood pressure (SBP) was observed in experimentally induced hypertension in rats by the indirect tail-cuff method [15]. Urethane was used as an anesthetizing agent, and adrenaline (0.1 ml) was injected intraperitoneally (I.P) into rats by 1 ml disposable syringe for 4 consecutive days to induce hypertension. Induction of hypertension was confirmed as the systolic pressure was detected and subsequently recorded with Data Acquisition System (Model: ML125,

Serial: NI0502) on a power lab (4/30 Model: ML866, Serial: 430-1612 AD Instruments, Australia).

Measuring of SBP using PowerLab with tail-cuff apparatus

SBP was measured by the tail-cuff method using the NIBP Controller with Data Acquisition System (Model: ML 125, Serial: NI0502 AD Instruments, Australia) and PowerLab (4/30 Model: ML866, Serial: 430-1612 AD Instruments, Australia). To reduce spontaneous variations in blood pressure, animals are adjusted to the experimental cage by bringing them into the restraining cage 3–4 times before the start of the experiment for a period of 30–60 min and the ambient temperature was maintained at 31–32 °C. The blood pressure was measured by a tubular inflatable cuff, placed around the base of the tail, and a piezo-electric pulse detector was positioned distal to the cuff. Normal blood pressures of animals before treatment were recorded as baseline blood pressure, and then, rats were treated with their respective treatments and again blood pressure was recorded. To evaluate anti-hypertensive effect of drugs, adrenaline was injected after an hour of the treatment in standard and test groups and again the blood pressure was recorded. This was made to find the protective effect of Saad Kufi on the SBP induced by the administration of adrenaline. Finally, the mean increase in SBP from the pre-treatment SBP and the percent inhibition of SBP with respect to negative control were calculated.

Experimental animals

Wistar albino rats (n = 6) of either sex weighing 220–300 g were used for the present study. The animals were procured from the animal house, Department of Pharmacology, JNMC, AMU, Aligarh, India, and were allocated randomly to treatment groups and kept in polypropylene cages with paddy husk as bedding. Animals were housed at a relative humidity of 30–70% and temperature of 24 ± 20 °C along with light and dark cycles. A standard balance diet and water ad libitum were provided.

After the experiments, all animals were alive and were gone in the washing out period.

Sample size

Animals were divided into 5 groups, and each group has 5 animals.

Allocating animals to the experimental groups

Animals were randomly allocated to the experimental groups.

Experimental outcome

Animals have an increased systolic blood pressure.

Statistical analysis

One-way ANOVA with Tukey-Kramer multiple pair comparison test was used to determine the degree of significance. The analysis was carried out by using the GraphPad version 8.4.3 InStat Software.

Result

Systolic blood pressure (SBP) was recorded in rats before and after respective treatment by the tail-cuff method using NIBP Controller with Data Acquisition System and PowerLab. Measurement of induction of SBP along with the percentage of inhibition in SBP indicated that 50% ethanolic extract of Saad Kufi (*Cyperus scariosus* R.Br) root has antihypertensive activity (Tables 1 and 2).

On continuous infusion of adrenaline for 4 days, increase in SBP from the initial SBP also increased. It was also found that the basal blood pressure rises on continuous infusion of adrenaline for consecutive 4 days.

Result data showed that induction of SBP in standard (metoprolol tartrate) and test groups (Saad Kufi) was significantly decreased as compared to the disease control (or negative control) which showed that standard and test drug has significant antihypertensive activity (Fig. 1).

The percentage of inhibition in SBP in the standard (metoprolol tartrate) and test group (Saad Kufi) was

Table 1 Induction of systolic blood pressure

	Day 1	Day 2	Day 3	Day 4
Normal control	8.68 ± 0.27 *b*c*d*e	7.56 ± 0.27 *b*c*d*e	7.81 ± 0.21 *b*c*d*e	7.62 ± 0.26 *b*c*d*e
Negative control	47.93 ± 0.70 *a *c*d*e	51.31 ± 1.02 *a *c*d*e	52.43 ± 2.20 *a *c*d*e	54.37 ± 2.15 *a *c*d*e*
Standard group	22.7 ± 0.88 *a*b*d*e	21.62 ± 1.13 *a*b*d*e	20.81 ± 1.04 *a*b*d*e	20.68 ± 1.03 *a*b*d*e*
Saad Kufi (15 mg/100 g)	39.91 ± 0.22 *a*b*c*e*	38.41 ± 0.36 *a*b*c*e	36.16 ± 0.08 *a*b*c*e*	34.5 ± 0.14 *a*b*c*e*
Saad Kufi (25 mg/100 g)	31.12 ± 0.59 *a*b*c*d*	29.87 ± 0.92 *a*b*c*d	27.87 ± 0.42 *a*b*c*d*	26.12 ± 0.32 *a*b*c*d*

Values as mean ± SEM, n = 5, one-way ANOVA followed by Tukey-Kramer multiple pair comparison test, where *p < .001
[a]Compared with normal control
[b]Compared with negative control
[c]Compared with standard
[d]Compared with Saad Kufi (15 mg/100 g)
[e]Compared with Saad Kufi (25 mg/100 g)

Table 2 Percent inhibition in systolic blood pressure

	Day 1	Day 2	Day 3	Day 4
Normal control	–	–	–	–
Negative control	–	–	–	–
Standard group	52.63	57.82	60.34	61.92
Saad Kufi (15 mg/100 g)	16.73	25.16	31.03	36.53
Saad Kufi (25 mg/100 g)	35.07	41.74	46.84	51.95

measured for four days. The percentage of inhibition in SBP in standard (metoprolol tartrate) and test groups (Saad Kufi) was significantly increased as compared to the disease control group which indicate that standard and test drugs decrease systolic blood pressure, and the percentage of inhibition was also consistently increased with each passing day (Fig. 2).

Discussion

Hypertension is a common debilitating disease in the population of both developed and developing countries. Community surveys in industrialized countries have shown a prevalence of 15–33% in people aged 30 years. The leading causes of morbidity and mortality are coronary artery disease and stroke. The available antihypertensive drugs lower the blood pressure to the normal level which is the primary step to manage coronary heart disease, cardiovascular disease, and other cardiovascular-related complications, but their concomitant risk factors remain uncontrolled [16].

In this regard, traditional herbal drugs are helpful and have encouraging results as compared to the synthetic drugs, and also, they have fewer or no side effects and are easily available. The discovery of safer and newer alternatives for the management of hypertension can be done by screening of various herbal drugs in compliance with their traditional uses and nutritional value along with their safety. One of the such traditional drug is Saad Kufi (*Cyperus scariosus* R.Br), which belongs to the family Cyperaceae, commonly known as Nagarmotha [8, 17]. It is mentioned in the Unani classical text for cardiac ailments. It contains many therapeutic phytochemical components (i.e., alkaloids, glycosides, tannin, flavonoids, phenols, resins) with no adverse effects [14].

Antihypertensive effect of Saad Kufi (*Cyperus scariosus* R.Br) root was investigated by adrenaline-induced hypertensive rat model. In this study, mild to moderate hypertension was induced with adrenaline (0.1 ml) by intraperitoneal injection [18].

Adrenaline was used to induce hypertension in rats because of three molecular mechanisms (1) by binding with G-protein coupled (Gs) α1-adrenergic receptors, adrenaline activates phospholipase-c (PL-C) which then convert phosphoinositol diphosphate (PIP2) to inositol triphosphate (ISP3), and Diacyl glycerol (DAG). The DAG thus formed, goes into endocrine glands and activates protein kinase-C (PK-C), and leads to vasoconstriction. ISP3 increases calcium influx from endoplasmic reticulum and causes vasoconstriction, and heart binding with G-protein coupled (Gs) to β1 receptors leads to activate adenylate cyclase (AC) which converts ATP to cAMP which activates protein kinase-A (PK-A), causing phosphorylation of calcium channels, causing increase calcium influx from the endoplasmic reticulum and thus leads to increase in force (positive inotropic effect) and frequency (positive chronotropic effect). By these mechanisms, namely vasoconstriction and increase in force and frequency of contraction in the heart musculature, adrenaline results in increase in blood pressure. (2) Adrenaline in the heart and the blood vessels activate natriuretic peptide and vasopressin which activate

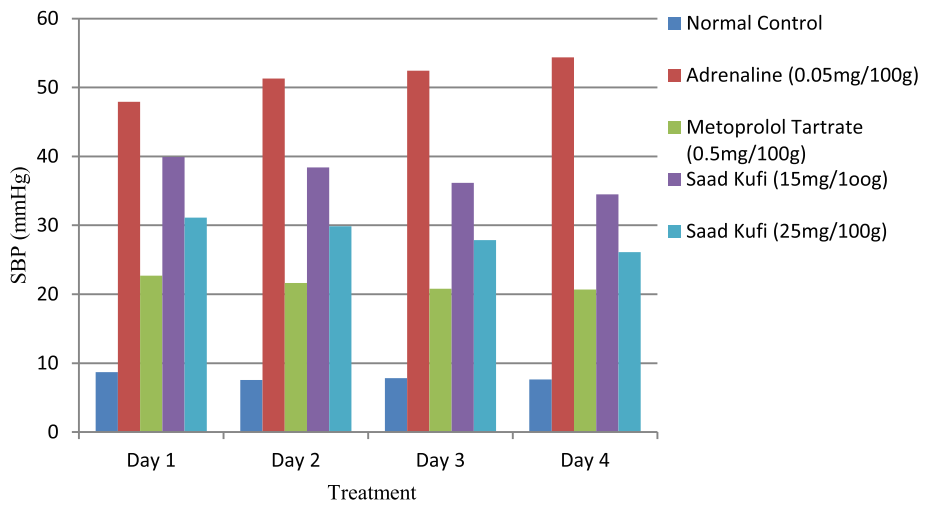

Fig. 1 Induction of systolic blood pressure

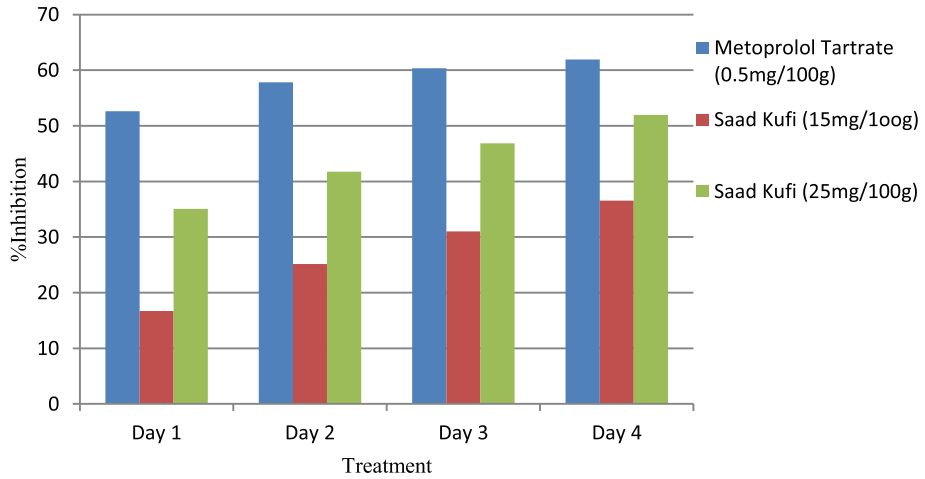

Fig. 2 Inhibition of systolic blood pressure

calcium channel in endothelium which causes the release of calcium and increase of calcium influx. This results in vasoconstriction which results in an increase in blood pressure. (3) Adrenaline binds to β1 receptors present on juxtaglomerular apparatus (JG cells) of the kidney and causes activation of adenylate cyclase (AC) which when activated converts ATP to cAMP, and this cAMP activates protein kinase-A, which causes renin release and so activate Renin Angiotensin Aldosterone System (RAAS) by activating ACE enzyme; this ACE enzyme so produced converts angiotensin-I to angiotensin-II which is a potent vasoconstrictor resulting in an increase in blood pressure [19]. β-adrenergic blocker—metoprolol tartrate—was used as the standard in this study, because of the described previous three mechanisms.

The result showed that SBP was significantly increased in adrenaline-treated animals as compared to normal rats in the tail-cuff method with AD instrument; this implies that significant hypertension was induced in treated animals.

The results data revealed that SBP was significantly decreased in the standard group and in the test group as compared to the disease control group. As the percentage of inhibition increases day by day in the test group, the study also suggests that the long-term use of Saad Kufi may be beneficial in controlling hypertension. Studies have shown that the good antioxidant activity of plants may be due to polyphenols, which helps in ameliorating endothelial dysfunction by increasing NO, prostacyclin formation, and endothelium-derived hyperpolarizing factor (EDHF)-mediated vasorelaxation and decreases LDL formation and endothelin-1 production. So, the polyphenols responsible for the beneficial effects of vasorelaxation have been accredited to blood pressure-lowering properties in rodents [20, 21]. In a study conducted by Kalim et al. [22], the 50% methanolic extract of *C. scariosus* showed a significant amount of polyphenols with potential antioxidant activity. Thus, the antihypertensive effect of Saad Kufi (*C. scariosus* R.Br) may be due to the polyphenols having an antioxidant effect.

Conclusion

The present study revealed that 50% of the ethanolic extract of Saad Kufi (*Cyperus scariosus* R.Br) root showed a dose-dependent and statistically significant inhibition of adrenaline-induced hypertension. The anti-hypertensive effect of the test drug went on increasing over 4 days. The use of Saad Kufi (*Cyperus scariosus* R.Br) root as medicine might have beneficial effects in the management of hypertension. Concomitant administration of the drug might be helpful in better management of hypertension along with available antihypertensive drugs; however, this has to be confirmed by clinical studies.

Abbreviations

ANOVA: Analysis of variance; EESK: 50% Ethanolic extract of Saad Kufi; i.p: Intraperitoneally; p.o: Per orally; SBP: Systolic blood pressure

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Plant authentication

The roots of Saad Kufi (*Cyperus scariosus* R.Br) was procured from the local market of Aligarh (Baradwari) and identified in the Pharmacognosy Section, Department of Ilmul Advia, A. K. Tibbiya College, Aligarh Muslim University. They were also authenticated by the National Institute of Science Communication and Information Resources, New Delhi (NISCAIR/RHMD/Consult/2017/3064-13-1). The sample of the test drug was submitted to Mawalid-e-Salasa Museum of the Department after identification, for future reference with the voucher no. SC-0220/17.
All other materials used in the study were provided by the Department of Ilmul Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh.

Authors' contributions

S N: Investigation, validation, writing the original draft, formal analysis, and data curation. S Z R: Co-supervision and resources. KMY A: Conceptualization,

methodology, and formal analysis. The authors have read and approved the manuscript.

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Availability of data and materials
All data and material are available upon request.

Ethics approval and consent to participate
All experimental procedures and protocols used in the study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) dated August 26, 2017, Jawahar Lal Nehru Medical College, Aligarh Muslim University, Aligarh, and care of laboratory animals was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The registration number is 401/GO/Re/S/2001/CPCSEA.

Consent for publication
Not applicable

Competing interests
No competing interests to declare.

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