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Study of Avicennan unani drug saad kufi (*Cyperus scariosus* R.Br) for cardiac activity on isolated Langendorff rat heart

Sana Nafees[1*], Huda Nafees[2], Kunwar Mohammad Yousuf Amin[1] and Syed Ziaur Rahman[3]

Abstract

Cardioprotective Unani drug, Saad Kufi (*Cyperus scariosus* R.Br.), mentioned by the intellectual colossus Ibn Sina (Avicenna) a thousand years back in his book, "*Kitab al-Adviya al-Qalbia*" and still widely used by Unani physicians, was selected for experimental study. The main objective of the study was to explore the trial drug ex vivo for validation, hemodynamic elucidation, and molecular mechanism identification for the improvement of Unani therapeutics and to identify a safe and effective novel cardiovascular drug for mainstream medicine. Five doses of 50% ethanolic extract of the physiochemically standardized root were studied in a normal rat heart, on a semi-automated Langendorff apparatus supported with an advanced data acquisition system, perfused with carbogenated non-recirculating Kreb's-Hensleit solution, at constant flow mode, by continuous recording. The effect of 6 repetitions ($n=6$) of each dose was studied with the following parameters on the Lab chart pro version: heart rate, left ventricular developed pressure, cardiac work, and coronary pressure. The significance of the difference was determined by the student's "t" test. Results of all doses of Saad Kufi showed a significant increase in the above parameters. The maximum increase was observed by Dose III. It could be concluded that Unani's clinical use of Saad Kufi (*C. scariosus* R.Br) as cardioprotective was validated and higher doses were indicated to be clinically optimal. Hemodynamically and molecular mechanism-wise, it is indicated to be a sympathomimetic or inotropic-like agent and possibly a direct vasodilator. Since existing long-term treatment of heart failure is not done by cardiac stimulants or positive inotropic and chronotropic agents, the possible future clinical demonstration of therapeutic improvement in heart failure will bring Saad Kufi as a novel drug in mainstream medicine.

Keywords Unani medicine, *Cyperus scariosus*, Cardiotonic, Langendorff heart, Avicenna (Ibn Sina)

*Correspondence:
Sana Nafees
dr.sananafees@gmail.com
[1]Department of Ilmul Advia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh, India
[2]Department of Saidla, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh, India
[3]Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India

Introduction

Cardiovascular diseases (CVDs) are the foremost cause of death globally, taking an estimated 17.9 million (31%) lives each year [1]. Therapeutic management of CVDs with optimum efficacy and a reasonable degree of safety by available market drugs is far from realization. The three major Traditional Medicines (TMs): Unani medicine, Ayurveda, and Chinese Traditional Medicine, are globally accepted as novel, effective, and safe medicines. However, they require evaluation by scientific methods to be used to their full potential. Treatments based on traditional medicines are used in various cardiovascular diseases like angina pectoris, congestive heart failure, atherosclerosis, arrhythmia, systolic hypertension, venous insufficiency, and cerebral insufficiency [2]. Ibn Sina (Avicenna, 980–1030 CE) the most significant thinker and writer of the Islamic middle age, first time systematized individual cardiac drugs based on pathophysiological and rational-empirical pharmacology in his medical book "The treatise on cardiac drugs" or *"Kitab al-Adviya al-Qalbia"*. It is one of Ibn Sina`s important medical books on simple and compound medicines used in the treatment of cardiac diseases [3]. In this monograph, he discussed 63 cardioactive drugs and their therapeutic effects on the cardiovascular system like "*tawahhush*", palpitation or "*khafaqan*", syncope or "*ghashy*" [4, 5], and heart weakness or "*za`af al-qalb*" as well as tried to connect (by novel correlations) his cardiac hypothesis with psychiatric ailments [6].

The present study was undertaken on normal rat Langendorff heart by semi-automated, DAQ-supported equipment (AD Instruments, Australia) to validate and elucidate the hemodynamic parameters and to some extent the molecular mechanism of Avicenna's cardiovascular drug, Saad Kufi (*Cyperus scariosus* R. Br.) that is still widely used by Unani physicians for conditions that overlap with congestive heart failure, angina and cardiac arrhythmias.

Ibn Sina described Saad Kufi as the root of a plant that is long and slender in shape with nodes on its surface [7–9]. It is a grass-like perennial plant that grows in warm temperate and tropical regions of the world [10, 11]. In Unani pharmacognostic literature, called *Mahiyat*, the roots are described as thick, elongated, slender, and black in colour with a pungent taste and aromatic smell [8, 9]. The Unani traditional actions (*Af'al*) or therapeutic uses (*Iste'malat e Ilaji*) ascribed to it are exhilarant [9], cardiotonic [12], stimulant [13], nervine tonic [12, 14–16], deobstruent [7–9], astringent, desiccant [17], carminative [7, 12, 15], appetizer [16, 18], anti-emetic [8, 9, 15], aphrodisiac [9, 18]. It also strengthens the urinary bladder [8, 9], attenuates palpitation [9, 14, 18], general weakness [12], chronic ulcers and ascites [18].

In cardiovascular research, the Langendorff heart model, better known as the "working heart" is an invaluable research tool as it uniquely allows to study inotropic, chronotropic, and coronary vascular effects of various interventions without interference of the endogenous substances such as hormones, catecholamines, autocoids, peripheral vasculature and in the absence of other organs thus solely examines the intrinsic properties of the heart [19, 20]. The Langendorff's isolated perfused mammalian heart depicts optimal compromise in the conflict between the quality and quantity of data that can be acquired from an experimental model versus its clinical relevance [21].

Materials and methods

Chemicals

Adrenaline, Propranolol, Heparin and all other chemicals were purchased from Aldrich Sigma Chemical Co. (USA).

Plant material

The test drug Saad Kufi (*C. scariosus* R. Br.) was procured from the local market of Aligarh in the month of April 2017. The proper identification was done according to the morphological features described in botanical and Unani classical literature which is then confirmed in the Department of Ilmul Advia at Pharmacognosy section, Aligarh Muslim University, Aligarh. It was also authenticated by the National Institute of Science Communication and Information Resources, New Delhi (NISCAIR / RHMD/ Consult/ 2017/ 3064-13-1). The sample of this test drug was submitted to *Mawalid-e-Salasa* Museum, Department of Ilmul Advia with the voucher number SC-0220/17 for future reference.

Preparation of extract

Roots of Saad Kufi (*C. scariosus* R. Br.) were cleaned from dust and earthy material, rinsed with double distilled water, dried at 45° C in a hot air oven, and powdered in an electrical grinder. Extraction was carried out in Soxhlet's apparatus by 50% ethanolic solvent for 6 h. The extract was filtered and dried on a water bath using evaporation. The yield was computed using the crude drug as a reference and was found to be 13.07%. During the experiment, a fresh suspension of the extract was prepared in distilled water (as per calculation w/v) and was infused on the Langendorff isolated heart.

Selection of animals

The present investigation used male Wistar albino rats (n=6) weighing 200–250 gm. These rats were procured from the Animal House of the Jawaharlal Nehru Medical College, Aligarh, India. The animals were placed at random and allocated to treatment groups in polypropylene

cages with paddy husk as bedding. The animals were kept at a temperature of 24 °C and a relative humidity of 30–70%. A light-dark cycle was observed. All animals were fed standard balanced meals and provided with water *ad libitum*.

Ethical statement

Jawaharlal Nehru Medical College's Institutional Animal Ethical Committee (IAEC) reviewed and approved all experimental procedures and protocols used in the study on August 26, 2017, and laboratory animals were cared for according to the guidelines laid by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). 401/GO/Re/S/2001/CPCSEA is the registration number.

Study design

This is an ex vivo study done on isolated/ excised rat hearts mounted on Langendorff apparatus with the physiological environment as shown in Fig. 1. To this day, the Langendorff heart model continues to be the stalwart investigational tool in cardiovascular and pharmacological research because of its simplicity and experimental reproducibility [22]. The test drug was screened

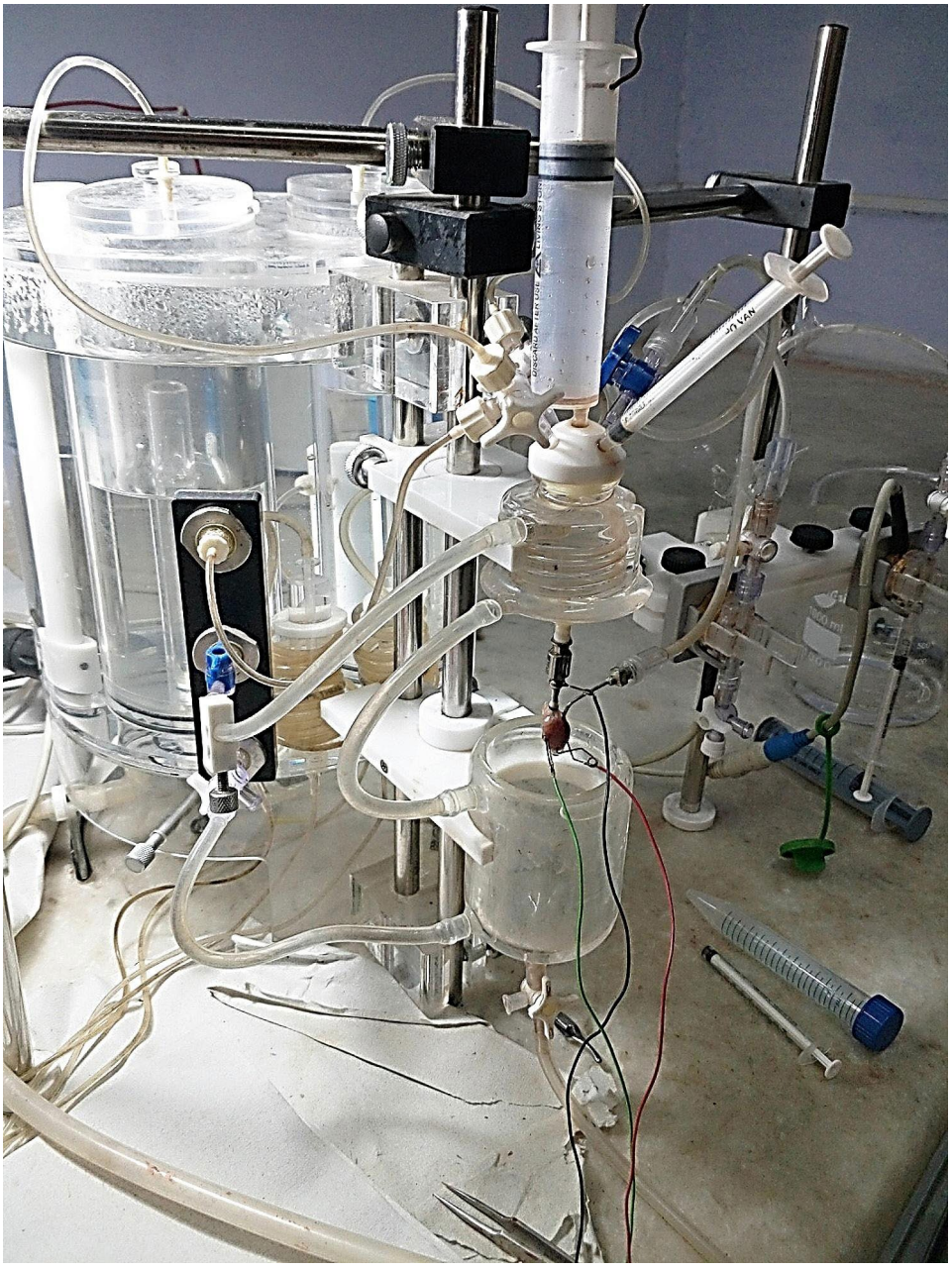

Fig. 1 Isolated rat heart mounted on Langendorff apparatus

for cardiovascular effects in male Wistar 6 albino rats weighed 200–250 g on isolated perfused rat hearts by using the Langendorff-perfused model and method, with modification [23]. The effect of 6 repetitions ($n=6$) of each dose of test drug without standard agonist or antagonist, 4 repetitions of standard drugs [agonist and antagonist ($n=4$)] and 4 repetitions ($n=4$) of test drug dose which shows maximum response with standard agonist or antagonist were studied on normal heart (Langendorff Preparation). Mean values of pre-treatment and post-treatment were calculated for all physiological parameters being assessed.

Doses

Doses of the test drug extract were determined by multiplying the Unani clinical dose by a conversion factor of 7 for rats [24]. It was found to be 15 mg/100 g (corresponding to the Unani clinical dose), Five doses of 50% ethanolic extract of Saad Kufi were studied. One dose was lesser than the clinical dose and four doses were higher than the clinical dose. Hence, the doses were Dose I: 7.5 mg/100 g, Dose II: 15 mg/100 g, Dose III: 25 mg/100 g, Dose IV: 35 mg/100 g and Dose V: 45 mg/100 g.

Animal and isolated heart preparation

Wistar Albino rats (200–250 g) were given Heparin sodium (100 IU, intraperitoneally), 30 min before anaesthetization by injecting urethane (1.5 g/kg I.P). Prepared animals were kept in a supine position, pericardium was removed after opening of the thorax. Rapid excision of heart was done and immediately placed in ice-cooled (4 °C) Krebs-Heinsleit solution (NaCl, 6.90 g/l; KCl, 0.35 g/l; NaHCO3, 2.10 g/l; CaCl2, 0.28 g/l; MgSO47H2O, 0.29 g/l; KH2PO4, 0.16 g/l; Glucose, 2.0 g/l) aerated with carbogen (95% O2%,5% CO2). On the Langendorff Apparatus (AD Instruments, Australia), the heart was cannulated at the aorta and promptly perfused retrogradely with oxygenated non-recirculating K-H solution for coronary perfusion. The temperature of the circulated water that surrounded the perfusate chambers, oxygenator, and chamber containing the heart was kept at 37^0C by a thermostatically controlled water-jacketed system and the perfusate was gassed with carbogen. Any extra tissue was trimmed and the aorta was tightly secured. A pressure transducer was put into a domestic wrap balloon, filled with water was inserted into the left ventricle via the left auricle (Mitral valve), balloon pressure was maintained at 12–18 mm Hg. In this investigation, the constant flow model was employed.

Treatment protocol

The drugs were administered in bolus form directly into the heated junction Block (SP 3797). The target coronary perfusion pressure range was 70–80 mmHg and was attained by appropriate adjustment of the pump flow rate. Predetermined quantities of test drug extracts were dissolved in and diluted with distilled water. Standard agonist and antagonist solutions were diluted with distilled water. All drug solutions were filtered and equilibrated and maintained at a temperature of 37 ± 0.5 °C for 15 min. The heart was perfused at constant flow with samples for a period not exceeding 10 min.

Effect of test drugs on normal heart (Langendorff Preparation)

Five doses of test drug were studied directly on the rat's normal heart, one lesser than the dose corresponding to the Unani clinical dose and 3 doses higher than the clinical dose. The doses thus determined were Dose I: 7.5 mg/100 g, Dose II: 15 mg/100 g, Dose III: 25 mg/100 g, Dose IV: 35 mg/100 g, Dose V: 45 mg/100 g. The independent effect of 6 repetitions of each dose of the test drug was seen and assessed on mean values from pre-treatment to post-treatment for all physiological parameters on the lab chart.

Effect of test drugs on the response to standard agonists and antagonists in normal heart (Langendorff Preparation)

The dose that showed a maximum response in the first test was taken for study in this test, which was Dose III i.e. 25 mg/100 g. The standard agonist viz. adrenaline and standard antagonists viz. propranolol was studied in the dose of 0.1 mg/100 g and 0.1 mg/100 g respectively. Four repetitions of standard ligand were studied alone then four repetitions of each ligand followed by test drug (Dose III) after 1 min of ligand administration were seen and assessed on mean values from pre-treatment to post-treatment for all physiological parameters.

Physiological parameters monitored

Coronary Pressure (CP), Coronary Flow (CF), Left Ventricular End-diastolic Pressure (LVEDP), Perfusate Temperature, Heart Rate (HR), and Left Ventricular Systolic Pressure (LVSP) were the physiological parameters that were continually observed and recorded. Subtracting LVEDP from LVSP yields Left Ventricular Developed Pressure (LVDP). Cardiac Work (CW) was determined by calculating the product of the LVDP and the HR.

A StathamR pressure transducer was linked to the side arm of the aortic cannula to detect CP, and a StathamR pressure transducer was connected to the water-filled domestic wrap balloon put into the left ventricle to monitor LVEDP. A computer-based Data Acquisition System-supported Langendorff Apparatus (AD Instruments, Australia) was used for continuous recording as shown in Fig. 2. From the various pressure readings, the software calculated the HR. A bespoke component of the software was used to analyze the raw data. The values for a certain

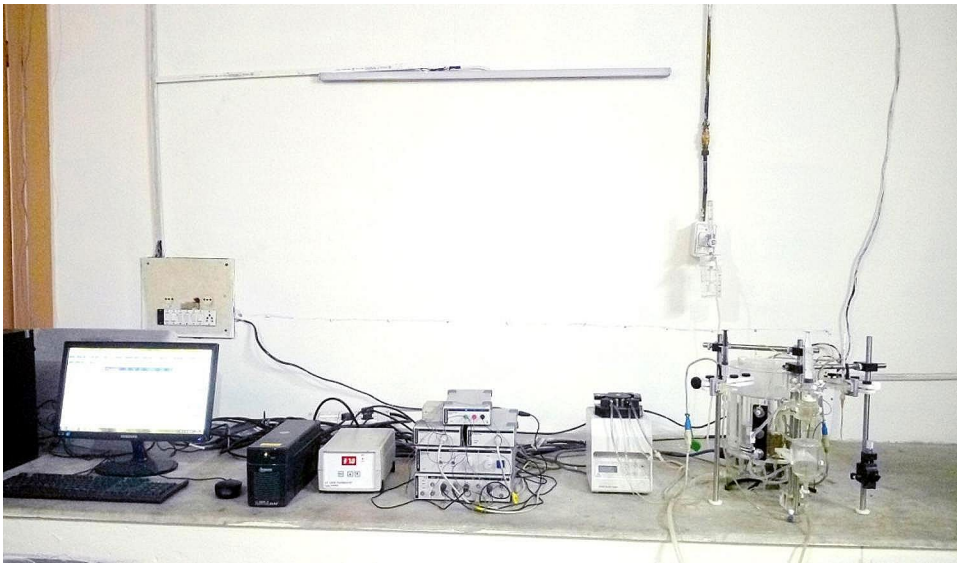

Fig. 2 Set up for recording the physiological parameters on lab chart

set of data points were averaged using this software. The data was relocated to Microsoft Excel, and the variable means were calculated. The percentage (%) change from pre-treatment to post-treatment was used to express the data.

Statistical test

The result obtained of each parameter in various groups of different dosing was statistically compared to determine the significance of difference by the Students "t" test. Values were compared from pre-treatment to post-treatment. Secondly, the post-treatment values of standard ligands plus each test drug were compared with the post-treatment values of the ligands given alone by One-way ANOVA analysis with the Tukey-Kramer multiple pair comparison test. The analysis was carried out by using GraphPad (8.4.3) InStat Software.

Results

Five doses of 50% ethanolic extract of Saad Kufi were studied and mean values from pre-treatment to post-treatment for all physiological parameters on the lab chart (Fig. 3) were calculated. One dose was lesser than the clinical dose and four doses were higher than the clinical dose (Dose I: 7.5 mg/100 g, Dose II: 15 mg/100 g, Dose III: 25 mg/100 g, Dose IV: 35 mg/100 g and Dose V: 45 mg/100 g).

Effect of test drugs on normal heart (Langendorff Preparation)

Results showed that Dose III of Saad Kufi (*Cyperus scariosus* R. Br.) increased the heart rate significantly ($p<0.01$) with an increase of 32.28% while Dose V significantly decreased the heart rate amounting to a decrease of 34.39% as shown in Fig. 4. Dose I, II, III, IV of Saad Kufi (*Cyperus scariosus* R. Br.*)* significantly increased the cardiac work, and the maximum increase was shown by Dose III with an increase of 85.04%. Dose V significantly decreased the cardiac work with a decrease of 25.84%) ($p<0.05$). All doses (Dose I, II, III, IV, V) of Saad Kufi (*Cyperus scariosus* R. Br.*)* significantly increased the coronary pressure while the maximum increase was by Dose III which is 65.35% increase ($p<0.05$).

Effect of test drugs in response to standard agonist and antagonist

The dose that shows the maximum response in the first test was taken for the study, that is Dose III of test drug (25 mg/100gm). The standard agonist viz. adrenaline and standard antagonists viz. propranolol were studied in the dose of 0.1 mg/100 g and 0.1 mg/100 g respectively. 4 repetitions of standard ligand were studied alone then 4 repetitions of each ligand followed by each test drug (Dose III) after 1 min of ligand administration were seen on mean values from baseline to post-treatment for all physiological parameters being assessed as shown in Fig. 5. Results showed that Dose III (25 mg/100gm) of Saad Kufi potentiates the studied cardiac parameters when treated with standard agonist- adrenaline, however test drug at the same dose reverses the effect of standard antagonist-propranolol.

Discussion

The Langendorff Perfused Heart Model is an experimental procedure in which an excised heart has a cannula inserted into its aorta so that the heart can be retrogradely perfused via the coronary artery. The procedure has been improved in recent times, and these

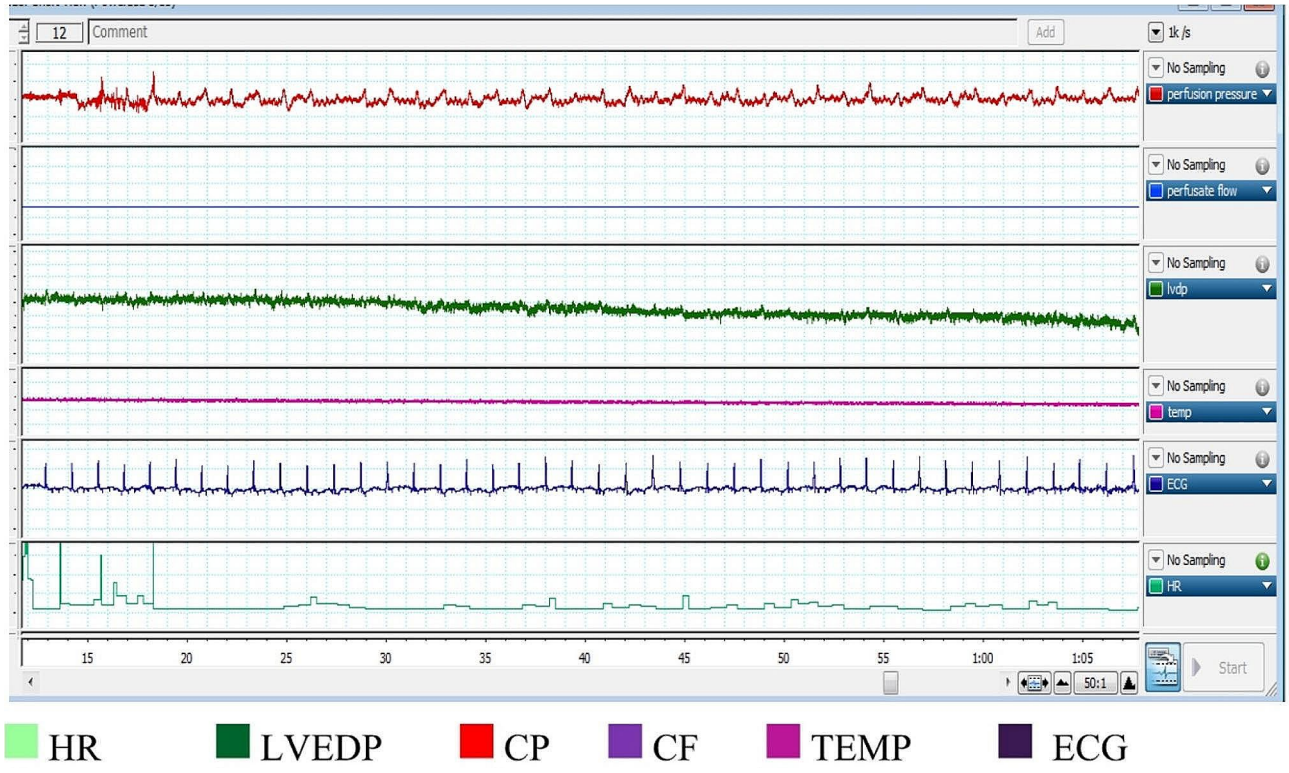

Fig. 3 Effect of Saad Kufi (*Cyperus scariosus*) On cardiac parameters on lab chart

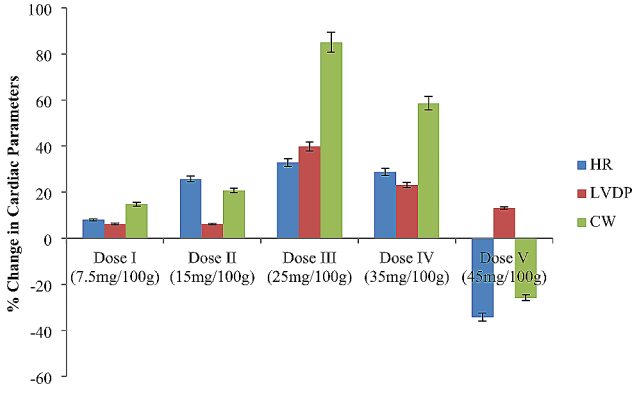

Fig. 4 Percent change in cardiac parameters of isolated rat hearts after test drug treatment at different doses. Values are given as Mean ± SD. Analysis by one-way ANOVA and values were considered significantly different at $p < 0.05$

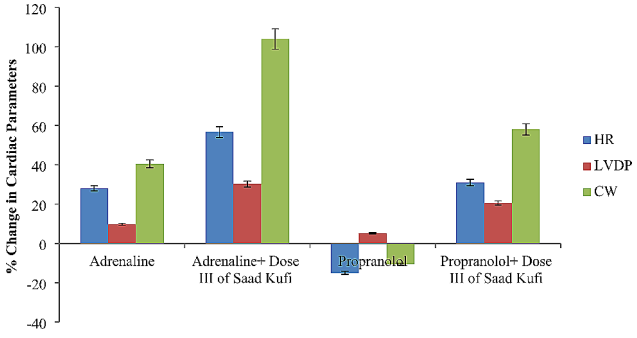

Fig. 5 Percent change in cardiac parameters of isolated rat hearts after treatment of test drug with standard agonist (adrenaline) and antagonist (propranolol). Values are given as Mean ± SD. Analysis by one-way ANOVA and values were considered significantly different at $p < 0.05$

improvements are used to evaluate the direct effect of medication on the heart as well as the effect of ischemia-reperfusion injury on heart function [25]. In this study, five doses of the test drug were used to investigate the direct effect on the rat normal Langendorff heart, one lesser than the dose corresponding to the Unani clinical dose (Dose II) and 3 doses higher than it, primarily for exploring the dose-response relationship. This higher dose of selection was taken because it had been reported in several studies that Unani drugs frequently failed to produce an appreciable degree of effect at traditionally described low doses taken for the concern of safety, however, they show a good degree of traditionally reported effect at higher doses [26]. Our study corroborates with this as the maximum effect was shown by Dose III-25 mg/100 g.

Saad Kufi (*Cyperus scariosus* R. Br.) increased the Left Ventricular Developed Pressure (LVDP) and Cardiac Work (CW) significantly, and increased the force of cardiac contraction thus it could be indicated to be cardiac stimulant rather than cardiotonic (in the Western Medicine sense i.e., digoxin like). It also significantly increased the heart rate (HR), which further supported the cardiac

stimulant effect. These effects are due to presence of cardiac glycosides as seen in previous study [27]. Sympathomimetic and inotropic drugs increase the cardiac work and heart rate along with force of cardiac contraction whereas cardiotonic drugs like digoxin increases the force of cardiac contraction without increasing cardiac work and heart rate.

The above findings and the indicated stimulant rather than cardiotonic effect could be due to sympathomimetic activity, particularly at β1-adrenoceptors. In the test for studying the interactions of Saad Kufi (*Cyperus scariosus* R. Br.) with the standard agonists and antagonists, the test drug along with adrenaline increases heart rate, left ventricular diastolic pressure, and cardiac work so from its hemodynamic pattern, namely, increase in cardiac contraction, cardiac work, and heart rate, it could be suggested that the test drug could possess sympathomimetic activity particularly at β1-adrenoceptors as previous studies shows that adrenaline produced stimulatory effect on both heart rate and force of contraction of isolated hearts, probably through an activation of beta-adrenoceptors [28, 29]. Saad Kufi increases the propranolol-induced reduction in HR and CW, thus it is a strong β-adrenoceptor agonist as it can reverse the effect of the standard and strong β1-adrenoceptor antagonist propranolol. Propranolol slows down the strength of the heart's contractions and reduces its oxygen requirements [30].

Although the present study is the first to evaluate the hemodynamic and cardiac receptor effects of Saad Kufi (*Cyperus scariosus* R. Br.) but the test drug and its sister species have been subjected to many allied beneficial cardiac effects as investigated in earlier studies, such as anti-lipidemic effect, anti-oxidant effect, anti-obesity effect, anti-hypertensive effect supports the use of this drug for cardiovascular diseases (CVDs). The demonstration of the anti-hypertensive effect [31] conflicts with the cardiac stimulant and possibly sympathomimetic effect shown in the Langendorff test of the present study. This discrepancy can be explained by reflex vasodilatation, reflex reduction in sympathetic outflow, or/and direct vasodilatory effect. Chawda et al., [32] investigated the lipid-lowering and antioxidant effects of root of *C. scariosus* R. Br. hydroalcoholic extract on guinea pigs that are fed with high cholesterol diet. Results show a decrease in serum lipid profile and atherogenic indices. Polyphenols with excellent antioxidant activity were found in the 50% methanolic extracts of *C. scariosus* derived from various plant sections, as demonstrated by the scavenging of DPPH, ABTS+, NO, OH, O2.-, and ONOO-. It had a lot of potential for reducing oxidative DNA damage and acting as a radical scavenger. The extracts had a large amount of total phenolic and total flavonoid content, both of which contribute to their antioxidant properties [33]. Barai conducted a clinical comparison of two source plants, Motha (*Cyprus rotundus* R. Br.) and Nagarmotha (*Cyprus scariousus*) in Sthaulya (Obesity). The study concluded that Nagarmotha (*C. scariousus* R. Br.) and Motha (*Cyperus rotundus* R. Br.) help lower the characteristic signs and symptoms of obesity in subjective criteria as well as weight and BMI in objective criteria [34].

Conclusion

The present study showed that the test drug increased cardiac contraction and possessed both positive inotropic and chronotropic effects as well as agonistic effects on β1-adrenoceptors. It, thereby, supports the Unani clinical use of Saad Kufi (*Cyperus scariosus* R. Br.) in heart failure by showing cardiac stimulant effects in this hemodynamic and receptor-based study. Its Unani clinical use as a cardiotonic agent (as understood in Unani medicine i.e., strengthening of heart) was not only validated by this study but a possible advancement was also indicated by suggesting that higher doses could be clinically more effective.

Since existing long-term treatment of heart failure is not done by cardiac stimulants or positive inotropic and chronotropic agents, on account of toxicity and long-term reversal of hemodynamic benefits, the possible future clinical demonstration of long-term therapeutic improvement in heart failure would bring Saad Kufi (*Cyperus scariosus* R. Br.) as a novel drug in mainstream medicine producing safe and stable long-term benefit despite being an inotropic agent.

Abbreviations

CP	Coronary Pressure
CW	Cardiac Work
HR	Heart Rate
IP	Intraperitoneally
IU	International unit
LVDP	Left Ventricular Developed Pressure
LVEDP	Left ventricular End-diastolic Pressure
LVSP	Left Ventricular Systolic Pressure

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Author contributions

Data gathering and idea owner of this study: SN, KMYA, Study design: SN, KMYA, Data gathering: SN, Writing and submitting manuscript: SN, SZR Editing and approval of final draft: SN, HN, SZR, KMYA.

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Data availability

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Declarations

Competing interests

There is no known conflict of interest associated with this publication.

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