Cardiotonic and vasoconstriction effects of aqueous methanolic extract of *Paspalidium flavidum* L.

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Abstract: The cardiovascular activity of aqueous methanolic extract of *Paspalidium flavidum* L. was evaluated on isolated rabbit heart and aorta. Heart rates, force of contraction and perfusion pressure were assessed in the presence of different concentrations of extract and adrenaline by using Langendorff's technique. Moreover, the vasoconstriction effects were studied in rabbit aorta using isolated organ bath. The results indicated that the extract (1ng-100µg/ml) exhibited a significant increase in heart rate, contractility and perfusion pressure of isolated rabbit's heart; with a maximum effect at 1ng/ml, which was comparable to adrenaline (1µg/ml). Similarly, adrenaline at doses from 1-10µg/ml produced a significant dose dependant increase in all the cardiac parameters. The cardiotonic effects of the extract were significantly blocked by propranolol (10^{-5}M) while an increase in perfusion pressure was completely antagonized by verapamil (10^{-6}M). Activity of cardiac marker enzymes was also significantly raised in the perfusate of isolated heart pretreated with the extract. In rabbit aorta, the extract exhibited a dose dependent vasoconstriction effect however it did not increase the tone of aorta when pre-treated with verapamil (10^{-6}M). It is conceivable therefore; that the cardiotonic and vasoconstriction effects of the extract might be due to its agonistic actions on β-receptors and Ca^{+2} channels.

Keywords: *Paspalidium flavidum* L., cardiotonic, propranolol, vasoconstriction, verapamil.

INTRODUCTION

Cardiovascular diseases (CVDs) have become a major problem and are increasing throughout the world (Isselbacher, 2001). The major risk factors for heart diseases include family history, sex, increased lipid levels, hypertension, obesity and cigarette smoking. Most of these risk factors are prevalent in developing countries due to the lack of resources and infra-structure (Trivedi and Nehra, 2004). Today, a large number of pure synthetic drugs are being used for the treatment of various CVDs, however most of the people of developing countries still rely on traditional herbal medicines for their health related needs. According to a survey by WHO, it has been estimated that about 60% of the world's population rely on traditional medicines. Moreover, it has also been reported that approximately 25% of these modern drugs are from plant origin and used either in their pure form or in natural form (Kutchan, 1995).

Now, recent attention has been focused on the herbal medicines as they can provide alternative remedies for the treatment and prevention of various cardiovascular problems. Ethnobotanical surveys of medicinal plants also indicate their extensive use in the treatments of cardiovascular disorders. For example, plants like *Syzygium guineense, Passiflora nepalensis* Wall, *Ginko biloba*, *Stephania tetandra* and *Uncaria rhynchophylla* have been used to treat hypertension and other cardiac diseases. Similarly, medicinal plants such as *Digitalis purpurea, Crataegus monogyna* (Hawthorn), *Allium sativum* and *Rauwolfia serpentina* have been used for the treatment of congestive heart failure, hypertension, arrhythmias and atherosclerosis (Jerie, 2007; Weng *et al*., 1984; Sutter *et al*., 2007). Traditional medicines, though effective and potent medicines for a number of diseases, require further scientific evaluation in order to be used to their full potential.

Paspalidium flavidum L. (Family: Poaceae) commonly known as Madhana Ghas is a plant indigenous to Pakistan that has been largely distributed in subcontinent region (Nazar *et al*., 2008). Ethnobotanical data has indicated that this plant has been used to treat various skin, teeth, liver and heart disorders (Hussain *et al*., 2010). Hence, this study was conducted to confirm and scientifically appraise the folkloric claim of *Paspalidium flavidum* L.

MATERIALS AND METHODS

Chemicals and drugs

Propranolol hydrochloride, verapamil hydrochloride, phenylephrine hydrochloride and heparin were purchased

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from Sigma Chemicals Co. Standard aspartate transaminase (AST), alanine aminotransferase (ALT), creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) kits (Merck Chemical Co, Germany) were also used. All other chemicals used in this study were of analytical grade.

Animals

Both male and female rabbits of local strain (*Oryctolagus cuniculus*) weighing 1-1.5 kg were used. All the animals were housed in controlled environment (23-25°C) and treated in accordance with National Institute of Health (Pakistan) guidelines. The study protocol was approved by the local ethical committee.

Plant material

Paspalidium flavidum L. (whole plant) was collected from a garden in Jauharabad; district Punjab, Pakistan during the month of August, 2011. The plant was identified and authenticated by Haider Ali Shakir (Assistant Director, Soil conservation).

Plant extraction

Aqueous methanolic (70:30) extract of powdered plant material was prepared by using cold maceration process (Alamgeer *et al*., 2012). The extract was then air-dried and a solid mass was obtained with a percentage yield of 16%.

Effect of crude extract on various cardiac parameters of isolated rabbit heart

The experiment was performed at constant flow mode according to the method prescribed by Langendorff (1895). Each rabbit (n=6) received intra-peritoneal injection of heparin (1000 units), 30 minutes before dissection. The animal was sacrificed and the heart was immediately removed from the pericardial sac, with 1 cm aorta. The heart was then cleaned of any excessive tissue and mounted on the Langendorff's apparatus containing Krebs-Henseleit solution maintained at 37°C (Radnoti isolated heart system, AD Instruments, Australia). The aorta was tied to the glass cannula with a pressure transducer. A clip was attached to the apex of heart to measure the force of contraction (g) by force-displacement transducer. Both the transducers were attached to the Power Lab data acquisition system and the recordings were measured using Chart 5.0 Pro software.

The preparation was then allowed to equilibrate for 30 minutes before starting the experiment. After stabilization, different doses of the extract (1ng, 10ng, 100ng, 1µg, 10µg, 100µg, 1mg and 10mg/ml) and adrenaline (1, 2.5, 5 and 10µg/ml) were applied to assess various cardiac parameters i.e., heart rate (beats/min), force of contraction (g) and perfusion pressure (mm Hg) with each heart serving as its own control. In order to elucidate the possible mechanism of action, the effect of selected dose of the extract was assessed both in the absence and presence of propranolol 10^{-5} M and verapamil 10^{-6} M (Farid *et al*., 1992; Shatoor *et al*., 2012; Mugabo *et al*., 2012).

Biochemical analysis

Rabbits (n=6) of either sex weighing (1-1.5 kg) were used for this study. The Isolated rabbit heart was allowed to work normally by Langendorff's mode (Langendorff, 1895). After stabilization, perfusate of the heart was collected in measuring cylinder before and after the administration of extract. Cardiac enzyme levels of AST, ALT, CK-MB and LDH were then estimated from the perfusate by enzymatic test kits (Merck Chemical Co., Germany) using Microlab 300 (Singh *et al*., 2009).

Effect of crude extract on isolated rabbit aorta

Rabbit of either sex (n=6) were sacrificed and the descending thoracic aorta was immediately removed. Aorta was then cleaned of any excessive tissue and cut into rings of 2-3mm width. Each ring was mounted in a 25 ml tissue organ bath containing Krebs solution. The tissue was allowed to equilibrate for a period of 1 hour and then stabilized by repeated doses of phenylephrine (10^{-6} M). The vasoconstrictive effects of the extract were studied by adding the doses in a cumulative manner. Changes in isometric tension of aortic rings were assessed with the help of force-displacement transducer attached with Power Lab data acquisition system. In order to determine the possible mechanism, the effect of aqueous methanolic extract of *Paspalidium flavidum* L. was assessed in the presence of verapamil (10^{-6} M) (Gillani *et al*., 2005).

STATISTICAL ANALYSIS

The results were expressed as means ± SEM. Statistical analysis was done by student's t-test using Graph Pad Prism 5 software. Probabilities of less than 0.05 were considered as statistically significant.

RESULTS

Effect of various doses of crude extract and adrenaline on perfusion pressure, force of contraction, heart rate of isolated rabbit heart

The results showed that the aqueous methanolic extract of *Paspalidium flavidum* L. at doses from 1ng/ml to 10 mg/ml exhibited a significant ($p<0.001$) increase in perfusion pressure of isolated rabbit heart. Similarly, the extract produced a significant ($p<0.05$-0.001) positive inotropic and chronotropic effects at doses from 1ng-100µg/ml. Interestingly, the increase in cardiac parameters was much more pronounced at lower doses. A prominent increase in all the three cardiac parameters was observed at 1ng/ml and these effects were comparable to that of adrenaline (1µg/ml); hence this dose (1 ng/ml) was selected to elucidate the possible mechanism of action.

Adrenaline also exhibited a significant ($p<0.001$) dose dependant increase in perfusion pressure, force of contraction and heart rate of isolated perfused rabbit heart (figs. 1 and 2).

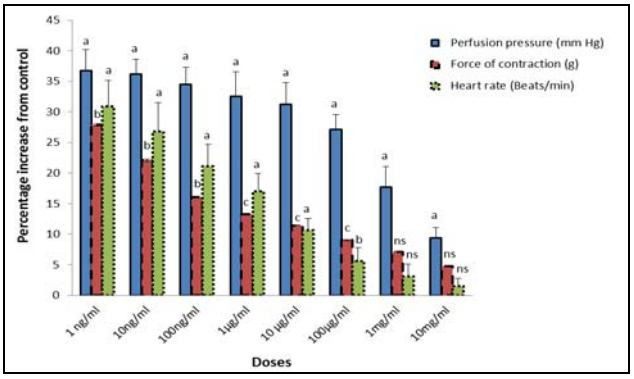

Fig. 1: The effect of different doses of *Paspalidium flavidum* L. (crude extract) on various cardiac parameters of isolated perfused rabbit heart (n=6), where a=($p<0.001$), b = ($p<0.01$), and c = ($p<0.05$) vs. control.

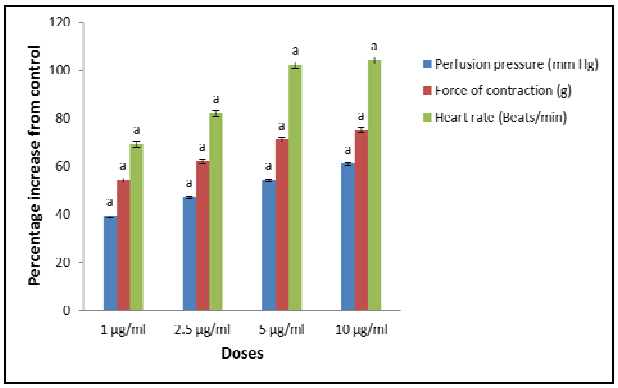

Fig. 2: The effect of different doses of adrenaline on various cardiac parameters of isolated perfused rabbit heart (n=6), where a = ($p<0.001$) vs. control.

Effect of crude extract on perfusion pressure, force of contraction, heart rate of isolated rabbit heart in the presence of both propranolol (10^{-5}M) and verapamil (10^{-6}M)

In the presence of propranolol (10^{-5}M), the extract (1ng/ml) produced a non-significant increase in force of contraction and heart rate while a significant increase in perfusion pressure was observed. Conversely, the rise in perfusion pressure was significantly reduced in heart pre-treated with verapamil (10^{-6}M), whereas force of contraction and heart rate remained raised even in the presence of verapamil (10^{-6}M) (fig. 3 and 4).

Effect of crude extract on cardiac enzyme levels of isolated rabbit heart

The extract also produced a significant ($p<0.001$) increase in cardiac enzymes (AST, ALT, LDH, CK-MB) of the perfusate when compared to normal control (fig. 5).

Effect of crude extract on rabbit aorta

In isolated rabbit aorta, the extract exhibited a dose dependent (0.1-10mg/ml) vasoconstriction effect. This rise in contractile response was completely blocked by verapamil (10^{-6} M) at most of the doses. However, at higher doses of 5 and 10 mg/ml, these vasoconstriction effects were only partially blocked (fig. 6).

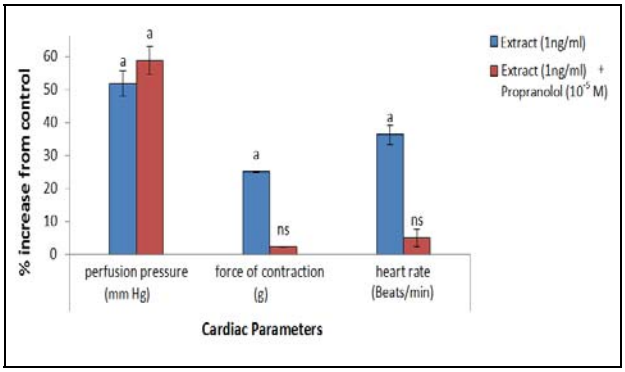

Fig. 3: The effect of *Paspalidium flavidum* L. (crude extract) on various cardiac parameters of isolated rabbit heart both in the absence and presence of propranolol (10^{-5} M) (n=6), where a = ($p<0.001$) vs. control.

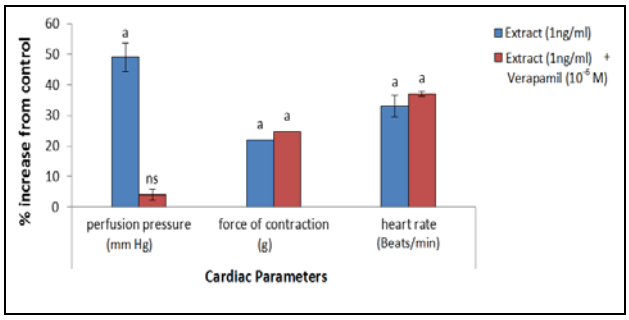

Fig. 4: The effect of *Paspalidium flavidum* L. (crude extract) on various cardiac parameters of isolated rabbit heart both in the absence and presence of verapamil (10^{-6} M) (n=6), where a = ($p<0.001$) and ns = Non-significant vs. control.

DISCUSSION

The present study revealed that the aqueous methanolic extract of *Paspalidium flavidum* L. produced a significant cardiotonic effect in isolated rabbit's heart, which was characterized by positive inotropic and chronotropic actions. A highly significant ($p<0.001$) increase in force of contraction and heart rate was observed at 1 ng/ml, which was comparable to adrenaline (1µg/ml). This positive inotropic and chronotropic effect was significantly blocked by propranolol (10^{-5} M).

It is well established that adrenaline, a sympathomimetic drug acts directly on β1 receptors and produces an increased automaticity, conduction and contractility. Propranolol blocks these receptors and produces a negative inotropic and chronotropic effect (Bain, 1929;

Magnussen and Kudsk, 2009). Hence the cardiotonic activity of the extract might be due to the involvement of β-adrenoceptors. Moreover, the extract produced a significant increase in perfusion pressure of the isolated heart, which was completely blocked by verapamil (10^{-6} M) indicating a vasoconstriction effect. Previously it has been documented that Langendorff's preparation involves perfusion of the heart through the aorta. As the perfusion fluid reaches the aorta, the aortic valve closes and the fluid is then delivered to the myocardium through left and right coronary blood vessels. Any constriction of coronary vessels has been reported to cause a significant rise of the perfusion pressure (Langendorff, 1895). The antagonizing effect of verapamil thereby illustrated that the extract produced constriction of coronary vessels probably through Ca^{+2} channels. This effect might be dependent on the Ca^{+2} entry through the voltage-dependent Ca^{+2} channels.

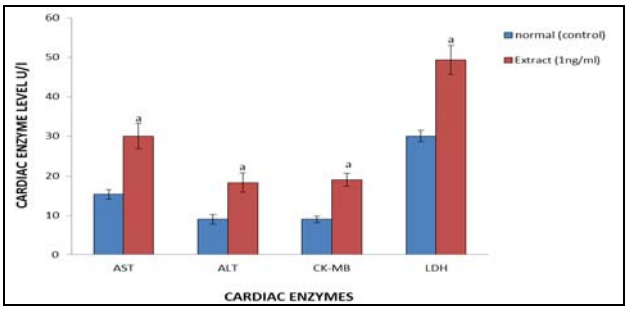

Fig. 5: The effect of *Paspalidium flavidum* L. (crude extract) on cardiac enzymes of the perfusate of isolated rabbit heart (n=6), where a=($p<0.001$) vs. normal (control).

The aqueous methanolic extract of *Paspalidium flavidum* L. also showed a significant increase in the cardiac enzymes levels (AST, ALT, CK-MB and LDH) of the perfusate signifying possible myocardial damage. Cardiac enzymes are generally released from the tissue into the perfusate due to myocardial injury by β agonists like adrenaline. Therefore, the level of these enzymes are raised in the perfusate and decreased in the tissue (Hearse and Leiris, 1979). Moreover, a rise in Ca^{+2} levels has also been involved in the release of these enzymes from the isolated heart (Opie *et al*., 1979). Hence, it could be inferred from the previous findings that the extract might had exerted its effects by β agonistic activity or through an interaction with Ca^{+2} channels.

In isolated rabbit aorta, the extract produced a significant dose dependant vasoconstriction effect. This effect was completely antagonized by verapamil at all the doses except at 5 and 10mg/ml, on which a partial blockade was observed. These results indicated that vasoconstriction was mainly dependent on the influx of (Ca^{+2}) through voltage-gated calcium channels. However, partial blockade at higher doses might be due to interaction with some other receptors (Gillani *et al*., 2005). The vasoconstriction effect in isolated aorta further confirmed the involvement of Ca^{+2} channels in constriction of coronary vessels of isolated perfused rabbit heart.

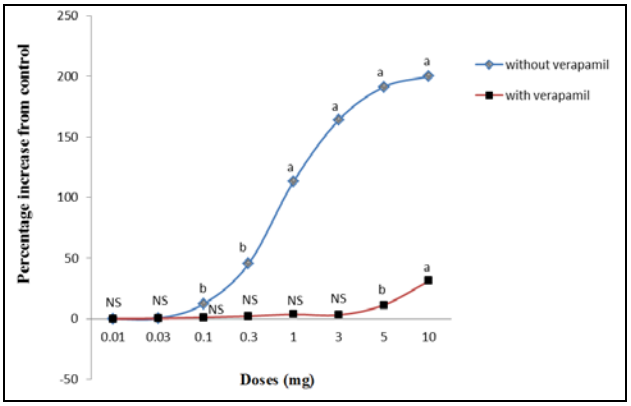

Fig. 6: Dose dependant vasoconstriction effects of aqueous methanolic extract of *Paspalidium flavidum* L. on basal tone with and without verapamil (10^{-6} M) in isolated rabbit aorta (n=6), where a=($p<0.001$), b=($p<0.05$) and ^{NS} = Non-significant vs. control.

It is therefore concluded from this investigation that the aqueous methanolic extract of *Paspalidium flavidum* L. showed remarkable cardiotonic and vasoconstriction effects, which might be attributed to its stimulating actions on β-receptors and Ca^{+2}channels. Further studies are however required to isolate the active principles responsible for these cardiac effects and to determine their exact mechanism of actions.

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