Prunus domestica alters functions of frog's heart

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Abstract: Prunes could exert cardiovascular protective effects. Trials have demonstrated antihypertensive effects of *Prunus domestica*. The aim of this study was to find out if prunes could alter cardiac functions that may help understanding the mode of control of hypertension. Changes in rate and contractile force of frogs' heart were recorded using Power Lab. Effects of prunes' extracts: aqueous (10, 20, 40%); methanolic, acetonic, ethanolic and chloformic (10%); were evaluated and compared with other drugs. We tested effects of acetylcholine and atropine (10^{-5}), adrenaline, propranolol, verapamil and diltiazem (10^{-3}); NaC1, KCl, $CaC1_2$, $MgC1_2$ (10% w/v) on frog's heart alone and with prunes/drugs. All extracts of prunes significantly reduced HR and contractile force. Prunes combined with acetylcholine, propranolol or verapamil significantly enhanced bradycardia; whereas it blocked tachycardia produced by epinephrine, atropine or calcium; moreover prunes blocked the significant increase in HR and cardiac contractility produced by $CaCl_2$; and reduced HR along with $MgC1_2$. NaCl and KCl alone or with prunes had non-significant effects on frog's heart. In conclusion, *Prunus domestica* plays a key role in modification of intracellular Ca^{+2} concentration resulting in negative ionotropic and chronotropic effects (similar to cholinergic stimulation and adrenergic or calcium channel blockade) that could lead to hypotensive effects.

Keywords: *Prunus domestica*, frog's heart, acetylcholine, adrenaline, calcium.

INTRODUCTION

Prescribing medications might become very difficult in near future with patients being better informed and expecting more from their physicians. In future, a non-drug medicine i.e. complementary and alternative medicine might be preferred more for treating different ailments (Groves, 2010). As plants show similarity to drugs and are environment friendly, therefore compared to synthetic molecules, they are a more suitable source for obtaining valuable ingredients for maintaining optimum health (Koehn and Carter, 2005).

Various types of prunes have been studied to see their cardiovascular effects. *Prunus serotina ssp*. contains secondary metabolites that promote vascular relaxation and display antioxidant activities. The vasodilating effects of *P. calyculatus* extract were higher than vasorelaxation achieved by acetylcholine, whereas this response was decreased by introducing a soluble guanylate cyclase activity inhibitor, suggesting a possible role of NO/cGMP pathway (Ibarra-Alvarado *et al*., 2010).

Extract of seeds of *Prunus cerasus* reduced the frequency of ventricular arrythmias from their baseline values and significantly accelerated recovery of the post ischemic cardiac function like coronary and aortic flow, left ventricular pressure during reperfusion. Furthermore, this induced protection of cardiac functions significantly reflected a decrease in infarct size (Bak *et al*., 2006).

Blood flow was significantly improved by a conventional Japanese fruit, *Prunus mume*. Active ingredients of this fruit i.e. mumefural, citric acid, malic acid, and furfuryl alcohol were isolated by HPLC (Chuda *et al*., 1999). The fruit-juice concentrate of *Prunus mume* (Bainiku-ekisu) markedly inhibited vascular remodeling by Ang II-induced stimulation of endothelial growth factor receptor and extra cellular signal-regulated kinase as well as resulted in decreased synthesis of reactive oxygen species. Together, these results suggest that the extract of *Prunus mume* is very beneficial in cardiovascular diseases (Utsunomiya *et al*., 2002).

Some researchers also observed inhibition of platelet aggregation induced by ADP, collagen and arachidonic acid possibly due to higher anthocyanin concentration in prunes (Santhakumar *et al*., 2015a)

Prunes are known to possess antioxidant and free radical scavenging activity (superoxide and peroxy radicals) (Morabbi Najafabad and Jamei, 2014), which could be due to higher levels of phenolic compounds than other fruits and vegetables in human diet (Shahidi, 2012). Noratto and his colleagues also observed anti-adipogenic and anti-inflammatory effects of prunes due to reduction of mRNA levels of peroxisome proliferator-activated receptor possibly due to higher concentration of polyphenols (Noratto *et al*., 2015).

One of the important natural products of genus *Prunus* are plums. Previously, in clinical trials, we found that fruit of *Prunus domestica* reduced blood pressure (BP), serum cholesterol and LDL (Ahmed *et al*., 2010).

In this study, we aim to explore possible mechanism of action of *Prunus domestica*. As we already know that BP

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is directly proportional to total peripheral resistance and cardiac output (CO= heart rate x force of contraction) (Katzung and Tervor, 2012), therefore this study would help to determine whether prunes have any role in altering cardiac functions which would further help in understanding the mode of control of hypertension.

MATERIALS AND METHODS

Preparation of prune extract

Dried prunes of plums (*Prunus domestica*) of good quality were collected from market of Islamabad, Pakistan. Three different concentrations of extract (10%, 20% and 40%) were prepared by soaking 10, 20 and 40 grams of prunes in 100ml of methanol, ethanol, acetone, chloroform and distilled water for twenty four hours. Extracted solution were filtered and stored at 4°C till further use.

Preparation of drugs and solutions

Aqueous solutions of different drugs were prepared by dissolving them in distilled water. $A10^{-5}$ strength of acetylcholine, atropine and 10^{-3} of adrenaline, propranolol HCl, verapamil and ditiazem respectively were prepared. In addition, 10% solutions of NaCl, $CaCl_2$, $MgCl_2$ and KCl (10% w/v- 10g in 100ml distill water) were prepared. 10% methanolic, acetonic, ethanolic and chloformic solutions were used.

Ringers' solution was prepared by dissolving 6.5 gm NaCl, 1.4 gm KCl, 0.4 gm NaH_2PO_4, 0.4 gm $NaHCO_3$, 1.08 gm $CaCl_2$ and 20 gm glucose ($C_6H_{12}O_6$) in one liter of distilled water.

Preparation of experimental animals

Frogs (*Hoplobatrachus tigerinus*) were purchased from Ezekiel Animal House Lahore, Pakistan. Approximately equal size of frogs weighing between 150-175 grams was selected for this study. After stunning the frog with a blow on the head, pithing was carried out with a sharp needle through the foramen magnum. After abdominal dissection, sternum was removed to expose the heart. The pectoral girdle was cut using a bone cutter and the pericardium was removed carefully. A pin was passed through the apex of the pericardium and connected to the transducer of Power Lab with the help of a thread. Ringers' solution was added continuously on the heart with a dropper throughout the experiment.

Working heart

We did our research experiments on the Power Lab 4/26 data acquisition system and connected the apex of the heart to a force transducer and bridge amplifier via a pulley system. Tension was applied to the thread and changes in contractile force and heart rate were examined. Effects of selected prune's extracts were evaluated and compared with other drugs.

Steps of the experiment

Different combinations of drug solutions and prunes extract were used to see the effects on cardiac muscles of frogs. Number and force of contractions were recorded before and after administration of every drug. Following are the general steps which are used in this study.

1. Record normal contractions for 30 sec.
2. Add five drops of drug(s) or prunes extract drop by drop on the heart and wait for 15 sec. Then record contractions for 30 sec.
3. Wash the heart with the ringer's solution. Record the normal contractions for 30 sec and add 5 drops of other drug(s) drop by drop on the frog's heart and wait for 15 sec. Then record the contractions for 30 sec.

Apparatus/ Equipment

Power Lab 4/26, Cat No: ML846; Ad Instruments, Australia

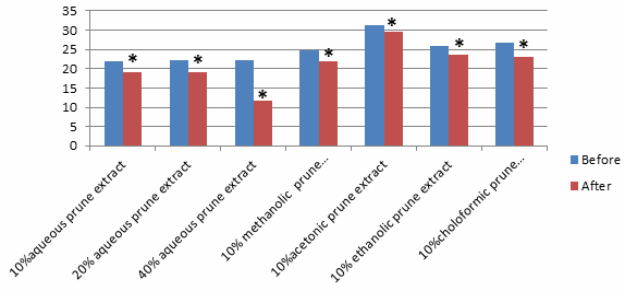
STATISTICAL ANALYSIS

Number and force of contractions were recorded before and after administration of every drug solution. Data was entered in SPSS version 15.0 and analyzed by paired samples T test. Graphs were prepared on Microsoft Excel. A value of $P<0.05$ was considered as statistically significant.

RESULTS

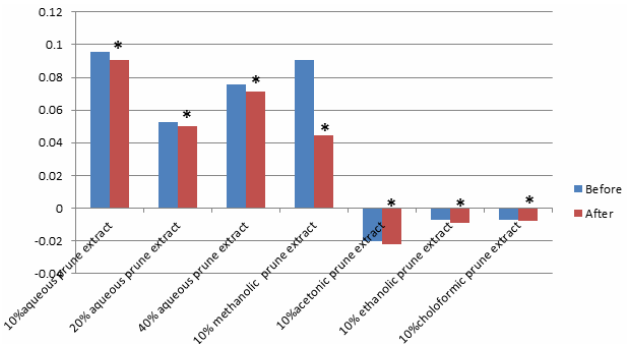
We took 228 frogs with average weight of 150-175 grams by random selection

There was a statistically significant ($p<0.05$) reduction in heart rate and force of contraction by all prune extracts: 10%, 20% and 40% of aqueous, 10% methanolic, 10% acetonic, 10% ethanolic and 10% chloroformic prune extracts. There was dose dependent reduction in myocardial contractility by10%, 20% and 40% of aqueous prunes extract (p 0.043, 0.004 and 0.001 respectively). Therefore further experiments were carried mostly on 10% prunes aqueous extract (table 1, graphs 1, 2, 3 & 4; fig 1a, 1b).



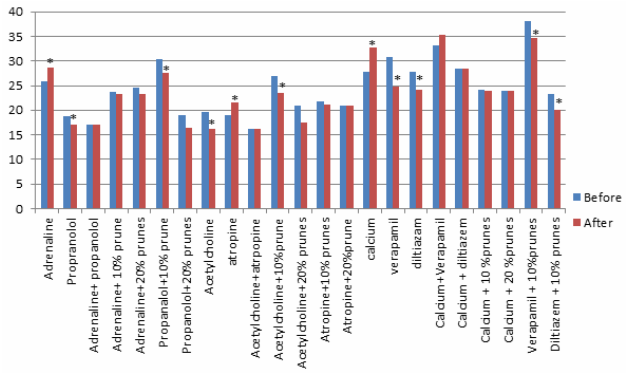
* = statistically significant

Fig. 1a: Effects of various Prune extracts on frog's heart rate



* = statistically significant

Fig. 1b: Effects of various Prune Extracts on force of contraction of frog's heart.



* = statistically significant

Fig. 2a: Effects of various agonists/antagonist on frog's heart rate

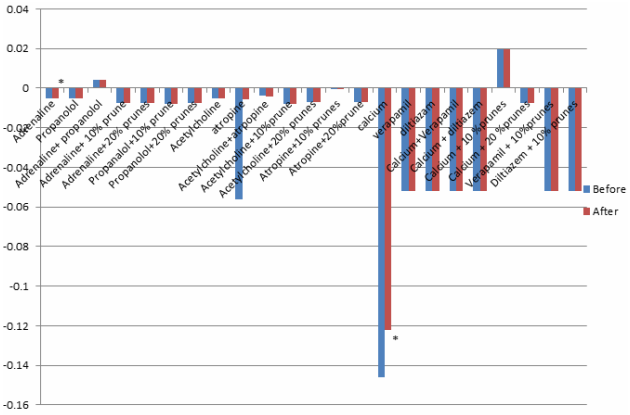
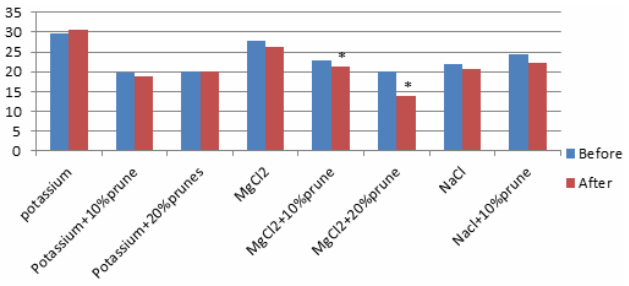


Fig. 2b: Effects of various Agonists /Antagonist on force of contraction of frog's heart

The significantly agonist effects of acetylcholine (ACh) and epinepherine were blocked by atropine and propranolol respectively. When given alone atropine caused significant tachycardia and propranolol significant bradycardia. Aqueous prunes extract produced significant bradycardia when combined with ACh or propranolol; and blocked the significant tachycardia when combined with epinephrine or atropine (table 2, fig. 2a & 2b).



* = statistically significant

Fig. 3a: Effects of various salts on frog's heart rate

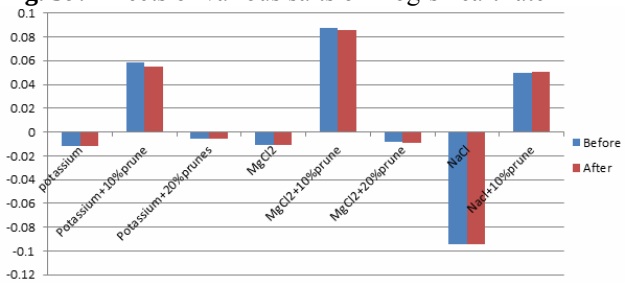


Fig. 3b: Effects of various salts on force of contraction of frog's heart

Calcium significantly altered the rate and force of contraction of heart and this effect was reversed by verapamil and diltiazem. There was no significant change in heart rate or contractility after combination of verapamil or diltiazem with calcium. Aqueous prunes extract significantly reduced heart rate when combined with verapamil or diltiazem. The significant increase in heart rate and cardiac contractility by calcium was converted to non significant reduction in HR and cardiac contractility after instillation of both 10% and 20% aqueous prune extract (table 2, fig. 2a & 2b).

There was no significant reduction in HR and cardiac contractility when $MgCl_2$ was given alone but significant dose related reduction of HR was recorded after combination with 10% and 20% prunes (0.02 and 0.008 respectively). There was no significant change in HR and cardiac contractility after KCl or NaCl given alone or in combination with 10% or 20% prunes (table 3, fig. 3a & 3b).

DISCUSSION

Prunus domestica significantly ($p < .05$) reduced heart rate (HR) and force of contraction by all prune extracts: 10%, 20%, and 40% of aqueous (dose related), 10% methanolic, 10% acetonic, 10% ethanolic and 10% chloroformic prune extracts. We tested the effects of NaC1, KCl, $CaC1_2$ and $MgCl_2$ (10% w/v) on frogs' heart alone and in combination with prunes.

A study on lobster heart has shown that the main inorganic ions of plasma such as sulphate, chloride,

sodium, calcium, potassium and magnesium are required for normal functioning of cardiac tissue. Diastolic arrest is caused by isotonic $MgCI_2$, NaBr, $CaC1_2$, $MgSO_4$, NaI and glucose whereas isotonic LiC1, KC1, NaC1 and urea could lead to systolic arrest. On the other hand, heart can survive for several hours in the absence of magnesium and sulphate (Cole, 1941).

In present study, we found that *Prunus domestica* blocked the myocardial stimulant effect of calcium, and reduced heart rate when combined with magnesium chloride.

Calcium dependent mechanisms control the chronotropic and ionotropic properties of pacemaker and cardiac myocytes. Voltage dependent L and T-type Ca^{+2} channels and Na^{+} /Ca^{+2} exchanger are the chief regulators for cardiac myocytes contraction (Shemarova *et al*., 2009; Wang *et al.,* 2013).

The protective actions of $MgCI_2$ can be achieved due to its ability to compete with Ca^{+2} for the binding sites in a number of proteins responsible for the rise in intracellular free Ca^{+2}, including Na^{+}/ Ca^{+2} exchangers. Variations of intracellular Mg^{++} could modify transmembrane Ca^{+2} movements and were ensured by Na^{+}/ Ca^{+2} exchangers (Levitsky & Takahashi, 2013). The responsiveness of muscle cells to isoproterenol (beta adrenergic agonist) was suppressed significantly by increasing the magnesium concentration. It is concluded that magnesium ion may have an antiarrhythmic effect on partially depolarized cardiac muscle cells (Hasegawa *et al*., 1989).

In a randomized double blind clinical trial, fatal arrhythmias due to acute myocardial infarction were not reversed by administration of $MgSO_4$ whereas occurrence of sinus bradycardia was significantly higher in the group receiving magnesium (Roffe *et al.,* 1994). Another study has shown that low serum magnesium is moderately associated with the development of atrial fibrillation in individuals without cardiovascular disease (Khan *et al*., 2013). Mean heart rate tended to increase from the lowest to the highest percentile (a third of the population to whole of population) of Ca^{+2} levels (p=0.081), whereas it decreased significantly with higher Mg^{++} levels (p=0.026). Associations of serum Mg level and Ca/Mg ratio with heart rate variability could be one of the mechanisms involved in cardiovascular diseases (Kim *et al.,* 2012). In our study *P. domectica* reduced HR when combined with Mg; perhaps it is due to alteration of Ca and Mg concentration ratio within cells.

Stimulation of calcium channels offers a vital role in sinus acceleration during beta adrenergic excitation, which accentuates with the voltage channels to amplify sinus rate (Joung *et al*., 2009). Ypey and his colleagues established a membrane mechanism of depolarization-induced automaticity of cardiomyocytes primarily based on L-type Ca^{+2} current, repolarizing current and inward rectifier properties (Ypey *et al*., 2012).

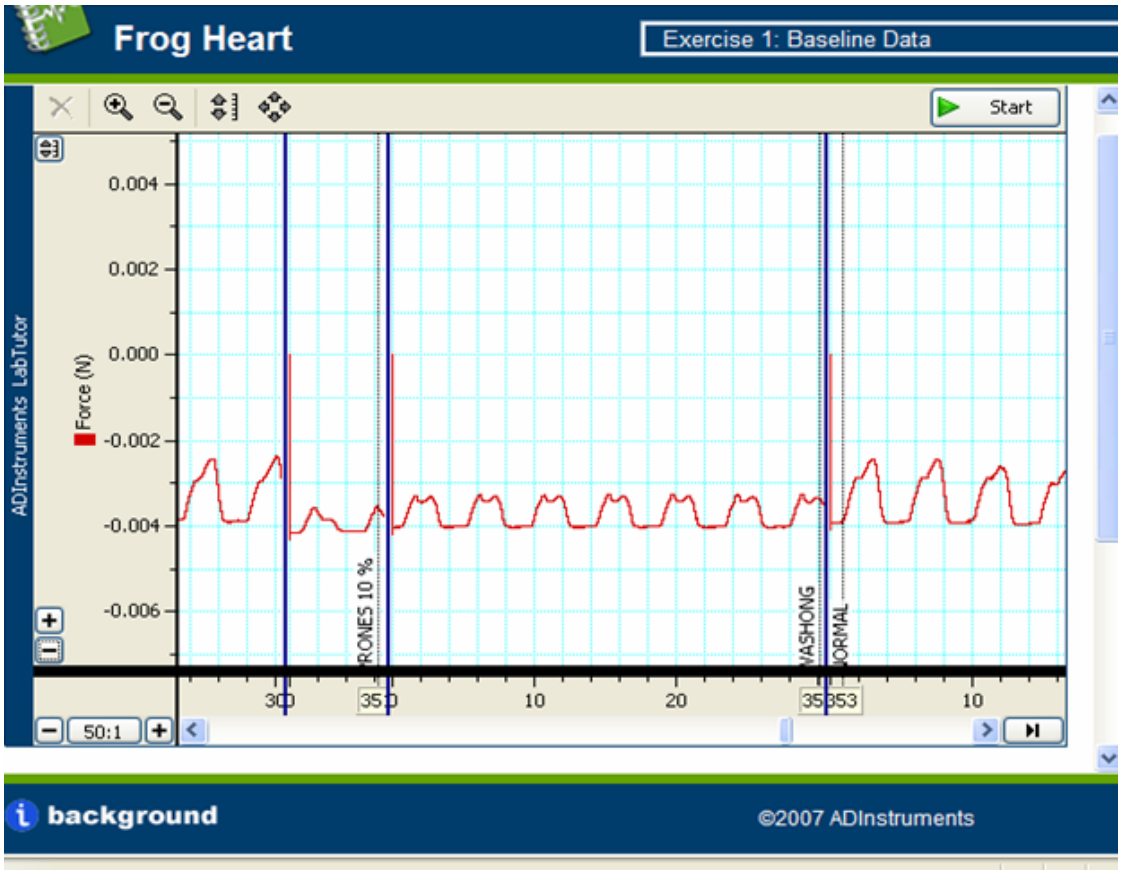
Suppression of cardiac functions could be due to malfunctioning of L-type calcium current and decreased transient intracellular calcium current leading to inadequate threshold potential responsible for β-adrenergic receptors stimulation (Cui *et al*., 2010).

A probable mechanism of Ca^{+2} dependent βeta receptors stimulation is presence of calcium activated adenyl cyclase isoform 1 (AC1) in sinoatrial node. AC isoforms also exhibit a physical and functional association with HCN2 (hyper polarization -activated cyclic nucleotide, ion channel family) pacemaker channel suggesting central role of calcium activated AC1 in β-adrenergic receptors functioning in heart (Kryukova *et al*., 2012; Morabbi Najafabad and Jamei, 2014).

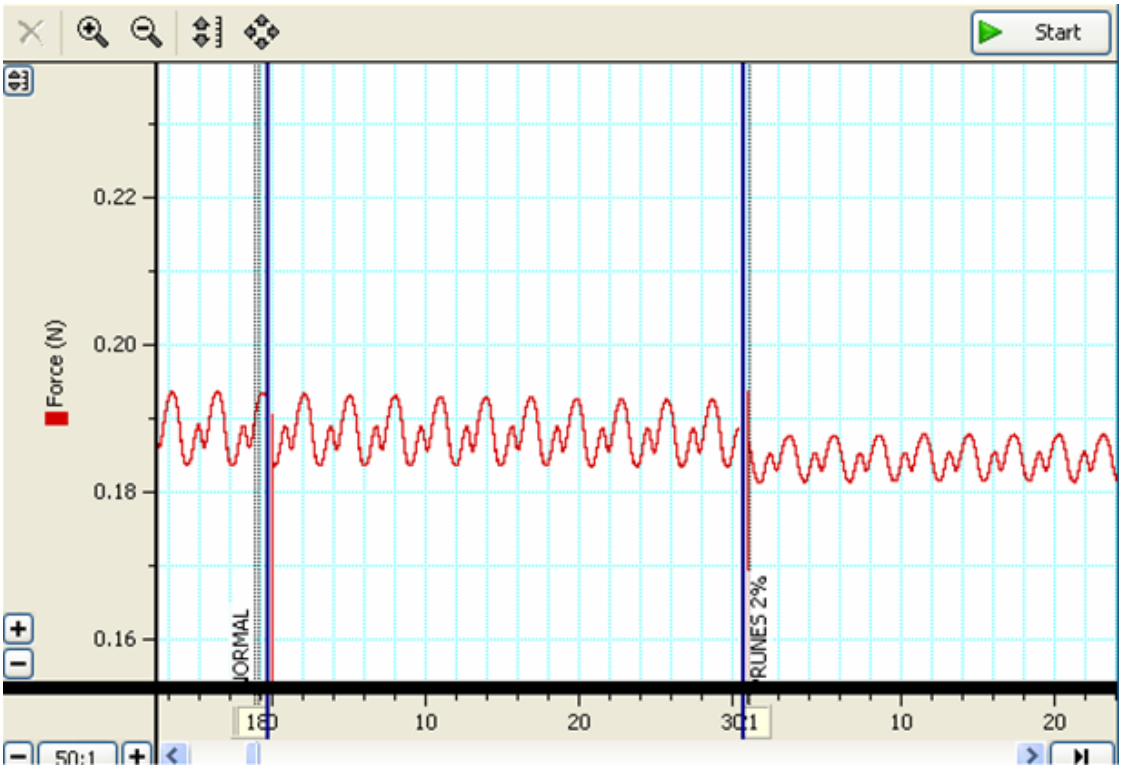
Both alpha and beta adrenergic receptor blockers completely inhibited the increase in myocardial Ca^{+2} during reperfusion in reversibly injured tissue whereas α - adrenergic blockers prevented the increase in myocardial intracellular Ca^{+2} specifically (Sharma *et al*., 1983). Inhibition of the β-adrenoceptor activity on the heart can lead to bradycardia and its chronic use reduces CO; therefore it can be used for the treatment of hypertension (Thadani, 1983). In present study we found that *prunus domestica* have myocardial depressant effects similar to β-adrenergic blockade.

The depletion of the intracellular inositol 1,4,5-trisphosphate-sensitive Ca^{+2} pool initiates calcium entry (Takemura *et al*., 1989). Alteration in action potential firing rate of sinoatrial node is caused by fluctuations in cytoplasmic and sarcoplasmic reticulum (SR) calcium loading which in turn is affected by mitochondrial changes in calcium flux (Yaniv *et al*., 2012).The main force for regulating heart rate is the interaction between sodium-calcium exchanger and calcium release by ryanodine receptors in the SR, leading to intracellular changes in calcium current. Another mechanism for calcium release in cells like cardiomyocytes is inositol 1,4,5-trisphosphate receptor (IP_3R) channel in sarcoplasmic reticulum. Calcium release from IP_3R may stimulate numerous membrane currents as well as a store operated calcium current thereby highlighting intricate heart rate control mechanisms (Ju *et al*., 2012; Santhakumar *et al.,* 2015a).

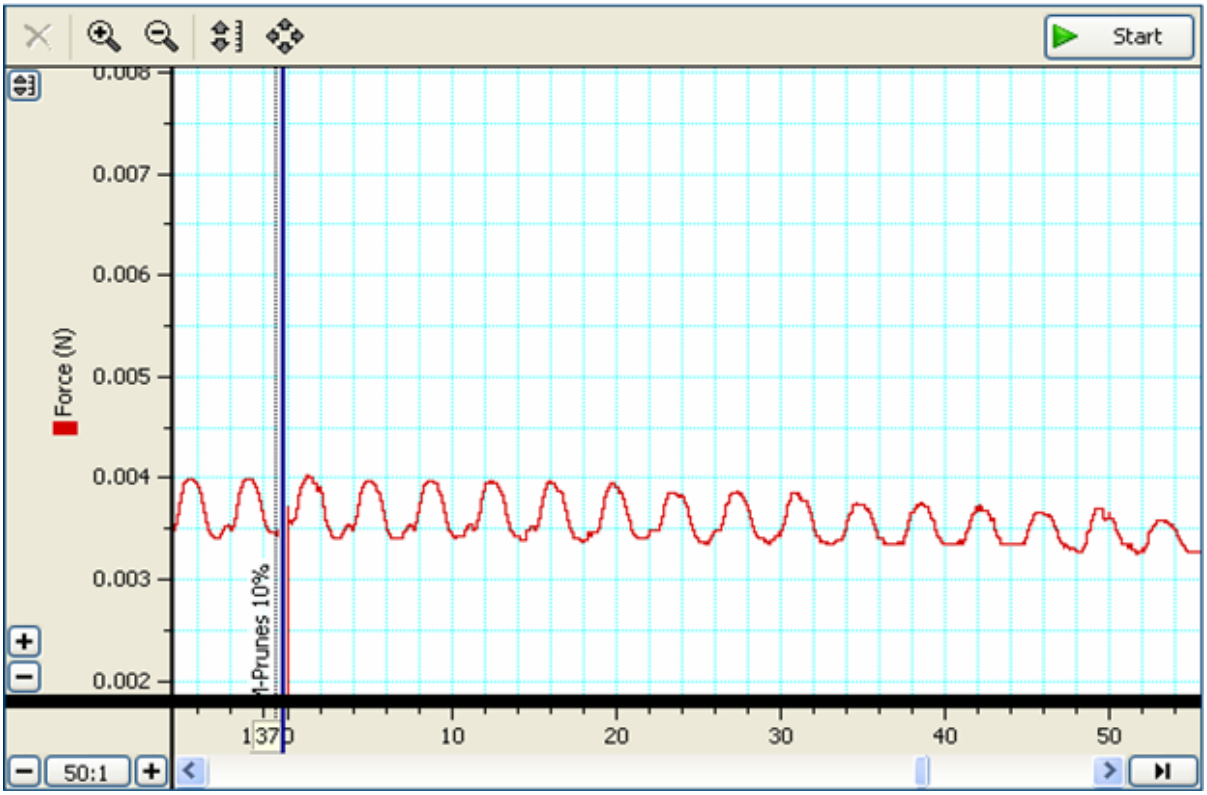
Activation of muscarinic receptors on the neurons stimulates phosphatidyl inositol turnover and induces calcium oscillations that are initiated and maintained by calcium release from caffeine/ryanodine-insensitive intracellular stores (Rathouz *et al*., 1995). The muscarinic drugs evoke intracellular Ca^{+2} responses probably by release of Ca^{+2} from intracellular stores (Harrison *et al*., 2002).



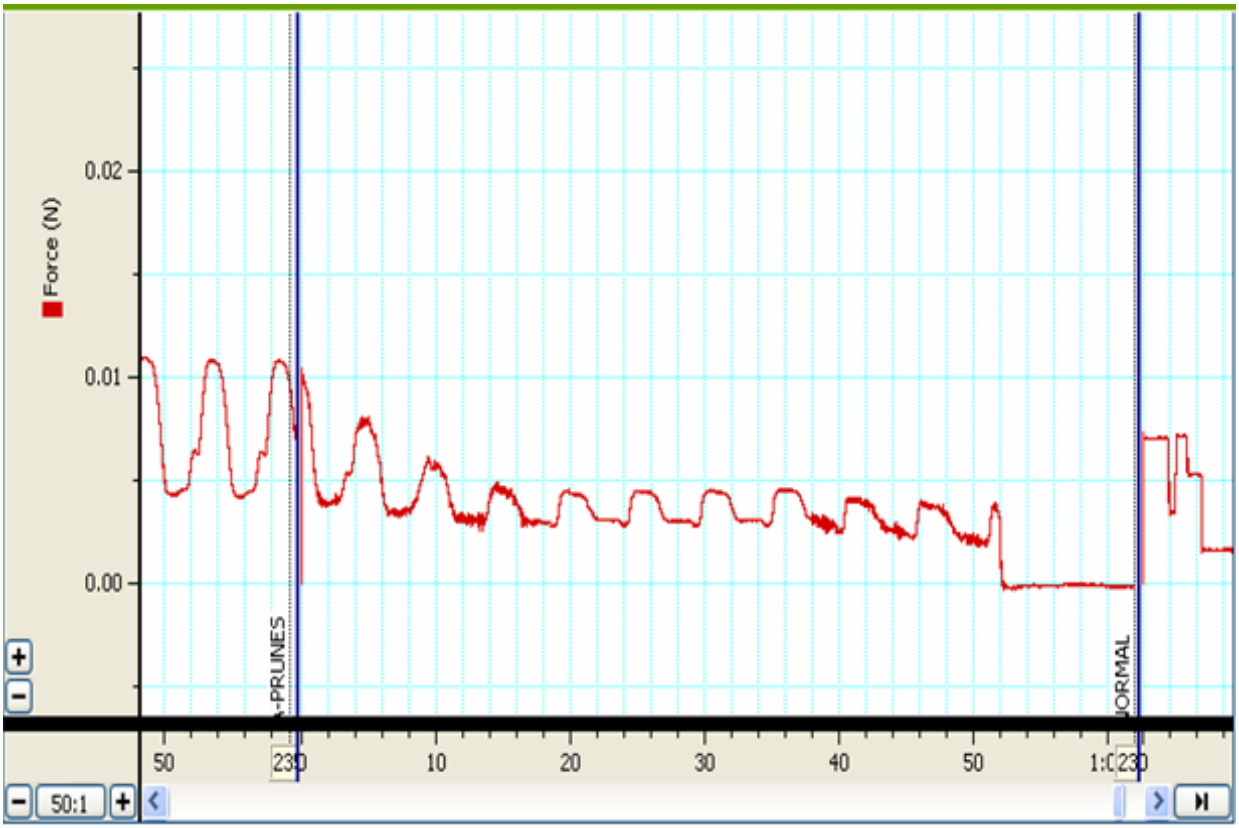
Graph 1: Force of contraction and heart rate is reduced by 10 % aqueous prune extract**.**



Graph 2: Effect of 20% Aqueous Prune Extract on frog heart.



Graph 3: Effect of 10 % Methanolic prune extract



Graph 4: Effect of 10 % Acetonic prunes extract

Table 1: Effects of various Prune Extracts on frog's heart rate and force of contraction

PAIR	Drug	Heart rate				Force of contraction			
		before drug Mean ±sd	after drug Mean ±sd	N	p	Before drug Mean ±sd	after drug Mean ±sd	n	P
1	10%aqueous prune extract	21.79 ±4.784	19.05 ±4.059	43	.000	.095667 ±.0891740	.090889 ±.0919014	41	.0463
2	20% aqueous prune extract	22.26± 3.945	19.12 ±3.877	42	.000	.052613 ±.0919256	.050129 ±.0912424	39	.004
3	40% aqueous prune extract	22.33 ±2.338	11.67 ±1.966	12	.000	.075415 ± .082841	.0713041± .0831542	12	.001
4	10% methanolic prune extract	24.77 ±3.811	21.77 ±3.370	13	.000	.0907 ±.044399825	.0445 ± .049146606	13	.0118
5	10%acetonic prune extract	31.33 ±6.022	29.50 ±5.128	16	.006	-.020429 ±.0215705	-.022039 ±.0225705	15	.0463
6	10% ethanolic prune extract	26 ±1.414	23.50 ±1.761	15	.007	-.007000 ±.00910500	-.008897 ±.0093050	15	.042
7	10%choloformic prune extract	26.83 ±5.601	23.00 ±6.419	16	.001	-.007033 ±.0001667	-.007835 ±.0004082	16	.0363

sd =standard deviation, n=sample size

Table 2: Effects of various Agonists /Antagonist on frog's heart

GROUP	PAIR	Drug	Heart rate				Force of contraction			
			Before drug Mean ± sd	After drug Mean ± sd	n	p	Before drug Mean ± sd	After drug Mean ± sd	n	P
B Adrenergic	1	Adrenaline	25.889 ±4.4845	28.667 ±4.330	28	0.00021	0.005333333 ±0.001581139	0.007888889 ±0.00600925	27	.0028 6
	2	Propranolol	18.83 ±4.569	17.17 ±4.152	12	.000	-.005333 ±0.0023484	-.005333± .0023484	12	
	3	Adrenaline+ propanolol	17.13 ±4.051	17.13± 4.051	8		.004143± .0020354	.004143 ± .0020354	7	
	4	Adrenaline+ 10% prune	23.67 ±3.670	23.33± 3.445	6	.175	-.007500 ±.0005477	-.007500± .0005477	6	
	5	Adrenaline+2 0% prunes	24.50 ±3.416	23.25 ±3.948	4	.278	-.007500± .0005000	-.007500± .0005774	4	
	6	Propanalol+1 0% prune	30.38 ± 6.760	27.50 ±6.990	8	.000	-.007875± .0003536	-.007875± .0003536	8	
	7	Propanolol+2 0% prunes	19.00 ± 1.414	16.50± 2.121	2	.126	-.007500 ±.0007071	-.007500± .0007071	2	
Musca Rini C	8	Acetylcholine	19.54 ±4.802	16.27 ±4.114	26	.000	-.005333 ±.0022435	-.005333± .0022435	21	
	9	atropine	18.90 ±4.847	21.67 ±5.314	21	.000	-.05600 ±.0022100	-.005500 ±.0022827	20	.163
	10	Acetylcholine +atrpopine	16.17 ±1.602	16.17 ±1.602	16		-.004000 ±.0017728	-.004100± .0019728	16	0.823
	11	Acetylcholine +10%prune	26.89 ±10.833	23.44± 10.406	13	.000	-.008000 ±.0000000	-.008000± .0000000	14	
	12	Acetylcholine +20% prunes	21.00 ±1.414	17.50 ±.707	2	.090	-.007000 ±.0000000	-.007000 ±.0000000	2	
	13	Atropine+10 % prunes	21.78 ±2.108	21.22 ±1.787	9	.139	-.000556 ± .0139294	-.000556 ±.0139294	9	
	14	Atropine+20 %prune	21.00 ±1.155	21.00± 1.414	4	1.000	-.007250± .0005000	-.007250 ±.0005000	4	

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Calcium Channels	15	Calcium	27.87 ±8.079	32.67 ±7.961	30	.000	-.14600 ±.0768262	-.122400 ±.0775127	30	.00382
	16	verapamil	30.89 ±11.624	24.89 ±14.709	9	.002	-.052000 ±.0000000	-.052000 ±.0000000	9	
	17	diltiazam	27.71 ±5.589	24.1429 ±5.24177	7	.000	-.052000 ±.0000000	-.052000 ±.0000000	7	
	18	Calcium+ verapamil	33.25 ±7.274	35.25 ± 5.252	4	.456	-.052000± .0000000	-.052000± .0000000	4	
	19	Calcium+ diltiazem	28.50 ±4.950	28.50 ±4.950	6		-.052000± .0000000	-.05200± .0000000	6	
	20	Calcium+ 10% prune	24.11 ±6.583	24.04 ±6.630	27	.808	.019500 ±.0591762	.019417 ±.0590121	24	.162
	21	Calcium+ 20% prune	24.00 ±4.195	23.83 ±3.920	15	.363	-.007600 ±.0005477	-.007600 ±.0005477	15	
	22	Verapamil + 10% prune	38.00 ±3.338	34.75 ±3.991	8	.008	-.052000± .0000000	-.052000± .0000000	7	
	23	Diltiazeam + 10% prune	23.33 ±2.733	20.17 ±3.656	6	.003	-.052000± .0000000	-.052000± .0000000	6	

sd =standard deviation, n=sample size

Table 3: Effects of various salts on frog's heart rate and force of contraction

Pair	Drug	Heart rate				Force of contraction			
		before drug Mean ±sd	after drug Mean ±sd	n	p	Before drug Mean ±sd	after drug Mean ±sd	n	p
1	potassium	29.75 ±11.841	30.75 ±10.846	8	.374	-.012000± .1007413	-.012000± .1007413	8	
2	Potassium+10%prune	19.88 ±2.031	18.88 ±3.796	8	.374	.058167± .0677803	.055167± .691821	6	.363
3	Potassium+20%prunes	20.00	20.00	5		-.005333± .0023484	-.005333± .0023484	5	
4	MgCl2	27.75 ±10.334	26.25 ±11.781	8	.265	-.010667± .1038897	-.010667± .1038897	6	
5	MgCl2+10%prune	23.00 ±4.980	21.17 ±4.355	6	.020	.087167± .867742	.086167± .853192	6	.363
6	MgCl2+20%prune	20.00	14.00	6	.008	-.007975± .0003536	-.008875± .0003536	6	.733
7	NaCl	22.00 ±3.464	20.67 ±1.155	3	.423	-.094000	-.094000	3	
8	Nacl+10%prune	24.25 ±4.787	22.25 ±3.862	4	.161	.050000± .0325269	.050500± .0332340	2	.500

sd =standard deviation, n=sample size

The hypothesis that muscarinic receptors blockade is achieved by the increased second messenger cyclic adenosine monophospate expression is in agreement with the finding that particular cAMP exhibition results in L-type calcium current opposition by muscarinic receptors (Imai *et al*., 2001).

On arrhythmia models, choline prevented arrhythmia by stimulating the cardiac M_3 receptor that may be related to alteration in Ca^{+2} concentrations (Liu *et al*., 2008). Stimulation of cardiac M_3-AChR by pilocarpine exerted antiarrythmic effects in aconitine- and ouabain-induced arrhythmias in animal models and this appears to be associated with intracellular calcium changes (Zhao *et al*., 2009). In present study, we found that *Prunus domestica* have myocardial depressant effects similar to cholinergic stimulation. *Prunus domestica* aqueous extract produced significant bradycardia when combined with Ach; and blocked the significant tachycardia produced by atropine when combined with atropine. The mode of action is probably changes in the intracellular Ca^{+2} concentrations.

Normally force of contraction is increased by intracellular calcium but in case it exceeds therapeutic window, it builds up inside the cells triggering arrhythmias as well as heart block (Seidler *et al.,* 2007) In the present study the significant increase in heart rate and force of contraction by calcium was made insignificant when calcium was combined with prunes; moreover prunes potentiated the myocardial depressant effects of calcium channel

blocking drugs. *Prunus domestica* might be acting at intracellular level by calcium related myocardial contractility and pace maker activity.

Resistance offered by blood vessels is controlled by store-operated calcium (SOC) channels and stretch-activated cation (SAC) channels in cell membranes which are an important source of activator calcium (William, 2000). *Prunus domestica* might cause dilation of these resistance blood vessels by reducing intracellular calcium. Cardiac output and total peripheral resistance are main determinants of changes in blood pressure (Li *et al*., 1998). The negative ionotropic and chronotropic effect of *Prunus domestica* similar to acetylcholine and opposite to epinephrine on the heart could contribute to its blood pressure lowering effect. Further research trials are required to elucidate active ingredients of *prunus domestica* and their effects on various cardiac parameters.

CONCLUSION

We conclude that *Prunus domestica* plays a key role in modification of intracellular calcium concentration resulting in negative ionotropic and chronotropic effects (similar to cholinergic stimulation, adrenergic blockade and calcium channel blockage) that could contribute to lowering of blood pressure. Highest levels of phenolic compounds, including flavonoids and anthocyanins in *prunus domestica* could be attributed to their cardiovascular protective effects.

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