**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); NaCl, KCl, CaCl₂, MgCl₂ (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 CaCl₂; and reduced HR along with MgCl₂. 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 Ca²⁺ 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 arrhythmias 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 10⁻⁵ of adrenaline, propranolol HCl, verapamil and diltiazem respectively were prepared. In addition, 10% solutions of NaCl, CaCl₂, MgCl₂ and KCl (10% w/v- 10g in 100ml distill water) were prepared. 10% methanolic, acetonolic, ethanolic and chloformic solutions were used.

Ringers’ solution was prepared by dissolving 6.5 gm NaCl, 1.4 gm KCl, 0.4 gm NaH₂PO₄, 0.4 gm NaHCO₃, 1.08 gm CaCl₂ and 20 gm glucose (C₆H₁₂O₆) 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 by 10%, 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).

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

* = statistically significant
The significantly agonist effects of acetylcholine (ACh) and epinephrine 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).

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 MgCl2 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% acetic, 10% ethanolic and 10% chloroformic prune extracts. We tested the effects of NaCl, KCl, CaCl₂ and MgCl₂ (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 MgCl₂, NaBr, CaCl₂, MgSO₄, NaI and glucose whereas isotonic LiCl, KC1, NaCl 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 inotropic properties of pacemaker and cardiac myocytes. Voltage dependent L and T-type Ca²⁺ channels and Na⁺/Ca²⁺ exchanger are the chief regulators for cardiac myocytes contraction (Shemarova et al., 2009; Wang et al., 2013).

The protective actions of MgCl₂ can be achieved due to its ability to compete with Ca²⁺ for the binding sites in a number of proteins responsible for the rise in intracellular free Ca²⁺, including Na⁺/Ca²⁺ exchangers. Variations of intracellular Mg²⁺ could modify transmembrane Ca²⁺ movements and were ensured by Na⁺/Ca²⁺ 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 MgSO₄ 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 Ca²⁺ levels (p=0.081), whereas it decreased significantly with higher Mg²⁺ 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. domestica 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 Ca²⁺ 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 beta-adrenergic receptors stimulation (Cui et al., 2010).

A probable mechanism of Ca²⁺ dependent beta receptors stimulation is presence of calcium activated adenyl cyclase isofrom 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 beta-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 Ca²⁺ during reperfusion in reversibly injured tissue whereas alpha-adrenergic blockers prevented the increase in myocardial intracellular Ca²⁺ specifically (Sharma et al., 1983). Inhibition of the beta-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 beta-adrenergic blockade.

The depletion of the intracellular inositol 1,4,5-trisphosphate-sensitive Ca²⁺ 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 current (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,R) channel in sarcoplasmic reticulum. Calcium release from IP,R 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 Ca²⁺ responses probably by release of Ca²⁺ 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.
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**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

<table>
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<th>PAIR</th>
<th>Drug</th>
<th>Heart rate before drug Mean ± sd</th>
<th>Heart rate after drug Mean ± sd</th>
<th>N</th>
<th>P</th>
<th>Force of contraction before drug Mean ± sd</th>
<th>Force of contraction after drug Mean ± sd</th>
<th>n</th>
<th>P</th>
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<td>.0463</td>
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<td>20% aqueous prune extract</td>
<td>22.26 ± 3.945</td>
<td>19.12 ± 3.877</td>
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<td>.000</td>
<td>.052613 ± .0919256</td>
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<td>40% aqueous prune extract</td>
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<td>.073415 ± .082841</td>
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<td>.001</td>
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<td>24.77 ± 3.811</td>
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<td>.0907 ± .0919256</td>
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<td>10% acetonic prune extract</td>
<td>31.33 ± 6.022</td>
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<td>10% ethanolic prune extract</td>
<td>26 ± 1.414</td>
<td>23.50 ± 1.761</td>
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<td>-.00897 ± .0093050</td>
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<td>10% chloroformic prune extract</td>
<td>26.83 ± 5.601</td>
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<td>-.007033 ± .0001667</td>
<td>-.007835 ± .000482</td>
<td>16</td>
<td>.0363</td>
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sd = standard deviation, n = sample size

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

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<th>Drug</th>
<th>Heart rate before drug Mean ± sd</th>
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<th>Force of contraction before drug Mean ± sd</th>
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<th>P</th>
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<td>B</td>
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<td>Adrenaline</td>
<td>25.889 ± 4.4845</td>
<td>28.667 ± 4.330</td>
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<td>0.00021</td>
<td>0.053333 ± 0.00158139</td>
<td>0.00788889 ± 0.00600925</td>
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<td>Propranolol</td>
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<td>-.00533 ± .0023484</td>
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<td>Adrenaline+propanolol</td>
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<td>.004143</td>
<td>-.00533 ± .0020354</td>
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<td>Adrenaline+10% prune</td>
<td>23.67 ± 3.670</td>
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<td>.175</td>
<td>-.00750 ± .0005477</td>
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<td>19.00 ± 1.414</td>
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<td>.126</td>
<td>-.00750 ± .0000701</td>
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<td>Acetylcholine</td>
<td>19.54 ± 4.802</td>
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<td>.000</td>
<td>-.00533 ± .0022435</td>
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The hypothesis that muscarinic receptors blockade is achieved by the increased second messenger cyclic adenosine monophosphate 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 M3 receptor that may be related to alteration in Ca^{2+} concentrations (Liu *et al.*, 2008). Stimulation of cardiac M3-AChR by pilocarpine exerted antiarrhythmic 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 blockers.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Drug</th>
<th>Heart rate before drug Mean ±sd</th>
<th>Heart rate after drug Mean ±sd</th>
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<td>-.007975 ±.003536</td>
<td>-.008785 ±.003536</td>
<td>6</td>
<td>.733</td>
</tr>
<tr>
<td>7</td>
<td>NaCl</td>
<td>22.00 ±3.464</td>
<td>20.67 ±1.155</td>
<td>3</td>
<td>.423</td>
<td>-.094000</td>
<td>-.094000</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>NaCl+10%prune</td>
<td>24.25 ±4.787</td>
<td>22.25 ±3.862</td>
<td>4</td>
<td>.161</td>
<td>.050000 ±.0325269</td>
<td>.050505 ±.0332340</td>
<td>2</td>
<td>.500</td>
</tr>
</tbody>
</table>

sd = standard deviation, n=sample size

Table 3: Effects of various salts on frog’s heart rate and force of contraction
blocking drugs. *Prunus domestica* might be acting at intracellular level by calcium related myocardial contractility and pacemaker 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.

REFERENCES


Groves T (2010). Therapeutic value (TV) of alternative medicine (non-drug CAM). Rough estimates for all clinical conditions based on Cochrane reviews and the ratio: Number Needed to Harm/Number Needed to Treat (TV=NNH total/NNT). Evidence based medicine.


Levitsky DO and Takahashi M (2013). Interplay of Ca(2+) and Mg (2+) in Sodium-Calcium Exchanger and in Other Ca(2+)-Binding Proteins: Magnesium,


