THE ELECTROPHYSIOLOGICAL EFFECT OF *TERMINALIA ARJUNA* BARK EXTRACT ON ACTION POTENTIAL OF GUINEA PIG PAPILLARY MUSCLE

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Abstract: The bark of *Terminalia arjuna* has been reported to have cardiovascular effect. The present study was designed to investigate the electrophysiological effect of various concentration of bark extract of *Terminalia arjuna* on electrically driven cardiac action potential of papillary muscle of guinea pig. Intracellular recording of cardiac action potentials were employed in the present study. The result of the present study showed extract caused significant increase in Vmax and amplitude of action potential. This may be due to Na+ channel enhancing property of *Terminalia arjuna*.

Key words: *Terminalia arjuna*; Papillary muscle; Action potential;

INTRODUCTION

Several botanicals including *Terminalia arjuna*, *Crataegus oxytanta*, *Inula racemosa* and *Asparagus membranaceus*, have been found to have therapeutics benefit for the treatment of cardiovascular disease. The bark of the *Terminalia arjuna* tree has a long history of use as cardiac tonic as well, and has been indicated in the treatment of coronary artery disease [1] heart failure [2], hypercholesterolemia, [3] and for relief of anginal pain [4]. Echocardiographically, *Terminalia* therapy was associated with significant improvements in stroke volume and left ventricular ejection fraction, with decreases in end-diastolic and end-systolic left ventricular volumes compared to placebo[5]. Improvement of cardiac muscle function and subsequent improved pumping activity of the heart seem to be the primary benefits of *Terminalia* [6]. *Terminalia*’s active constituents include tannins, triterpenoid saponins (arjunicacid, arjunolic acid, arjungenin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), phytosterols, calcium, magnesium, zinc, and copper[7]. It is thought that the saponin glycosides might be responsible for the inotropic effects of *Terminalia*, while the flavonoids and OPCs provide free radical antioxidant activity and vascular strengthening [8].

In order to characterize the electrophysiological effect of the bark extract of *Terminalia arjuna* on myocardium, intracellular action potential potential were conducted Electrically driven ventricular action potentials were recorded using classical microelectrode technique. Extract concentration ranging from 1µg/ml. to 16 µg/ml. was added cumulatively to the bath and effect was seen (Table 1).

MATERIALS AND METHODS

Chemicals: All chemicals used in the study were of analytical grade and procured from Sigma, chemicals Co., St. Louis, MO, USA. The bark powder of *Terminalia arjuna* was obtained from local herbal traders. The powder was dissolved in triple distilled water by vortexing for 10 minutes. The insoluble particles were removed by filtering thorough syringe filter. The subsequent dilution was made in physiological buffer solution (PBS) (in mM: 137 NaCl, 5 KCl,
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1.8 CaCl\(_2\), 1 MgCl\(_2\), 11 Glucose and 3 HEPES, pH adjusted to 7.4 with NaOH).

**Animals:** Guinea pigs (350-400 g) of either sex English albino, laboratory animals division of our institute (CDRI) were used as per permission and guidelines of animal ethical committee of the institute.

**Electrophysiology:** The animal was sacrificed under deep anesthesia & heart was removed quickly and placed in a dissecting chamber containing oxygenated PBS. Thin papillary muscles (n=5) of about 1-mm diameter were removed from the right or the left ventricle and placed in a 2 ml perspex electrophysiological chamber with a DC-controlled heating device to maintain the bath temperature (Hugo Sachs electronics, March-Hugstetten, Germany) at 35\(^\circ\)C. The preparation was superfused with oxygenated PBS maintained at 35\(^\circ\)C and driven electrically with rectangular pulse of twice the threshold potential and 1-to-2-ms duration delivered at 1Hz unless otherwise stated using teflon coated Ag-AgCl bipolar electrodes that are connected to out put of pulse generator (Isolator -ISO –100, Experimetria, Hungry) and operated through software “advanced intrasys”.

The preparation was allowed to equilibrate for 30 minutes before carrying out electrical recording. Papillary muscle showing arrhythmia or missed beats under control conditions were not used in the experiments.

Action potentials (APs) were recorded with conventional intracellular recording technique using 3M KCl – filled glass microelectrodes (FMG -20 glass tubing, Dagan Corporation, U.S.A) of 10-20 M\(\Omega\) tip impedance coupled to the input stage of a capacitance-compensated preamplifier (Intracellular Amplifier-Intr-01, Experimetria, Hungry). APs signals displayed on computer were digitized, and saved for subsequent offline analysis using “advanced intrasys”. Bark extract of various concentrations (1, 2, 4, 6, 8, 10, 12, 12, 18\(\mu\)g) was applied cumulatively to circulating oxygenated perfusion solution to study dose and time dependent effect of its action on action potential.

To test the reversibility of the effect, the organ bath was then perfused with extract free PBS for 15 minutes after final dose of the experiments and effect was seen. Experiments with stable impalements only were included in this study.

**Statistical analysis:** Dunnetts multiple comparison test was used for determination of significance. P<.01 being considered significant.

**RESULTS**

The effect of extract on the following parameters of action potentials were analyzed using intrasys programme :RP- Resting potential; APA- Action potential amplitude; APD 25%- The time period corresponding to 25% return; APD 90%- The time period corresponding to 90% return; Vmax- The positive maximum of the derivatives signal (Upstroke velocity).

The mean value of resting potential at 1Hz was 78.8±0.9 (range -75 to -81 mv) in normal papillary muscle preparation. APD 25% and AP duration at 90% repolarization (APD 90%) had mean value of 108.90± 2.63 and 162.0 ± 1.98 ms respectively in normal preparation. The mean value of +Vmax, which reflects the activity of fast sodium channels was 85.75±0.5 V/sec. in normal preparation. Application of extract of *Terminalia Arjuna* caused 24% increase in Vmax with 18 \(\mu\)gm and 10% increase in amplitude with 10 \(\mu\)gm (Table 1). None of the changes were truly dose dependent. In addition, the application of extract caused slight increase in APD 25% and APD 90%.

**DISCUSSION**

The cardiac action potential is a specialized electrical signal in the heart for propagation of impulse, with unique properties necessary for its function. It is clearly evident from the present observation that the application of various concentration of aqueous extract of *Terminalia arjuna* caused significant increase in +Vmax of action potential. Vmax which represent the maximum rate of depolarization of the cell and is due to the opening of the fast Na\(^+\) channels causing a rapid increase in the membrane conductance to Na\(^+\) (\(G_{Na}^+\)) and thus a rapid influx of Na\(^+\) ions (\(I_{Na}^+\)) into the cell. The increase in Vmax with application of this extract indicates the effect of extract on Na\(^+\) channel activity. In addition to Vmax, the increase of amplitude of action potential with extract strongly support the increase of Na channel
Raghu

Table 1: Effect of various cons. of bark extract of *Terminalia arjuna* on amplitude (APA), maximum upstroke velocity (Vmax), duration of action potential measured at 25% of repolarization (APD 25%) and 90% of repolarization of action potential (APD 90%) of guinea pig papillary muscle. The data are mean values ± s.d. with % changes respect control value.

<table>
<thead>
<tr>
<th>Concentration (gm/ml)</th>
<th>APA (mV)</th>
<th>Vmax (V/sec)</th>
<th>APD 90% (ms)</th>
<th>APD 20% (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110.3 ± 0.78 (100)</td>
<td>85.75 ± 0.50 (100)</td>
<td>162.0 ± 1.99 (100)</td>
<td>108.9 ± 2.62 (100)</td>
</tr>
<tr>
<td>2</td>
<td>116.5 ± 0.92 (105.62)</td>
<td>92.0 ± 0.70 (107.28)*</td>
<td>170.5 ± 1.74 (105.24)</td>
<td>108.8 ± 3.05 (100)</td>
</tr>
<tr>
<td>4</td>
<td>121.00 ± 0.26 (109.7)*</td>
<td>95.8 ± 0.44 (111.7)*</td>
<td>171.7 ± 1.80 (105.88)</td>
<td>113.7 ± 2.86 (104.40)</td>
</tr>
<tr>
<td>8</td>
<td>119.7 ± 0.0 (108.5)*</td>
<td>100.4 ± 0.54 (111.72)*</td>
<td>170.9 ± 1.46 (104.9)</td>
<td>111.8 ± 3.78 (102.66)</td>
</tr>
<tr>
<td>10</td>
<td>122.00 ± 0.44 (110.6)*</td>
<td>98.2 ± 0.83 (114.52)*</td>
<td>170.6 ± 0.89 (105.3)</td>
<td>113.0 ± 2.48 (103.76)</td>
</tr>
<tr>
<td>12</td>
<td>121.5 ± 0.45 (110.15)</td>
<td>97.8 ± 1.09 (114.05)*</td>
<td>171.0 ± 1.125 (105.5)</td>
<td>113.0 ± 3.59 (103.76)</td>
</tr>
<tr>
<td>14</td>
<td>121.8 ± 0.41 (110.42)*</td>
<td>100.8 ± 0.44 (117.55)*</td>
<td>171.0 ± 1.24 (105.5)</td>
<td>113.0 ± 2.17 (103.76)</td>
</tr>
<tr>
<td>16</td>
<td>122.2 ± 0.55 (109.8)*</td>
<td>101.8 ± 2.16 (118.71)*</td>
<td>169.5 ± 0.28 (104.6)</td>
<td>112.4 ± 3.6 (103.21)</td>
</tr>
<tr>
<td>18</td>
<td>122.00 ± 0.62 (110.6)*</td>
<td>106.5 ± 1.0 (124.19)*</td>
<td>168.5 ± 0.49 (104.01)</td>
<td>113.0 ± 3.05 (103.76)</td>
</tr>
</tbody>
</table>

* indicates significant changes compared respective control at p<0.01

The slight increase in plateau phase of action potential (APD 25%) and final stage of repolarization (APD90%) may be due to very slow inactivation of sodium channel thereby increasing net Na influx. The increased intracellular Na+ concentration, in turn activates reverse mode Na/Ca exchange to augment intracellular Ca2+, leading to +ve inotropic effect [12] and *Terminalia arjuna* has been reported to increase contraction of myocardium. On the basis of these results it is concluded that Na+ channel enhancing activity of this herb is responsible for +ve inotropic property of this medicinal plant.

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**REFERENCES**