PHARMACODYNAMIC STUDY OF CARDIOTONIC PROPERTY OF A POLY HERBAL DRUG (MUYM) IN PITHED FROGS

S. SUMBUL AND S.I. AHMAD*
Faculty of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
*Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences, Hamdard University, Karachi

ABSTRACT
This study was performed to evaluate the cardiac effects of a Poly herbal drug Mufarreh Yaqooti Motadil (MUYM). The product is being manufactured by Hamdard Laboratories (waqf) Pakistan for the last several years and has produced immense effects on cardiovascular system. These tests were performed by using kymographic tracing method and results were evaluated statistically by using students-t-test and one way ANOVA. The results obtained on the cardiac force, heart rate and cardiac cycle were compared with Digoxin and confirmed the positive inotropic and negative chronotropic property of MUYM.

Keywords: Mufarreh Yaqooti Motadil (MUYM), Digoxin, positive inotropic, negative chronotropic, Acetylcholine, Atropine

INTRODUCTION
The task of revival of the old system of medicine however, is not an easy one at the present time. Advances in knowledge are so fast in every time that an investigator is overwhelmed by the application of new matter with which is confronted. There comes a time, however, when we feel like going back to the ancient page and leave where secrets remain to be revealed and unfold. We are fortunately endowed with a very rich flora, because of the size of our country and varieties of climatic and soil conditions, obtained in the different parts and as such there is wonderful opportunity for working on plant products. Another fortunate factor is that herbal medicines do not produce many side effects commonly seen after long-term administration of synthetic drugs, resulting in a revival of interest in their use all over the world in both developing and developed countries (Jain and Nagra, 1990).

Mufarreh Yaqooti Motadil (MUYM) is a herbal product being prescribed for strengthening the heart so acts as a Cardiac tonic and as such is traditionally used as specified in Hamdard pharmacopoeia and in Unani Qrabadin. The product is being manufactured by Hamdard Laboratories (Waqf.) Pakistan for the last thirty years and has shown significant therapeutic effects. No side effects have been observed (Said, 1979).

The need for understanding the mechanism by which this product would be acting on heart, lead to this research work.

MATERIAL AND METHODS
Mufarreh Yaqooti Motadil
The drug Mufarreh Yaqooti Motadil (MUYM) is a poly herbal product prepared by Hamdard Laboratories (Waqf.) Pakistan. It contains 45 ingredients (Said 1979). The drug was provided by the manufacturers against the order.

Correspondence to: E-mail: shamim.drsumbul@gmail.com
Reference Drug
Digoxin (Lanoxin) 0.25 mg (Glaxo Smith Kline®)
Acetylcholine Chloride (Merck®, Germany)
Atropine Sulfate (Boehringer ingelheim®)

Chemicals
Normal Ringer solution (NaCl=115mM/L, KCl=2.5mM/L, CaCl_2= 1.8mM/L, Na_2HPO_4=2.15 mM/L, Na H_2PO_4=0.8mM/L) (Daher C. F. 2006).

Experimental Protocol
Pharmacodynamic study was carried out on exposed and intact frog’s heart by using the method described by (Xiang-Chun et al., 2006, Kimura et al., 1986). The experimental protocols were approved by the Institutional animals Ethical Committee. For each dose of the tested sample, 12 frogs were taken. Frogs were made unconscious by pithing procedure and the unconscious frog was fixed on frog board. An incision was made on ventral side of body, the skin was carefully removed by using scalpel and thoracic region was cut down with scissors to expose out the heart. The pericardium was removed by using forceps (Fig.1). Exposed heart was then connected to the lever of Harvard Apparatus (Cat. No. 50-0660) using nylon thread and hook, inserted to the heart apex. During recording of heart movement, normal ringer solution was continuously poured on the heart to maintain vitality and prevent drying. The heart activity was recorded by using Harvard Kymograph Universal Model (Cat. No.50-7392). Its drum revolved at the speed of 10mm/sec (Talalay et al., 2001).

Preparation of Dilutions of Drugs:
The *Carissa carandas* (C.C.L.E.) extract in doses of 5mg/ml – 45mg/ml, Herbal Product Mufarrehyaqooti Motadil ( MUYM ) in doses of 5mg/ml-45mg/ml and Digoxin (0.008mg/ml) were freshly prepared by dissolving in warm distilled water before each experiment. In addition cholinergic agent i.e., Acetylcholine Chloride (10^{-3} M) and Anticholinergic agent i.e., Atropin Sulfate (10^{-3} M) were used for comparison with the tested drugs. Digoxin (0.008mg/ml) (Wójcicki et al., 1997, Tripathi and Das 1983, Mikhailov et al., 2004) was used to compare the results on heart. All dilutions were freshly made in 0.65% saline solution (Isotonic for Amphibions) before each experiment (Azmat et al., 2005, Erum et al., 2004).

Calculations:
The records obtained were used for the measurement of Heart Rate (per min.), Cardiac Force (g), Cardiac Cycle (Sec) at the Kymograph speed (10mm/sec) and heart Lever Calibration at 2g weight (Azmat et al., 2005)
The force of contraction and the rate of contraction was counted and tabulated (Ahmed et al., 2004).

Heart Rate:
The Calibrated Value is, 10mm = 1Sec.
If the tracing shows, 47 beats = 255 mm
Known value is, 10mm = 1 Sec.
Therefore, 255mm= 25.5 Sec.
And 25.5 Sec. = 47 beats
So 60 Sec.=110.5 beats
or 110.5beats/ min.

Cardiac Force:
The Calibrated Value is, 29mm = 2 g
If the tracing shows, 1 beat = 3 mm
Known value is, 29mm=2 g
Therefore, 3mm= 0.206 g
So 1 beat. = 0.206 g

Cardiac Cycle:
The Calibrated Value is, 10mm = 1Sec.
If the tracing shows, 1 Cycle = 6.5 mm
Known value is, 10mm =1 Sec.
Therefore, 6.5mm= 0.65 Sec.
So 1 Cycle = 0.65 Sec.
RESULTS

Effects of Mufarreh Yaqooti Motadil (MUYM) on Intact Heart of Frog:
The effects of MUYM in different doses, exhibited on intact heart of frog, are shown by the following tracings (Figs. 2-9).

Fig. 2: Tracing for Control (left) and 5mg/ml Treated (right) Frog’s heart.

Fig. 3: Tracing for Control (left) and 10mg/ml Treated (right) Frog’s heart.

Fig. 4: Tracing for Control (left) and 15mg/ml Treated (right) Frog’s heart.

Fig. 5: Tracing for Control (left) and 20mg/ml Treated (right) Frog’s heart.

Fig. 6: Tracing for Control (left) and 25mg/ml Treated (right) Frog’s heart.

Fig. 7: Tracing for Control (left) and 30mg/ml Treated (right) Frog’s heart.
Effects on Heart Rate:
This herbal product found to cause a decrease in Heart Rate. The pattern of Heart Rate reduction was calculated as follows:

1. The drug Mufarreh Yaqooti Motadil (MUYM) was found to cause a non significant decrease in heart rate at the doses 5mg/ml and 10mg/ml (P>0.05) when compared with their controls.
2. While the dose of 15mg/ml showed a statistically significant decrease i.e., 8.89% ± 0.36 (P<0.05) when compared with its controls.
3. 20mg/ml and 25mg/ml caused a significant decrease in Heart Rate (P<0.025) with percentage of decrease 8.44% ± 0.85 and 8.63% ± 1.55 respectively when compared with their controls.
4. 30mg/ml, 35mg/ml has been found to present a highly significant reduction in heart rates 8.18% ± 1.29 and 8.99% ± 1.85 respectively with the p-value as P<0.01, when compared with its controls.
5. 40mg/ml dose produced a highly significant decrease in heart rate with P<0.005 (14.45% ± 2.39) as compared with its controls (Table 1, Fig. 10).

Effects on Cardiac Force:
The effect on Cardiac Force produced by MUYM was found to have an increasing pattern when compared with control, and calculated from the tracings as follows:

1. At the doses 5mg/ml, 10mg/ml, 15mg/ml and 20mg/ml, the drug caused a non significant increase in cardiac force, with percentage increase as 9.461% ± 1.259, 11.116% ± 2.476, 15.00% ± 3.542 and 16.796% ± 6.605 respectively (P>0.05) as compared with their controls.
2. While the doses of 25mg/ml and 30mg/ml caused a significant increase in cardiac force with percentage increase as 30.971% ± 40.436 and 31.635% ± 3.077 respectively (P<0.05) when compared with their controls.
3. At the doses 35mg/ml and 40mg/ml the increase in cardiac force produced by MUYM was statistically highly significant (P<0.025) with the percentage increase as 32.533% ± 4.577 and 37.61%
Effects on Cardiac Cycle

MUYM effects on Cardiac Cycle showed a dose dependent increase when compared with control:

1. In the doses 5mg/ml, 10mg/ml, 15mg/ml and 20mg/ml, the drug caused a non significant increase in cardiac cycle, with percentage of decrease as 4.54% ± 3.03, 8.88% ± 7.02, 11.16% ± 3.95, 11.95% ± 6.49 respectively (P>0.05) when compared with their controls.

2. While the doses of 25mg/ml and 30mg/ml, caused a significant increase in cardiac cycle when compared with their controls i.e., 14.96% ± 1.83, 14.37% ± 6.27 (P<0.05) respectively.

Table-1
Effect of different doses of MUYM on heart rate of frog’s heart

<table>
<thead>
<tr>
<th>MUYM</th>
<th>Heart Rate (Beats/min.) Control</th>
<th>Heart Rate (Beats/min.) treated</th>
<th>% Fall in Heart Rate</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5mg/ml</td>
<td>55.75±3.14</td>
<td>53.39±2.63</td>
<td>4.07±2.46</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>10mg/ml</td>
<td>57.3±2.23</td>
<td>52.67±4.04</td>
<td>8.28±4.68</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>15mg/ml</td>
<td>67.79±2.6</td>
<td>61.75±2.29</td>
<td>8.89±0.36</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>20mg/ml</td>
<td>68.2±0.93</td>
<td>62.47±1.41</td>
<td>8.44±0.85</td>
<td>P&lt;0.025</td>
</tr>
<tr>
<td>25mg/ml</td>
<td>72.25±1.49</td>
<td>65.9±0.45</td>
<td>8.63±1.55</td>
<td>P&lt;0.025</td>
</tr>
<tr>
<td>30mg/ml</td>
<td>53.89±0.83</td>
<td>49.46±0.55</td>
<td>8.18±1.29</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>35mg/ml</td>
<td>67.92±0.98</td>
<td>61.78±1.11</td>
<td>8.99±1.85</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>40mg/ml</td>
<td>72.01±1.74</td>
<td>61.36±1.06</td>
<td>14.45±2.39</td>
<td>P&lt;0.005</td>
</tr>
</tbody>
</table>

n= 12, Mean ± S.E.M., F(12.25)= 47.78; P<0.01, F(6.99)= 31.57; P<0.01

Fig. 10: Effect of different doses of MUYM on heart rate of frog’s heart
* =P < 0.05, ** = P<0.025, *** =P< 0.01, ****=P<0.005

± 9.852 respectively, compared with their controls (Table-2, Fig. 11).
3. 35mg/ml and 40mg/ml produced a highly significant increase in cardiac force of frog’s heart when compared with their controls i.e., $14.2\% \pm 2.37$, $16.34\% \pm 3.54$ and with $P$-value $<0.025$ (Table 3, Fig. 12).
Fig. 12: Effect of Mufarreh Yaqooti Motadil (MUYM) on Cardiac Cycle of frog in different doses, compared with control. 
* =P <0.05  ** =P<0.025

Fig 13a Tracing for control frog’s heart activity  
Fig 13b: Effect of MUYM (40mg/ml) on intact

Receptor Activity for Effect on Heart
Effect on cardiac force:
The dose of 40mg/ml MUYM has demonstrated an increase in the cardiac force that was found to be highly significant (P<0.025) i.e., 37.61% ± 9.85 higher than its control (Fig. 14). On the other hand similar dose of MUYM had increased the cardiac force by only 12.18%±4.21 in the presence of atropine (10^{-3}M), that was statistically non significant (P>0.05) when compared with their controls (Fig. 15 and Table 4).

Table-4
Receptor Activity of MUYM for the Effect on Cardiac Force

<table>
<thead>
<tr>
<th>Dose</th>
<th>Cardiac force (g) control</th>
<th>Cardiac force (g) treated</th>
<th>% increase</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>40mg/ml MUYM</td>
<td>0.439±0.035</td>
<td>0.600±0.051</td>
<td>37.61±9.852</td>
<td>P&lt;0.025**</td>
</tr>
<tr>
<td>Atropine (10^{-3}M) + MUYM (40mg/ml)</td>
<td>0.439±0.035</td>
<td>0.49±0.032</td>
<td>12.18±4.21</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

n= 12, Mean ± S.E.M.
Effect on Heart Rate:

MUYM (40mg/ml) has been found to decrease the heart rate by 14.45±2.39% (P<0.005) than its control Fig. 16. While on pretreatment with atropine (10^{-3}M) in the presence of atropine, MUYM could not show its effect and tried to decrease the heart rate but a nonsignificant decrease i.e., 3.69±0.93 decrease was observed when compared with its controls (Fig. 17, Table 5).

Table 5
Receptor Activity of MUYM for the Effect on Heart rate

<table>
<thead>
<tr>
<th>Doses</th>
<th>Heart rate (beats/min.) (control)</th>
<th>Heart rate (beats/min.) (treated)</th>
<th>% decrease</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>40mg/ml MUYM</td>
<td>72.01±1.74</td>
<td>61.36±1.06</td>
<td>14.45±2.39</td>
<td>P&lt;0.005****</td>
</tr>
<tr>
<td>Atropine (10^{-3}M) + MUYM (40mg/ml)</td>
<td>72.01±1.74</td>
<td>69.37±1.96</td>
<td>3.69±0.93</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

n= 12, Mean ± S.E.M.

Fig. 14: Effect of MUYM (40mg/ml) on Cardiac force of frog’s heart. **=P<0.025

Fig. 15: After Atropine administration effect of MUYM (40mg/ml) could not be produced on Cardiac force of frog’s heart.

Fig. 16: Effect of MUYM (40mg/ml) on Heart rate of frog’s heart. **** =P<0.005

Fig. 17: Effect of MUYM (40mg/ml) on Heart rate of frog’s heart was not produced after Atr. Administration.
CONCLUSION

In the present study the drug MUYM in different doses i.e., 5mg/ml, 10, 15, 20, 25, 30, 35, 40 mg/ml has been tested for its effects on cardiac parameters. The results presented shows that the drug tested in different doses has the ability to increase the cardiac force in dose dependent manner. This is found to be highly significant at the dose of 40mg/kg (37.61%±9.852, P<0.025) it is also observed that the drug has the ability to decrease the Heart rate. It is important to note that heart rate reduction is associated with an increase in cardiac force. It suggests that this drug MUYM is increasing the efficacy of heart by reducing the heart rate, increasing the cardiac force and the duration of cardiac cycle.

Receptor Activity of C.C.L.E. for Effect on Heart:

The receptor activity of MUYM for the action on intact heart of frog has been tested by using cholinergic agonist Ach (10^{-3}M) and cholinergic competitive antagonist Atropine (10^{-3}M). The results demonstrate that Ach and MUYM both when administered alone have significantly reduced the heart rate. On the other hand the use of Ach and MUYM on Atropine pretreated heart did not show such decline in heart rate as shown earlier without atropine pretreatment. These results clearly indicate that Drug MUYM’s effect is mediated through same receptors and mechanisms as established for Ach. and decreases heart rate by activation of Muscarinic receptors (Erum et al., 2004).

REFERENCES