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Cardiovascular Activities of an Ayurvedic Preparation Amalaki Rasayan in Rat Model

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Abstract

The importance of traditional or alternative medicines including Ayurvedic drugs are increasing day by day because of their necessary pharmacological actions and fewer side effects. Previous investigators showed hypolipidemic and cardioprotective effects of Amla which is the active ingredient of Amalaki Rasayan. It is an ayurvedic formulation that contains extracts of *Phyllanthus emblica* plant. Therefore, this study has been conducted to investigate the scientific evidence of its cardioprotective effects in rats by recording electrocardiographic tracings. Rats, of either sex, were treated with preparation containing extracts of *Phyllanthus emblica* in doses 800 µg/kg bodyweight, 1600 µg/kg body weight and 3200 µg/kg body weight through intraperitoneal route. Electrocardiogram along with heart rate and other parameters were measuredin rat model before and after administration of Amalaki Rasayan. It was evident that Amalaki Rasayan, at a dose of 800 µg/kg, was safe but 1600 µg/kg and 3200 µg/kg produced abnormal activities in the heart. It can be inferred from the study that, the ayurvedic preparation Amalaki Rasayan possesses significant cardioprotective effect.

Key words: Amalaki Rasayan, ECG, Heart disease, Rat model, Traditional medicines.

Introduction

Medicinal plants are one of the principal healthcare resources for the majority of people all over the world. The healing properties of herbal medicines have been recognized in many ancient cultures. The traditional systems such as Ayurveda, Siddha and Unani are part of a time-tested culture and honored by people till today. Pharmaceutical importance of plants has led to adoption of plant extracts commonly known to be used in traditional medicine, as alternative source of remedy (Suresh *et al*., 2011). Herbal medicines, also called botanical medicines or phytomedicines, refer to the use of any plant seed, berries, roots, leaves, bark or flower for medicinal purposes (Baquar, 2001). The economic significance of medicinal plants stems from the fact that the number of patients suffering from chronic ailments is on the rise and drugs from medicinal plants are more effective in treating such disorders (Deshpande *et al.*, 2006). Medicinal and aromatic plants (MAPs) are produced and offered in a wide variety of products, from crude materials to processed and packaged products like pharmaceuticals, herbal remedies, teas, spirits, cosmetics, sweets, dietary supplements, varnishes and insecticides (Ohrman, 1991; Gorecki, 2002; Lange, 1996). Ayurvedic medicine still are of main choices of the world's populations for primary healthcare because of better cultural acceptability, compatibility with the human body and fewer side effects. Now a days, many Ayurvedic preparations are used for the treatment of different diseases but

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there are little proven scientific evidence about their proper action and lethal dose. In the present days, the World Health Organisation (WHO) emphasizes on concomitant use of traditional drugs, which are bsed on plant materials, to ensure the total health coverage. A large number of plants are known to be used in the treatment of cardiovascular disorders in different corners of the world. Ayurveda is a Sanskrit term, made up of the words ayus and veda. Ayus means life and Veda means knowledge or science. The term Ayurveda thus means the knowledge of life or the science of life (Laksmi *et al.*, 2011).

Ayurveda is a traditional system of medicine (Courson, 2008) and used as a wide range of modalities to create health and wellbeing. It is used to restore the physical, mental and emotional balance in patients, thereby improving health, preventing disease (prophylaxis) and also treating any current illness (Mazumder *et al.*, 2011).The World Health Organization and National Institute of Health, USA have also recommended the use of Ayurvedic drugs in the name of complementary/alternative medicine (CAM) system, because these drugs have fewer side effects and give necessary pharmacological actions (Chopra and Doiphode, 2002; Dodds, 2008; Gogty *et al.*, 2002).

Amalaki Rasayan is prepared from whole fruit of *Phyllanthus emblica*, also known as emblic myrobalan, Indian gooseberry, Malacca tree, or amla of the family Phyllanthaceae. It is known for its edible fruit of the same name. Amalaki Rasayan is an Ayurvedic formulation which is beneficial for brain, eyes, heart, liver, skin and hair. It improves the functions and health of these organs. It is manufactured as liquid by arista process (BNFAM, 1992). In this paper we highlighted the effects of this formulation on cardiac system.

Materials and Methods

Drug: Amalaki Rasayan was purchased from Shree Kundeshwari Oushadhalaya, Dhaka. It was presented as 500 ml in glass bottle.

Instruments: Veterinary ECG machine (EDAN VET 300 model) was used to carry out the experiment. The EDAN VET-300 is suitable equipment for laboratory experiments with animals for measuring ECG. It is simple and easy to use, lightweight and portable. Interpretative analysis included in the form of automatic measurement calculation.

Selection of animals: A total of 40 rats of either sex, weighing about 130-150 g, aged 2 months were purchased from the animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. The rats were divided into four groups:

Group I: Rats were given only normal food and water. This group of rats is referred as Control rats.

Group II: Rats were given normal food and water. They received Amalaki Rasayan at a dose of 800 µg/kg body weight.

Group III: Rats were given normal food and water. They received Amalaki Rasayan at a dose of 1600 µg/kg body weight.

Group IV: Rats were given normal food and water. They received Amalaki Rasayan at a dose of 3200 µg/kg body weight.

All the rats were acclimatized to the new environment for a period of one week. During the experimental period, the rats were kept in a well ventilated animal house maintained at 25°C. They were supplied with standard pellets and fresh drinking water. All the rats were kept in cage and maintained with natural 12 h light and dark cycle in the animal house of Institute of Nutrition and Food Science, University of Dhaka, Bangladesh.

Preparation of dose: The dose was calculated from adult human dose of 10 ml as a single dose. The dose calculation is given in the Table 1.

Experimentation: For anesthesia, 50 mg/kg Ketamine was administered intraperitoneally. The electrodes were connected to the left arm, right arm, left leg, right leg and rib joint. Auto option was selected to get rhythm from standard limb lead I, II, III, avR, avL, avF, V. Finally standard limb lead I and II were used for characterization of ECG. Rhythm mode was also used and standard limb lead

II recording were used for calculation of ECG parameters.

ECG parameters: ECG parameters were recorded and analyzed to find out the effects of the test drug. The ECG parametersare summarized in Table 2. A schematic tracing of ECG record is shown in Figure 1. These tracings were used as standard to calculate the measured values of ECG parameters.

Table 1. **Calculation of dose ('X' is body weight of the rats) where normal human dose is 200 mg/kg.**

Concentration (as expressed by "X")	Concentration (as expressed by μg/kg)	Action sought
1/16 X	100	Less action
1/12 X	133	Less action
1/8 X	200	Less action
1/4 X	400	Less action
½ X	800	Proper action
X	1600	Slightly toxic
2 X	3200	More toxic
4 X	6400	Lethal dose
8 X	12800	Lethal dose

Table 2. **ECG Parameters.**

Parameters of ECG	Duration (ms)
P wave	≤100
QRS complex	80-110
Q wave	40
R wave	200
S wave	60-10
T wave	160
U wave	80
PR interval	120-200
ST segment	≤200
R-R interval	600-1200
Q-T interval	350-430

ECG paper speed: The paper moved at a rate of 25 mm/second. Time was measured horizontally. Each small block is 1 mm which is equal to 0.04 seconds and 0.1 mV. Each bold block is equal to 0.2 seconds. Amplitude was measured vertically in mV.

Results

ECG tracings were recorded before and after administration of the test drug and it was found that in control group the normal heart rate of rats was within 195-282 bpm (normal range being 250-350 bpm)and ECG tracing showed that every parameters were within the normal range (Figure 2).

When Amalaki Rasayan was administered at a dose of 800 μg/kg, no change in the heart rate and other ECG parameters were observed. It was indicated that 800 μg/kg is quite safe dose for the rats (Figure 3).

After administration of 1600 μg/kg, it showed some abnormalities such as SA nodal block which was an evident from missing beats (Figure 4).

After administration of 3200 μg/kg of the drug, the heart rate decreased to about 67 bpm. It indicated that Amalaki Rasayan produced bradycardia and ultimately leading to the deathof the animals (Figure 5a-d) producing various types of severe arrhythmias.

It indicated that 3200 μg/kg is a very lethal dose and this dose of the drug produced all types of arrhythmias.

Data from auto mode: The auto mode of the ECG machine was that mode where heart rate, P wave, PR interval, QRS duration was shown and measured. Using the auto mode it could be known about increase or decrease in heart rate and other

parameters as listed in Table 2. The data obtained from the ECG machine after administration 800 µg/kg in auto mode showed in the Table 3.

Table 3 showed the ECG parameters after administration of 800 µg/kg of Amalaki Rasayan. Pretreatment row shows the values in control condition. ECG parameters were calculated after 10 min of administration of drug. At this dose, the heart rate increased, P duration decreased, PR interval decreased followed by increased for some time and then came to normal value. All these indicated that 800 µg/kg was safe dose for the animal.

The data of auto mode obtained from ECG tracings after administration 3200 µg/kg in auto mode shown in the Table 4.

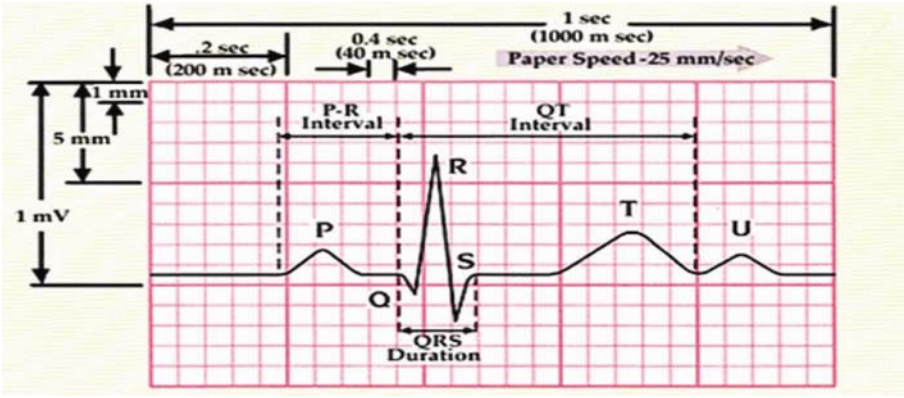

Figure 1. Schematic presentation of an ECG tracing. In the vertical axis,1 small square = 1mm (0.1 mV), 1 large square = 5 mm (0.5 mV) and 2 large square = 10 mm (1 mV), and in the horizontal axis, 1 small square = 0.04 s (40 ms), 5 small square = 0.2 s (200 ms) and 25 small square = 1 s (1000 ms).

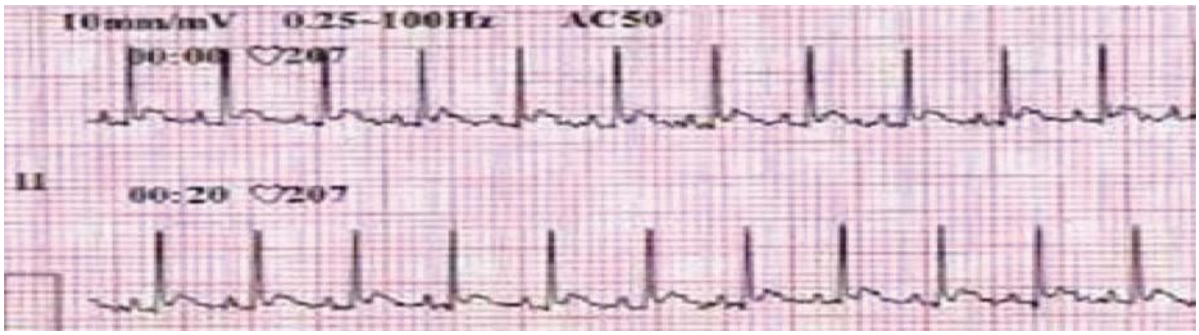

Figure 2.Normal ECG tracings of the rat heart.

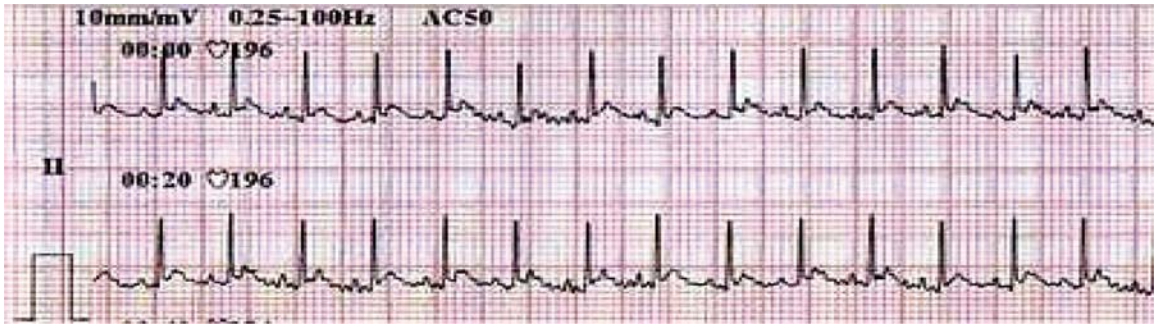

Figure 3. ECG tracing after administration of 800 µg/kg of Amalaki Rasayan.

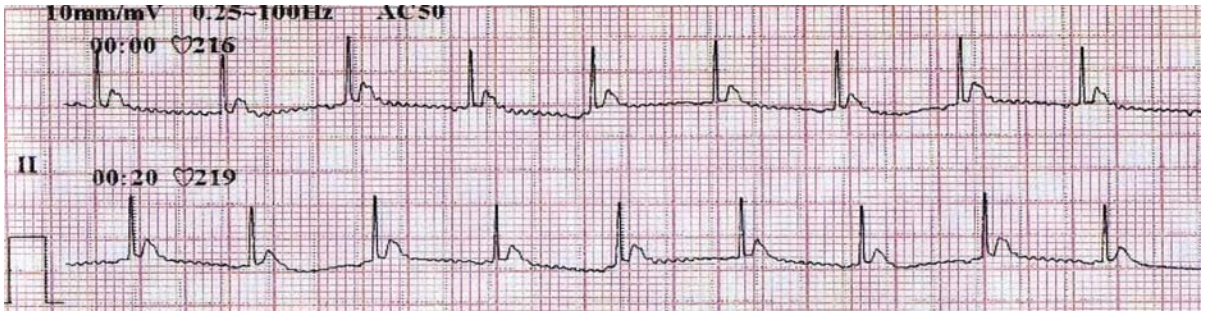

Figure 4. Typical ECG tracings of the standard limb lead I and II after intraperitoneal administration of 1600 µg/kg of drug.

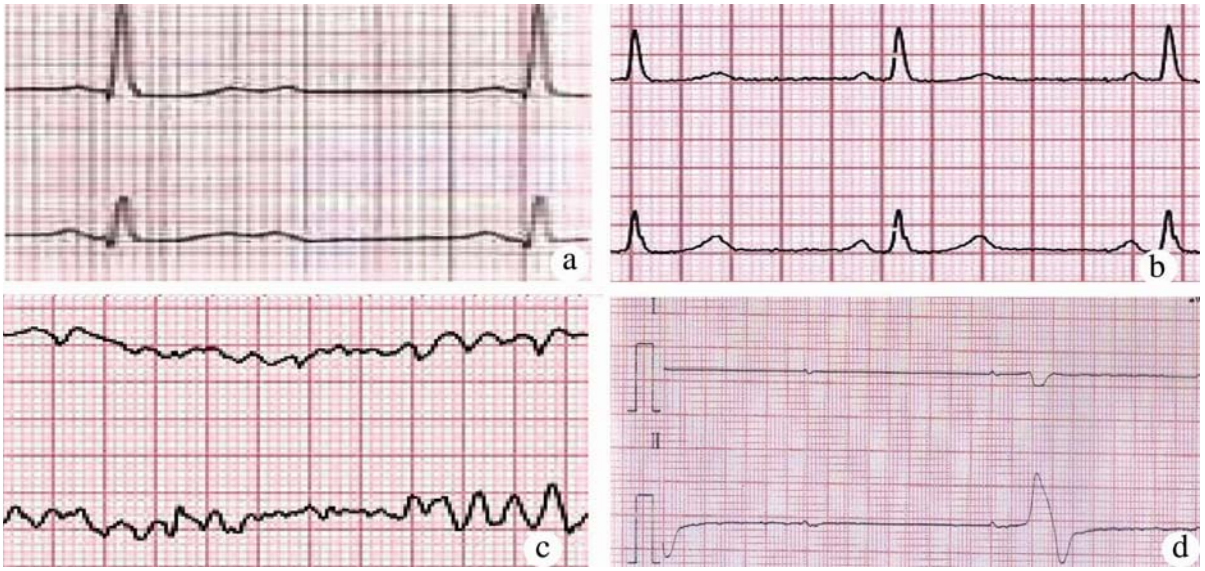

Figure 5. Typical ECG tracings of the standard limb lead I and II after intraperitoneal administration of 3200 µg/kg of drug. Panels (a) and (b) show AV nodal block as well as SA nodal block, Panel (c) and Panel (d) show ventricular fibrillation and dying conditions of the animal, respectively. The vertical line indicates mV and the horizontal line indicates time in second (s).

Table 3. Different ECG parameters after administration of Amalaki Rasayan at a dose of 800 µg/kg. The data were shown as mean of 9 similar experiments (n=9) in autoMode. (ms = mili second, bpm = beat per minute).

Time (min)	HR (bpm)	P duration (ms)	PR interval (ms)	QRS duration (ms)
Pretreatment	216.5	42.3	90	103.6
10	280.3	42	78.3	126
15	241	25.6	80.3	115.5
20	259.5	21.3	83.5	126.8
25	245	55.6	78	138.3
30	230.5	57.5	90.6	159.2
35	242.6	27.3	87.5	154
40	255.5	31.5	95.3	142.5
45	262.5	28.6	98.5	156

Table 4. **Different ECG parameters after administration of Amalaki Rasayan at a dose of 3200 µg/kg. The data were shown as mean of 9 similar experiments (n=9) in auto mode.**

Time (min)	HR (bpm)	Pduration (ms)	PR interval	QRS duration (ms)
Pretreatment	216.5	42.3	90	103.6
10	273.5	42	64	170
15	302.5	37	55.6	184.5
20	229.5	75.6	110	145.3
25	106.5	98.5	156.3	172.8
30	95.5	51	183	118.5
35	106.5	37	156	118
40	67	0	104	104
45	75	0	0	92

Table 4 showed the numerical values of different ECG parameters after administration of 3200 µg/kg of Amalaki Rasayan. Pretreatment row shows the normal values. The ECG parameters were calculated after 10 min. It was observed that heart rate decreased severely; P duration, PR interval and QRS duration also decreased dramatically. All these indicated that 3200 µg/kg was a lethal dose leading to dyeing condition of animals.

Data from rhythm mode: The rhythm mode was that mode where leads I, II, III, aVR, aVL, aVF, V were located. This mode showed RR average interval, RR maximum interval and RR minimum interval. The data obtained from machine after administration 800 µg/kg in rhythm mode is shown in the Table 5.

Table 6 displayed abnormal total R number, RR average, RR maximum interval and RR minimum interval. In the ECG interpretation curve shown in control mode, the normal heart rate of rats which was 238-282 bpm. But when Amalaki Rasayan was administered at a dose of 800 µg/kg, no change in the heart rate was observed. After administration of 1600 µg/kg, it revealed some abnormalities such as SA nodal block, atrial fibrillation, AV nodal block, right bundle branch block andleft bundle branch block. But after administration of 3200 µg/kg of the drug, the heart rate decreased to 67 bpm. It indicated that Amalaki Rasayan produced marked bradycardia and ultimately leading to the death of the animals.

Table 5. Different ECG parameters after administration of Amalaki Rasayan at a dose of 800 µg/kg. The data were shown as mean of 9 similar experiments (n=9) in rhythm mode.

Time (min)	Total R number	HR (bpm)	RR avg interval (ms)	RR max interval (ms)	RR min interval (ms)
Pretreatment	189.6	239	255.3	546.3	182.5
10	236.8	238.8	254.17	519.4	152.4
15	247.6	246.3	255.6	462.6	195.7
20	217.3	252.5	246.6	423.83	166.5
25	260.8	263.4	238	359.4	161.3
30	245.33	270	233.5	379	182
35	282.2	282	213.4	405.2	195.5
40	263.6	267.5	227.8	347.33	197.5
45	248.8	251.5	242	391.16	189.8

In Table 6, the pretreatment column showed the results in control condition. The calculation of data started after 10 min. It was observed that total R number, RR average interval, RR maximum interval and RR minimum interval all remained static after administration of 800 µg/kg of the drug.

Table 6 highlighted the results of ECG parameters after administration of 3200 mg/kg. It was observed that the total R number decreased but RR average interval, RR maximum interval and RR minimum interval increased drastically. These results suggested that 3200 µg/kg was a lethal dose and it affected all parameters of ECG tracings which indicated that the dose produced all types of arrhythmia.

The data obtained after administration 3200 µg/kg in rhythm mode were shown in the Table 6.

Table 6. Different ECG parameters after administration of Amalaki Rasayan at a dose of 3200 µg/kg. The data were shown as mean of 9 similar experiments (n=9) in rhythmmode.

Time (min)	Total R number	HR (bpm)	RR avg interval (ms)	RR max interval (ms)	RR min interval (ms)
Pretreatment	189	239	255.3	546.3	182.5
10	286.7	289	212.6	1161	217
15	220.5	223	296.6	1425	205
20	162.5	163	379.8	593.5	164.5
25	95.6	92	646.6	1067	510
30	53.8	54	1098	1200	531
35	33.6	46	1308	2614.5	682
40	49.6	47	1203	1839	217
45	60	67	566.6	754	640

Discussion

The experiments were performed in rat model with the drug at different doses using the intraperitoneal route and ECG tracing were recorded to explore the cardiac activity. Prolong use of traditional medicine and usage at large dose may cause injury to the related organ (heart, liver, kidney; heart was considered in this study) and produce drug induced effects which might prove lethal to the subjects. We observed that formulation of *Phyllanthus emblica* offered cardioprotection at 800 µg/kg. This finding is similar to our previous findings with other traditional medicines (Rahman *et al.*, 2013; Islam *et al.*, 2014).In another study in rats it was observed that *Phyllanthus emblica* dissolved in water preserved cardiac tissue during ischemia-reperfusion injury. It also increased the production of nitric oxide which is vasodilator, decreased the low density lipoprotein, total cholesterol and triglyceride (Thirunavukkarasu *et al.*, 2015).

Conclusion

It was found that 800 µg/kg was normal dose, 1600 µg/kg was slightly toxic but 3200 µg/kg was lethal to the rats. We, therefore, concluded that Amalaki Rasayan is safe to cardiac muscle at a dose of 800 µg/kg. But at long time use or at a high dose it could cause different cardiac abnormalities. Similarly, the effects of this drug to other vital organs of the body need to be evaluated. Study at cellular and molecular level with this drug is necessary to get more insight into the pharmacological actions of this drug.

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Conflict of Interest

The authors declare that no conflict of interest exists.

Limitation of Study

We could not use any standard product.

Ethical Approval

All authors hereby declared that all experiments were examined and approved by the Ethical review committee, Faculty of Pharmacy, University Dhaka, Dhaka-1000, Bangladesh and were therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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