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Cardiotonic effects of *Terminalia arjuna* extracts on guinea pig heart *in vitro*

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Abstract

We investigated the effects of fruit and bark extracts of *Terminalia arjuna* on the rate and contraction of guinea pig heart *in vitro*. Immediately after killing, the heart was connected to a perfusion apparatus containing oxygenated double-dextrose McWins solution with fresh arjun fruit and bark experimental extracts. The apex of the heart was then connected to a recording drum through a metallic hook and the rate and height (force) of contractions were recorded on a smoked drum. The heights of contraction for fruit extract and bark extract were significantly higher than that for normal saline ($p<0.001$), while they were significantly lower than that for adrenaline ($p<0.01$). Addition of verapamil caused a significant blockade in the heights of contraction produced by extracts of *T. arjuna* ($p<0.01$), while heart rate was not affected. The cardiotonic effect of *T. arjuna* was probably mediated through high concentration of Ca^{++} present in the plant.

Introduction

Terminalia arjuna (neer maruthu in Tamil and Malayan, commonly called aurjuna or the arjun tree in English) is about 20-25 meters tall, usually has a buttressed trunk and forms a wide canopy at the crown from which branches drop downwards. The Arjun is usually found growing on river banks or near dry river beds in West Bengal, South and Central India and in Bangladesh (Ali et al., 1966; Ali et al., 1979; Basu and Kirtikar, 1987; Biswas et al., 2011; Dwivedi, 2007; Ramchandran, 1992).

Since the studies in mice showing its leaves to have analgesic and anti-inflammatory properties, *T. arjuna* has found its way in traditional/alternative medicine for treatment of a wide range of human ailments. The arjuna was introduced into Ayurveda as a treatment for heart disease, wounds, hemorrhages, fractures, ulcers, tuberculosis, cough, chronic fever particularly tuberculosis, hemoptysis, urinary tract infections, renal stones, acne, bleeding piles and diarrhea (Ali et al., 1966; Dwivedi, 2007; Chopra et al., 1958; Dastur, 1982). Of these treatments our research interest was related to the reported anti-ischemic, cardiac protective and cardiotonic effects of *T. arjuna* on the cardiovascular system (Ali et al., 1979; Ramchandran, 1992). In an attempt to elucidate the cardiotonic properties, we investigated the effects of fruit as well as bark extracts of *T. arjuna* on the rate and contraction of guinea pig heart *in vitro*.

Materials and Methods

Animal

The study was conducted on guinea pigs of both sexes weighing 500-600 g. Immediately after killing by a blow on the head the heart along with great vessels were taken out and kept on a petri dish containing

McWins solution bubbled with oxygen. The heart was then cleaned of fat and other connective tissues, and was then connected to a perfusion apparatus (Langendorff's apparatus) containing oxygenated double–dextrose McWins solution. The apex of the heart was then connected to a recording drum through a metallic hook and the rate and force of contraction were recorded on a smoked drum. The height of contraction was measured in millimeter and the rate was counted per min by a timer.

Preparation of T. arjuna samples by extraction

Fresh arjun bark and fruit were collected from some trees (approximately 25 years old) within the premises of BCSIR at Dhaka. Various procedures were followed to prepare samples for pharmacological studies, e.g. i) crude water extraction of fruit; ii) crude powder bark; iii) water extraction of the fresh bark, and iv) extraction of the bark and fruit by various organic solvents like petroleum ether (bp 40-80°C), benzene, rectified spirit, etc.

Crude powder preparation of bark and fruit

Fresh barks and fruits were collected, chopped to small pieces and air dried. It was powdered in iron mortar-pestle and sieved by different types of standard sieves. The well dried powdered products were made ready for pharmacological experiments.

Water extraction of the bark and fruit

Chips of freshly collected barks and fruits were crushed and extracted with water at low temperature (50-60°C). The total extractive was concentrated on water bath and finally dried in desiccators containing $CaCl_2$/silica gel. It was then powdered and sieved.

Extraction of the bark and fruit with organic solvents

Chips of bark and fruits separately (4 kg each) were extracted exhaustively with rectified spirit. The total extractive were concentrated to about 1.5L and it was kept standing in a conical flask. After three days, heavy precipitates from it settled at the bottom which were filtered and dried over $CaCl_2$ in a desiccator. After separation of the solid from the solution, they were concentrated, dried and powdered. They were soluble in water. All chemicals were from BDH chemicals, London and sigma chemicals, London.

Grouping of animals and treatments

The animals were divided into groups receiving treatments as the following: Group I: Consisting of four guinea pigs; The heart received normal saline (0.2 mL/mL of bath fluid). This served as the control group. Group II: Consisting of four guinea pigs; The heart received crude fruit extract of *T. arjuna* (0.2 mL/mL of bath fluid). Group III: Consisting of three guinea pigs; The heart received fruit extract of *T. arjuna* (0.8 mL/mL of bath fluid). Group IV: Consisting of three guinea pigs; The heart received fruit extract of *T. arjuna* (1.2 mL/mL of bath fluid). Group V: Consisting of three guinea pigs; The heart received bark extract of *T. arjuna* (0.4 mL increasing to 0.8 mL/mL of bath fluid). Group VI: Consisting of five guinea pigs; The heart received calcium ions. Group VII: Consisting of five guinea pigs; The heart received verapamil (10 mg/mL of bath fluid) and alcoholic extract of *T. arjuna*. Group VIII: Consisting of two guinea pigs; The heart received calcium ions and verapamil.

These experiments were carried out in the Department of pharmacology of the then IPGMR, Shahbag, Dhaka 1000, in collaboration with BCSIR, Dhaka and Department of Medicine, IPGMR, Dhaka, during the period of 1983-85.

Results

The effects of normal saline and the fruit extract of *T. arjuna* on isolated guinea pig heart are stated in Table I. The mean height of contraction produced by crude fruit extract was significantly higher compared to normal saline as control ($p<0.01$). The mean ± SE heart rate was 110 ± 5.2/min and 112.0 ± 6.4/min for normal saline and crude fruit extract respectively, which were not significantly different ($p>0.2$).

The comparative effects of normal saline and bark extract of *T. arjuna* on guinea pig heart contraction *in vitro* are presented in Table II. Compared to fruit extract, the significant effect of bark extract on contraction was more pronounced ($p<0.01$ and $p<0.01$).

Table I

Effect of fruit extract of *T. arjuna* on isolated guinea pig heart

Drug	Dose (mL)	n	Height of contraction (mm)	%control
Normal saline	0.2	4	3.2 ± 0.6	100.0
Fruit extract	0.2	4	7.4 ± 0.8[a]	231.2
	0.8	3	8.2 ± 0.5[a]	250.0
	1.2	3	9.0 ± 0.6[a]	281.2

Data are mean ± SE; [a]$p<0.01$

Table II

Effect of bark extract of *T. Arjuna* on isolated guinea pig heart

Drug	Dose (mL)	n	Height of contraction (mm)	%control
Normal saline	0.2	3	6.1 ± 0.7	100.0
Bark extract	0.4	3	14.3 ± 1.4[a]	234.4
	0.8	3	12.2 ± 1.6[a]	200.0

Data are mean ± SE; [a]$p<0.01$

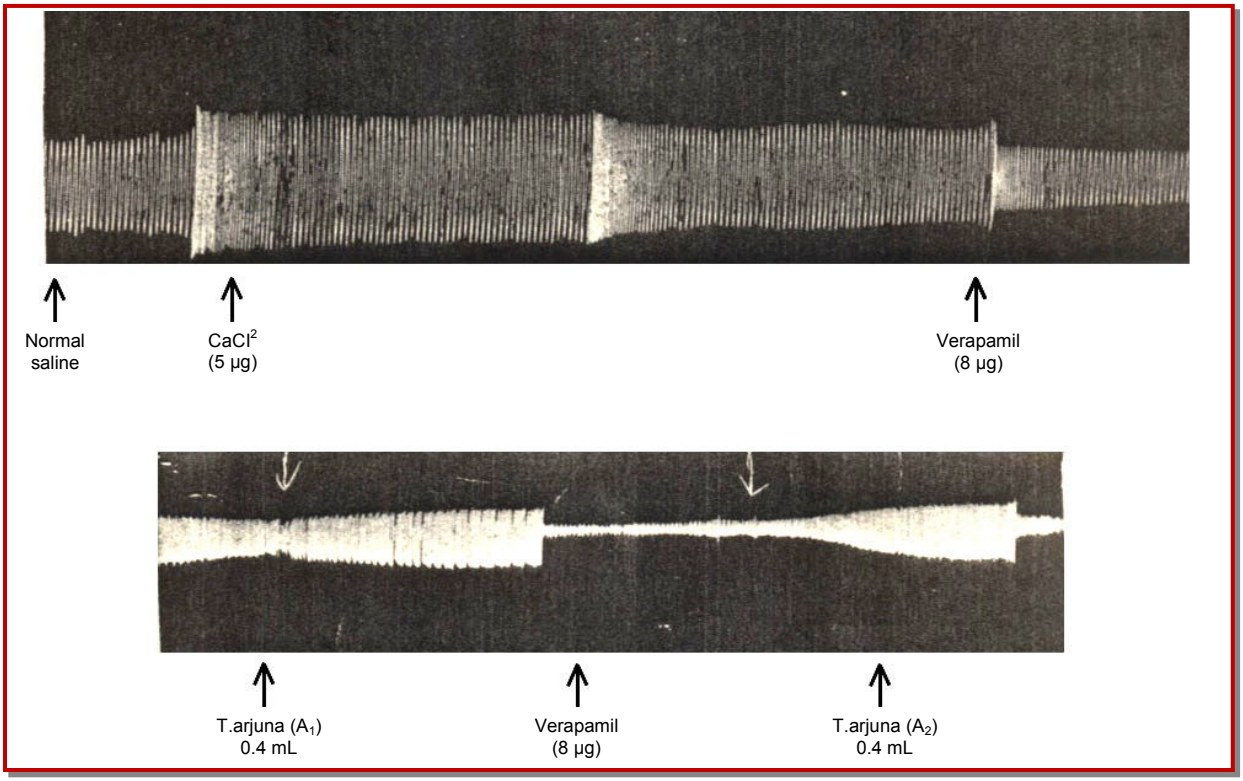

Figure 1: Tracing showing contractions of guinea pig heart after exposure to calcium, verapamil and different concentrations of *T. arjuna* extracts (A1: Water soluble part from the alcoholic extract of powdered bark dried earlier; A2: Water soluble part from the alcoholic extract of chopped bark without drying)

However, the mean heart rate was 114.0 ± 7.3/min which not significantly different from control (p>0.2).

The comparative effects of normal saline, adrenaline, *T. arjuna* (fruit extract) and *T. arjuna* (bark extract) are shown in Table III. The height of contraction (mean ± SE) was highest for adrenaline (p<0.001) and the heart rate was 115.0 ± 8.6/min which was significantly higher (p<0.01). The heights of contraction for *T. arjuna* (fruit extract) and *T. arjuna* (bark extract) were significantly lower than adrenaline (p<0.05), while they were significantly higher than that for normal saline (p<0.01).

The comparative effects of normal saline, $CaCl_2$ and verapamil on guinea pig heart *in vitro* are shown in Table IV and Figure 1. The mean ± SE of the heights of contraction for $CaCl_2$ alone was highest (p<0.001) and the heart rate of 134 ± 6.7/min were also significantly higher compared to control (p<0.01). Addition of verapamil (1 mg/mL of bath fluid) produced a significant (p<0.001) blockade in the height of contraction produced by calcium ions.

The effects of *T. arjuna* and verapamil on isolated guinea pig heart are shown in Table V and Figure 1. Addition of verapamil (1 mg/mL of bath fluid) caused a significant (p<0.01) blockade in the height of contraction produced by bark extract of *T. arjuna*.

Table III

Effect of adrenaline and extract of *T. Arjuna* on isolated guinea pig heart

Drug	Dose	n	Height of contraction (mm)	% control
Normal saline	0.2 mL	3	6.8 ± 0.8	100
Adrenaline	5.0 µg/mL	2	23.2 ± 2.4[a]	342
T. arjuna (fruit extract)	0.4 mL	3	12.3 ± 1.2[b]	180.9
T. arjuna (bark extract)	0.4 mL	3	16.4 ± 1.7[a]	241.1

Data are mean ± SE; [a]p<0.01; [b]p<0.05

Discussion

In the present study, cardiotonic properties was observed which was more pronounced with the water soluble part of alcoholic extract of *T. arjuna*. The force of contraction of the cardiac muscle was increased without any appreciable change in the rate. The bark of *T. arjuna* on frog heart and found an increase in the amplitude of contraction without increasing the rate which closely agrees with our observations. In the present experiment it was observed that the increased force of contraction was blocked by veraparmil, a calcium antagonist. This

Table IV

Effect of calcium chloride and verapamil on isolated guinea pig heart

Drug	Dose	n	Height of contraction (mm)	% control
Normal saline	0.2 mL	2	12.4 ± 2.1	100.0
Calcium chloride	5.0 µg/mL	2	30.0 ± 3.6[a]	241.9
Calcium chloride + verapamil	5 µg/mL	2	8.0 ± 0.6[a]	64.5

Data are mean ± SE; [a]p<0.001

Table V

Effect of *T. arjuna* extract and verapamil on isolated guinea pig heart

Drug	Dose	n	Height of contraction (mm)	% control
Normal saline	0.4 mL	2	6.1 ± 0.4	100.0
Bark extract	0.4 mL	2	11.4 ± 0.7[a]	186.8
Bark extract + verapamil	8 µg/mL	2	5.0 ± 0.3[b]	81.9

Data are mean ± SE; [a]p<0.01; [b]p<0.05

suggests that the cardiotonic effect of *T. arjuna* is likely to be mediated through the release of intracellular Ca^{++} ions. It has been found that arjun bark contains high calcium along with other organic acid (arjunic acid, etc.) and glycosidal substances (arjun extract) (Basu and Kirtikar, 1987). Bark is prescribed in traditional system of medicine in heart diseases. The cardiotonic property observed in the present study as well as by others, may therefore be due to this high calcium content and presence of glucosidal and other organic substances in *T. arjuna*.

Koman in 1920, on the other hand, failed to demonstrate the cardiotonic effect of *T. arjuna* when a decoction was used in the treatment of valvular heart disease. This may be due to completely different method of preparation from *T. arjuna* and also experimental situation. Caius et al., 1930 observed cardiotonic property of *T. arjuna* along with diuretic properties similar to cardiac glycosides (Biswas et al., 2011). The cardiotonic properties observed by these investigations are also compatible with our observations. This cardiotonic property of *T. arjuna* may be useful in the treatment of congestive heart failure (Basu and Kirtikar, 1987; Biswas et al., 2011).

However, compounds present in *T. arjuna* have anti-oxidant and hypocholesterolemic effects. In a randomized trail, it was shown that *T. arjuna* bark powder has significant anti-oxidant action that is comparable with vitamin E and it had significant cholesterol lowering effect (Gupta et al., 2001). Another study clearly demonstrated that bark extract of *T. arjuna* decreases platelet activation and may therefore possess anti-thrombotic properties (Malik et al., 2009). Many other studies demonstrated cardio perspective effect in myocardial neurosis, protection against cancer, benefits in angina pectoris, reversal of impaired endothelial function in chronic smokers, etc. (Barani et al., 2004; Dwivedi et al., 2005; Gauthamana et al., 2005; Karthikeyan et al., 2003; Sivalokanathan et al., 2006). There was no significant side effects of *T. arjuna* have been reported in medical journals (Sahelian, 2011).

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