Research Article

Cardiodepressant activity of 90% alcoholic extract of Terminalia arjuna and its probable mechanism of action

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ABSTRACT

Background: *Terminalia arjuna is being used in various cardiovascular diseases as cardiotonic, diuretic & in hypercholesterolemia. Studies conflict each other for its mechanism of action. This study aims to investigate effect of 90% alcoholic extract of Terminalia arjuna on in vitro isolated rabbit's heart & to find its probable mechanism of action.*

Objective: *To study the preliminary pharmacological effects of 90% alcoholic extract of Terminalia arjuna in-vitro on isolated heart, coronary blood flow, and to study its probable mechanism of action.*

Material & Methods: *Effect of Terminalia arjuna was observed on heart rate, coronary blood flow, amplitude on in vitro isolated perfused rabbit's heart mounted on langendorff apparatus & further cholinergic & adrenergic blockers were used to study the mechanism of action. Six experiments were conducted for each parameter & data was analysed using Student's t test.*

Results: *Terminalia arjuna causes mean percentage decrease of 7.26%, 9.31% & 20.51% in heart rate, decrease of 10.34%, 16.64%, 20.51% in coronary blood flow & decrease of 15.11%, 12.61%, 11.65% in amplitude at 25µg, 50µg & 100µg doses respectively. The decrease in heart rate, coronary blood flow & amplitude persists even after cholinergic & adrenergic blockers suggesting that cholinergic & adrenergic receptors are not involved in mechanism of Terminalia arjuna.*

Conclusion: *Terminalia arjuna cardiodepressant effect does not involve cholinergic & adrenergic receptors.*

Keywords: *Terminalia arjuna, alcoholic extract, aqueous extract, cardiac depressants*

Introduction

Cardiovascular diseases (CVD) / Coronary artery diseases (CAD) are the major cause of morbidity & mortality throughout the world especially in developed countries. It accounts for 30% of deaths worldwide. By 2001, CVD was responsible for 29% of all deaths. By 2030, when the population is expected to reach 8.2 billion, 32.5% of all deaths will be the result of CVD. [1] CAD is typically defined as presence of > 50% stenosis of any epicardial coronary artery. Underlying pathology is atherosclerotic changes in coronary vasculature. It manifests as angina pectoris, myocardial infarction, cardiac failure, sudden death. Though age, gender, family history, genetics can't be modified, it is necessary to treat modifiable risk factors like smoking, hyperlipidemia, hypertension, obesity, diabetes, physical inactivity. The prognostic indicators in patients of CAD include age, the functional state of the left ventricle, the location(s) and severity of coronary artery narrowing, and the severity or activity of myocardial ischemia, angina pectoris of recent onset, unstable

angina, early postmyocardial infarction angina, angina unresponsive or poorly responsive to medical therapy or accompanied by symptoms of congestive heart failure indicate an elevated risk for adverse coronary events. First line therapy of CAD depends upon modification of risk factors. Drugs used in CVDs include nitrates, β blockers, calcium channel blockers (CCB), aspirin, hypolipidemic drugs - statins. Nitrates, β blockers, CCB decrease the oxygen demand. Statins regulate the lipid profile of CVD patients. Aspirin, Clopidogrel interfere with platelet function. Use of these drugs is associated with side effects & drug drug interactions. Throbbing headache, tachycardia, orthostatic hypotension are major acute toxicity of nitrates. β blockers are contraindicated in asthma, severe bradycardia, atrioventricular block, severe unstable left ventricular failure. CCBs need careful titration when used in combination with nitrates, β blockers. Aspirin is blamed to be responsible for gastrointestinal bleeding, allergy and dyspepsia. Statins may cause elevation in serum aminotransferases, myopathy. From ancient times botanicals have been an integral part in medicine. In all ancient civilizations like the Egyptian, the Chinese, the Indian, the Roman and Greek civilizations, the most important sources of medicines were plants that were trusted to have healing powers. In recent years the botanicals are again gaining momentum for treatment of various diseases. Reason being botanicals/herbs are claimed to have minimum/ no adverse effect. In CVDs botanicals claimed to be beneficial are Terminalia arjuna, Crataegus oxycantha, Inula racemosa, and Astragalus membranaceus. Terminalia arjuna has a long history of its use as a cardiac tonic, [2] diuretic, [3] in heart failure, hypercholesterolemia and for relief of anginal pain. Terminalia arjuna is a large deciduous tree attaining a height of 60 -80 feet. It is common throughout sub Himalayan tracts of the Uttar Pradesh & in Deccan, southern Bihar, Chota Nagpur, Burma & Ceylon. The bark is considered by the Sanskrit writers to be cardiotonic. Vagbhatta was the first to prescribe the bark of TA in heart disease. Later Chakradutta, the great Indian physician, described it as a tonic and astringent & used it in heart disease. The practitioners of Hindu medicine use it for all sorts of conditions of cardiac failure and dropsy. [4] Singh [5] et al observed that hypotensive and bradycardia effects of TA are predominantly of central origin. Srivastav [6] et al concluded that hypotensive response of alcoholic extract of TA is due to muscarinic action on heart in variety of in vitro preparations. Nammi [7] et al observed that 70% alcoholic extract of TA produced dose dependant hypotension in anaesthetized dog which was blocked by propranolol but not by atropine & concluded that 70% alcoholic extract possess adrenergic β2 receptor agonist action. Some studies report that beneficial effect of TA is due to augmentation of endogenous antioxidant activity. [8, 9] In light of above conflicting reports this study is an endeavor to find the effect of TA extract on in-vitro isolated rabbit heart, coronary blood flow, and to find its probable mechanism of action.

Material and methods

Animal: Adult healthy rabbits of either sex of weight between 1.5-2.5

Kg were used as experimental animal. Permission was taken from Institutional Animal Ethics Committee for conducting the experiments on rabbits. In order to provide uniform experimental conditions, care was taken about the environment and diet of rabbits. The diet comprised of green vegetables, grass, soaked grams and milk given at libidum.

Procedure:

Study on in vitro isolated perfused rabbit's heart:

The rabbit's heart was mounted as per the scientific methods described by Burn [9] (1952) and Perry [10] (1970). The animal was bleeded through the carotid arteries. The chest was opened and heart along with an inch of ascending aorta was cut and immediately transferred in a china dish containing oxygenated Ringer Locke solution at 37˚C. All the blood from the ventricles was squeezed out to prevent the development of thrombi in the vessels.

The heart was mounted in the langendorff's assembly. A polyethylene capillary tube was used for administration of drug solution. The heart was connected through the apex to a modified starling's heart lever with a writing point. Oxygenated Ringer Locke solution was used to perfuse the heart. The preparation was allowed to stabilize for 15 minutes. The drug was administered through the indwelling polyethylene tubing in the arterial cannula. Administration of drug solution was followed every time by injection of 0.2ml of Ringer Locke solution to ensure complete displacement of the drug. The sensitivity of the heart was tested by giving adrenaline 2µg. The effect of test compound was observed on heart rate (HR), Amplitude, coronary blood flow (CBF) and further adrenergic and muscarinic receptor blockers were used to observe any change in HR, CBF, Amplitude caused by extract of Terminalia arjuna (TA). The dried 90% alcoholic extract was dissolved in distilled water to make aqueous solution of extract of TA. Six such experiments were performed with alcoholic extract of bark stem powder of TA and mean value calculated.

The data was pooled for each parameter & was statistically analyzed using student's t test. The results were displayed in tables and graphs. Mean value and standard error for all the parameters were determined separately and put in tables as Mean ± SE and as percentage change.

Results

Effect of aqueous solution of 90% alcoholic extract of bark stem powder of TA was measured on heart rate, coronary blood flow, amplitude. The doses used were 25 µg, 50 µg and 100 µg. The extract showed decrease in HR by 7.26%, 9.31% & 20.51% significantly at all the three doses i.e 25, 50 & 100 µg respectively, decrease in CBF by 10.34%, 16.64%, 20.22% at 25, 50, 100 µg (the decrease in CBF is significant at 50 & 100 µg doses) & decrease in amplitude by 15.11%, 12.61%, 11.26% at 25, 50, 100 µg significantly only at 25 µg (Table1) (Fig.1)

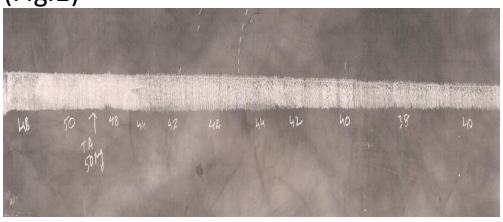

Fig. 1 Effect of extract of Terminalia arjuna on heart rate

After giving blocker the effect of TA was observed with 25 & 50 µg doses. After giving atropine, the extract showed decrease in HR by 3.58%, 17.18% at 25, 50 µg, decrease in CBF by 7.81%, 8.15%, decrease in amplitude by 22.45%, 33.42%.

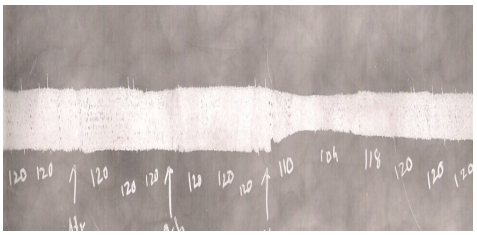

Fig. 2 Effect of extract of Terminalia arjuna after atropine on heart rate

After giving propranolol & Dihydroergotamine (DHE), the extract showed decrease in HR by 13%, 6.88% at 25, 50 µg, decrease in CBF by 3.59%, 3.18% & decrease in amplitude by 3.59%, 3.18% at 25, 50 µg respectively. The depressant effect was persistent even after administration of cholinergic blocker (atropine) (Table 2), (Fig 2) & adrenergic blocker (propranolol & Dihydroergotamine) (Table 3). suggesting that cholinergic & adrenergic receptors are not involved in TA action.

Table 1

Mean effect of Doses of 90% Alcoholic Extract of bark stem powder of Terminalia Arjuna on HR, CBF And Amplitude of Isolated Perfused Rabbit's Heart

Dose	*Mean %age decrease in HR*	*Mean %age decrease in CBF*	*Mean%age decrease in amplitude*
25 µg	7.26*	10.34	15.11*
50 µg	9.31*	16.64*	12.61
100µg	20.51*	20.22*	11.65

*significant, ** highly significant

HR -heart rate, CBF- coronary blood flow, µg- microgram

Table 2: Mean Effect of 90% Alcoholic extract of Bark Stem Powder of Terminalia Arjuna after administration of Atropine on HR, CBF & Amplitude of Isolated Perfused Rabbit's Heart

Dose	*Mean %age decrease in HR*	*Mean %age decrease in CBF*	*Mean %age decrease in AMPLITUDE*
25 µg	3.58*	7.81*	22.4
50 µg	17.18*	8.15*	33.42*

*significant, ** highly significant

HR –heart rate, CBF- coronary blood flow, µg- microgram

Table 3
Mean effect of 90% alcoholic extract of bark stem powder of terminalia arjuna after administration of propranolol & DHE on HR, CBF & Amplitude of isolated Perfused Rabbit's Heart

Dose	*Mean %age decrease in HR*	*Mean %age decrease in CBF*	*Mean %age decrease in AMPLITUDE*
25 µg	13*	3.59*	9.56
50 µg	6.88	3.18*	43.21*

*significant , ** highly significant
DHE- dihydroergotamine, HR–heart rate, CBF-coronary blood flow, µg-microgram

Discussion

The Aim of this study was to observe the effect of 90% alcoholic extract of bark stem powder of TA on HR, CBF & amplitude on in vitro isolated perfused rabbit's heart & to study probable mechanism of action. In this study the extract showed decrease in HR significantly at all the three doses i.e 25, 50 & 100 µg, decrease in CBF significantly at 50 & 100µg & decrease in amplitude significantly only at 25 µg (Table 1, Fig 1, 2). Dose dependant decrease in BP & HR by aqueous solution of 70% alcoholic extract was reported by Singh [5] et al on anaesthetized dog. Srivastav [6] et al also reported negative chronotropic & inotropic effect of 95% ethanolic extract of TA on isolated rat, frog atria, perfused frog heart & rabbit heart. The extract exerted negative chronotropic effect in all the preparations in dose dependant fashion and myocardial depression was easily reversible by washout in-vitro preparations. In frog atria, ventricular contractility sometimes culminate in stand still at 50- 200 mg dose. Radhakrishnan[12] et al has reported negative chronotropic & inotropic effect with chloroform extract of TA on isolated rat atria but positive inotropic effect with aqueous extract of TA. Studies on CBF are meagre. Bhatia [13] et al has reported increase in CBF with aqueous extract of TA contrary to this study where decrease in CBF with 90% alcoholic extract is significant at 50 & 100 µg. Aqueous and alcoholic extracts might contain different constituents depending upon the solubility of various constituents of bark stem of TA in different solvents.

In this study the decrease in HR with TA at 25, 50 µg was significant even after administration of atropine suggesting that mechanism of TA is not through muscarinic receptors. CBF & amplitude also decreased with TA after atropine. Srivastav[6] et al has reported that hypotensive effect of aqueous solution of 95% ethanolic extract of TA on anaesthetized dog BP is probably through muscarinic action as hypotensive response was abolished by pretreatment with atropine. In this study isolated rabbit heart preparation was used and negative chronotropic effect of TA was not blocked with atropine. Srivastava [6] et al has reported blockade of negative chronotropic effect of TA with atropine but that was in an anaesthetized dog (in- vivo) where

other confounding factors could also be present. Takahashi [14] et al observed hypotensive effect of aqueous extract of TA was not affected by pretreatment of rats with propranolol, but was attenuated by pretreatment with atropine. This suggested that an aqueous extract of TA containing tannin-related compounds has a hypotensive effect in the rat, which may be mediated through muscarinic receptors. Again this study was in whole animal.

In the present study the depressant effect of TA was persistent even after adrenergic blocker (propranolol & Dihydroergotamine) suggesting that adrenergic receptors are also not involved in TA action. Nammi [7] et al reported that hypotension produced by 70% alcoholic extract in anaesthetized dog BP was blocked by propranolol suggesting β agonistic activity but again in this case also the effect found by them was in the whole animal with 70% alcoholic extract. The possible mechanism of hypotension caused by aqueous solution of 70% alcoholic extract of TA was reported to be of central origin by Singh [5] et al. They observed that 70% alcoholic extract given in graded doses neither modified nor-adrenaline response on dog BP nor affected the pressor response induced by the splanchnic nerve stimulation in anaesthetized cats. But the extract inhibited carotid occlusion response thereby indicating the involvement of central nervous system in the hypotensive action. In this study the effects were observed on in vitro isolated perfused rabbit heart.

In Wistar albino rats, Gauthaman [15] et al observed that the beneficial effects of dried pulverized bark of TA on cardiovascular disease are exerted by its antioxidant activity by increase in the endogenous compounds- superoxide dismutase, glutathione. Endogenous antioxidant activity is also observed to be augmented with alcoholic extract of TA by Munasinghe[8] et al & Karthikeyan [9] et al. Increase in endogenous antioxidant activity may be the mechanism of beneficial effect of TA in cardiovascular disease but this study did not include assessment of endogenous activity which is needed to be explored. Chaturvedi [16] reported that TA increases prothrombin time and decreases the platelet count which may be contributing to its beneficial effect in CAD. Researchers have quoted that TA has lipid lowering potential that may be useful for CAD treatment. Chander [17] et al reported that ethanolic extract of bark powder of TA at dose of 250 mg per oral decreases plasma levels of total cholesterol, triglycerides, & phospholipids by 31, 40 and 18% in hyperlipidemic rats respectively more significantly than petroleum ether & water extract. Shaila [18] et al has also concluded that TA is potent hypolipidemic agent. In addition to experimental studies, many clinical studies have reported beneficial effect of TA in CAD. Gupta [19] et al observed significant decrease in total cholesterol and LDL cholesterol in patients of CAD receiving finely pulverized TA tree bark-powder (500 mg) in capsules daily. No significant changes in total, HDL, LDL cholesterol and triglycerides levels were seen in patients on placebo & on vitamin E. Dwivedi & Agarwal [20] has reported 50% reduction in angina episodes with TA in stable and unstable angina patients.

The drug also lowered systolic BP and it was concluded that monotherapy with TA is fairly effective in patients with stable angina. Bharani [21] et al reported that TA 500mg 8 hourly compared to placebo, was associated with improvement in symptoms and signs of heart failure, improvement in NYHA (Newyork Heart Association) Class (Class III vs. Class IV), decrease in echo-left ventricular end diastolic & end systolic volume indices, increase in left ventricular stroke volume index, & increase in left ventricular ejection fractions in patients of refractory chronic congestive heart failure (Class IV NYHA) & on 20-28 weeks continued therapy improvement in symptoms & signs continued. Dwivedi & Jauhari [22] reported that TA, in dose of 500 mg 8 hourly, is effective in angina pectoris, congestive heart failure patients. These patients were also on conventional treatment comprising of nitrates, aspirin, and/ or calcium channel blockers. Reduction in anginal frequency was observed however only patients on both conventional as well as TA treatment showed improvement in left ventricular ejection fraction and reduction in left ventricular mass on echocardiography following 3 months of therapy. Also TA did not show any adverse effects on renal, hepatic & haematological parameters. Bharani [23] et al observed that TA therapy 500 mg 8 hourly was associated with significant decrease in the frequency of angina and need for isosorbide dinitrate in males with chronic stable angina. The treadmill exercise test parameters improved significantly during therapy with TA compared to those with placebo. Dwivedi [24] et al reported that patients receiving TA 500mg in addition to anti anginal treatment showed significant decrease in ischaemic mitral regurgitation, improvement in E/A ratio (ratio of early & late diastolic flows) and considerable reduction in anginal frequency & concluded that TA possess antianginal, decongestive and hypolipidemic effect. Benefits of TA seen clinically can be due to its hypolipidaemic effect, antioxidant activity, diuretic action, [3] decrease in platelet count. [16] Further research on the effects & mechanism will help in establishing role of TA in CAD. To conclude in this study, aqueous solution of 90% alcoholic extract of TA has myocardial depressive effect on in-vitro isolated heart & probably does not involve muscarinic & adrenergic receptors. The status of TA in treatment of CVD needs further assessment and evaluation.

References

1. Antman EM, Andrew P, Selwyn AP, Braunwald E, Loscalzo J. Ischemic heart disease. In Fauci AS, Kasper DL, Longo DL, Braunwald E, Hauser SL, Jameson JL et al editors. Harrison's Principle of Internal Medicine. 17th ed. New York: McGraw hill Companies; 2008.p.1514-27.
2. Ghoshal M. Terminalia arjuna [Ph.D. Thesis] Calcutta: Calcutta University; 1909.
3. Cauis JS, Mhaskar KS, Issacs M. A comparative study of the dried barks of the commoner Indian species of genus Terminalia. Indian Med Res Memoirs 1930;16:51-75.
4. Chopra RN. Terminalia Arjuna W & A. In Chopra IC, Handa KL, Kapur LD editors. Chopra's Indigenous drugs of India. 2nd ed. Calcutta: Academics Publishers; 1982.p.421-24.
5. Singh N, Kapur KK, Singh SP, Shanker K, Sinha JN, Kohli RP. Mechanism of

Cardiovascular action of Terminalia arjuna. Planta Med 1982;45(6):102-4.
6. Srivastava RD, Dwivedi S, Sreenivasan KK, Chandrashekhar CN. Cardiovascular effects of Terminalia species of plant. Indian drugs 1990; 29:144-49.
7. Nammi S, Gudavalli R, Babu BSR, Lodagala DS, Boini KM. Possible mechanisms of hypotension produced by 70% alcoholic extract of Terminalia arjuna in anasthetized dogs. BMC 2003;3:5.
8. Munasinghe TC, Seneviratne CK, Thabrew MI, Abeysekera AM. Antiradical & antiproliferative effects of some plants extracts used by Sri Lankan traditional medical practitioners for cardioprotection. Phytother Res 2001; 15(6):519-23.
9. Karthikeyan K, Sarala Bai BR, Gauthaman K, Sathish KS, Devar NS. Cardioprotective effect of the alcoholic extract of Terminalia arjuna bark in an in vivo model of myocardial ischemic reperfusion injury. Life Sciences 2003;73(21): 2727-39.
10. Burn J H. Practical pharmacology. Edinburgh: Oxford Black well scientific publication Ltd; 1952.p.25.
11. Perry WLM editor. Experiments with heart muscle. In Pharmacological experiments on isolated preparations. 2nd ed. Edinburgh: E & S living stone; 1970.p.112-119.
12. Radhakrishnan R, Wadsworth RM, Gray AI. Terminalia arjuna, an ayurvedic cardiotonic, increases contractile force of rat isolated atria. Phytotherapy Research 2006;7:266-68.
13. Bhatia J. Study of the possible cardioprotective role of Terminalia arjuna in experimental animals and its clinical usefulness in coronary artery disease [MD thesis]. Delhi: University of Delhi;1998.
14. Takahashi S, Tanaka H, Hano Y, Ito K, Nomura T, Shingenobu K. Hypotensive effect in rats of hydrophilic extract from Terminalia arjuna containing tannin-related compounds. Phytotherapy research 1998;11(6): 424-27.
15. Gauthaman K, Maulik M, Kumari R, Manchanda SC, Dinda AK, Maulik SK**.** Effect of chronic treatment with bark of Terminalia arjuna: A study on the isolated ischemic-reperfused rat heart. J Ethnopharmacol 2001;75:197-201.
16. Chaturvedi PN. A study on the effect of an indigenous drug Terminalia arjuna on arterial thrombosis and ischaemic heart disease. [Thesis Doctor of ayurvedic Medicine]. Varanasi: Banaras Hindu University; 1967.
17. Chander R, Singh K, Khanna AK, Kaul SM, Puri A, Saxena R et al. Antidyslipidemic and antioxidant activities of different fractions of terminalia arjuna stem bark. Indian Journal of Clinical Biochemistry 2004; 19(2):141-148.
18. Shaila HP, Udupa SL, Udupa AL. Hypolipidemic activity of three indigenous drugs in experimentally induced atherosclerosis. Int J cardiol 1998;67:119-24.
19. Gupta R, Singhal S, Goyle A, Sharma VN. Antioxidant and hypocholesterolaemic effects of Terminalia arjuna tree-bark powder: a randomised placebo-controlled trial. J Assoc Physicians India 2001;49:231-5.
20. Dwivedi S, Agarwal MP. Antianginal and cardioprotective effects of Terminalia arjuna, an indigenous drug in CAD. JAPI 1994;42(4):287-89.
21. Bharani A, Ganguly A, Bhargava K. Salutary effects in patients with severe

refractory heart failure. International Journal of Cardiology 1995;49(3):191-199.

22. Dwivedi S, Jauhari R. Beneficial effects of Terminalia arjuna in coronary artery disease. Indian Heart Journal 1997;49: 507-10.
23. Bharani A, Ganguli A, Mathur LK, Jamra Y, Raman PG. Efficacy of Terminalia arjuna in chronic stable angina: a double-blind, placebo-controlled, crossover study comparing Terminalia arjuna with isosorbide mononitrate. Indian Heart J 2002; 54:170-175.
24. Dwivedi S, Aggarwal A, Agarwal MP, Rajpal S. Role of Terminalia arjuna in ischaemic mitral regurgitation. Int J Cardiol 2005;100(3):507-8.

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