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Original Research Article

**BRONCHODILATOR, CARDIOTONIC AND SPASMOLYTIC
ACTIVITIES OF THE STEM BARKS OF *TERMINALIA ARJUNA***

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ABSTRACT

Terminalia arjuna, Roxb barks has a folkloric repute to possess spasmolytic, bronchodilator and cardiotonic, properties. The present study was undertaken to evaluate possible effects of methanolic extract of barks of *Terminalia arjuna* (Ta.Cr) in gastrointestinal, respiratory and cardiovascular systems via *in vitro* experiments to rationalize its use in traditional system of medicine. The phytochemical analysis of crude methanolic extract of *Terminalia arjuna* barks revealed the presence of tannins and saponins as methanol soluble extractable constituents. The spontaneous contractions in isolated rabbit jejunum were relaxed at tissue bath concentrations of 0.01-5.0 mg/ml, however, addition of propranolol to the tissue bath reduced the extent of relaxation and same level of effect was achieved at increased concentrations, i.e., 0.1-10.0 mg/ml. The Ta.Cr showed a weak relaxant effect on high K⁺(80 mM) low K⁺ (25 mM) and carbachol (1 μM)-induced contractions. Similarly, it also caused partial relaxation of high K⁺ (80 mM) and low K⁺ (25 mM)-induced contractions in isolated rabbit tracheal preparations. However, it relaxed completely the carbachol (1 μM)-induced contractions, but addition of propranolol (1 μM) to the tissue bath minimized the relaxant effect. Moreover, the Ta.Cr caused partial relaxation of the phenylephrine (1 μM)-induced contractions in isolated rabbit aorta preparations. Furthermore, the Ta.Cr showed a positive inotropic and positive chronotropic effects at lower tissue bath concentrations in isolated rabbit paired atria preparations but these effects were found to be antagonized by the presence of propranolol in the tissue bath. Further addition of Ta.Cr to the tissue bath caused a decrease in the magnitude of observed inotropic and chronotropic effects in isolated rabbit paired atria; which was found to be abolished on addition of atropine. Thus; in conclusion, the crude methanolic extract of barks of *Terminalia arjuna*, Roxb exhibited spasmolytic, bronchodilator and cardiotonic properties which are likely to be mediated through presence of dominant β-adrenergic agonistic activity along with presence of some weak muscarinic agonistic activity.

Key words: *Terminalia arjuna*, bronchodilator, cardiotonic, spasmolytic,

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INTRODUCTION

Terminalia arjuna, Roxb. (Combretaceae) is commonly known as Arjun (Khare, 2007) and is found throughout India and Pakistan (Kiritkar and Basu, 1987). It is a large evergreen tree with buttressed trunk and drooping branches, having smooth grayish bark. Leaves are simple, oblong and unequal sided, green from above but pale brown beneath. Flowers are white in panicles of spikes with linear bracteoles. Fruits are ovoid or oblong with 5-7 short, hard angles or wings (Kumar and Prabhaka, 1987).

Phytochemical investigations on different plant parts revealed the presence of constituents like triterpenoid (arjunolic acid) (Ramesh *et al.*, 2011), triterpenes diglucoside terminolitin (23-deoxyarjunolitin) (Singh *et al.*, 1995), oleanane-type triterpene (terminoside A), from stem bark (Ali *et al.*, 2003a), triterpene glycoside like arjunetoside, oleanolic acid, arjunic acids from root barks (Upadhyay *et al.*, 2003), oleanane type triterpene glucosyl esters like arjun glucosides IV and arjun glucosides V from bark (Wang *et al.*, 2010a), 18,19-secooleanane-type triterpene glucosyl esters, namely arjunasides A, arjunasides B, arjunasides C, arjunasides D and arjunasides E from the bark (Wang *et al.*, 2010b). Cardenolides like 14, 16 dianhydrogitoxigenin-3- β -D-xylopyranosyl (1 \rightarrow 2)-O- β -D-galactopyranoside from seeds (Yadav and Rathore, 2000) and 16,17-dihydroneridienone 3-O- β -D-glucopyranosyl-(1 \rightarrow 6)-O- β -D-galactopyranoside from roots (Yadav and Rathore, 2001). Similarly, naphthanol glycoside, i.e., arjunaphthanolside from the stem barks (Ali *et al.*, 2003b) and ursane type triterpene glucosyl esters including 2 α ,3 β -dihydroxyurs-12,18-dien-28-oic acid 28-O- β -D-glucopyranosyl ester from the bark (Wang *et al.*, 2010c). Moreover, ellagitannins like arjunin (Kandil *et al.*, 1998) and ellagic acid (Bajpai *et al.*, 2005), gallitanins, (gallic acid and ethyl gallate) (Petit *et al.*, 1996), hydrolyzable tannin like casuarinin (Kuo *et al.*, 2005ab), flavones (luteolin) (Petit *et al.*, 1996) and flavonoids (Bhuyan and Saikia, 2005).

In indigenous systems of medicine, the barks of *Terminalia arjuna*, Roxb are found to be useful in asthma (Gruenwald *et al.*, 2000);, blood diseases (Kirtikar and Basu, 1975; Gruenwald *et al.*, 2000), dysentery (Kirtikar and Basu, 1975; Gruenwald *et al.*, 2000; Jain and deFillips, 1991), ulcers, tumors (Hartwell, 1982), and relieves fatigue (Kiritkar and Basu, 1987). Moreover, it helps to manage anaemia (Kirtikar and Basu, 1975; Gruenwald *et al.*, 2000), diabetes (Hansel *et al.*, 1998; Gruenwald *et al.*, 2000), cardiopathy (Burkill, 1966; Kirtikar and Basu, 1975; Bone, 1996), cirrhosis of the liver (Bone, 1996; Gruenwald *et al.*, 2000), hypertension (List and Hohammer, 1969-1979; Kapoor, 1990; Bone, 1996; Gruenwald *et al.*, 2000), leucorrhoea (Gruenwald *et al.*, 2000), otalgia (Kirtikar and Basu, 1975; Jain and deFillips, 1991), inflammations (Bone, 1996), internal and external blood loss (Kumar and Prabhaka, 1987)

Scientific investigations revealed inhibition of platelet function (Malik *et al.*, 2009), improve gastric ulcer healing (Devi *et al.*, 2007ab), protective effect against CCl₄- induced oxidative stress to heart (Manna *et al.*, 2006; Manna *et al.*, 2007) and cardio-protective effect (Karthikeyan, *et al.*, 2003).

Aims and objectives

Terminalia arjuna, Roxb has traditionally been used for the relief of asthma, bronchitis, and dysentery, but it has not been thoroughly investigated to rationalize its use in gastrointestinal and

respiratory tract ailments. The present study was undertaken to explore its therapeutic potential in the management of ailments pertaining to gastrointestinal and respiratory systems to validate its folkloric use in native systems of medicines.

MATERIAL AND METHODS

Plant Material

The plant material pertaining to *Terminalia arjuna*, Roxb. was collected from the residential area of the Bahauddin Zakariya University Multan, Pakistan. The plants was authenticated by the kind cooperation of an expert taxonomist (Prof. Altaf Hussain Dasti), at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan and a voucher specimen was deposited at the herbarium of the Institute. The plant material was rendered free from adulterated materials and vegetative debris through manual picking and grinded to coarse powder with the help of a special herbal grinder.

Crude extract

The powdered plant material (1 kg) was subjected to maceration in 70% aqueous-methanol in amber coloured bottle at room temperature for 8 days with occasional shaking. (Harborne, 1973). The soaked material was passed through muslin cloth to remove the vegetative material and the fluid obtained was filtered through Whatman-1 filter paper. The filtrate was evaporated on a rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) at 37°C under reduced pressure to thick paste like consistency (approximate yield was 12%) and the extract obtained was stored at -4°C in air tight jars. The plant extract (0.3 g) was dissolved in 1ml of distilled water to produce stock solution. From this stock solution further dilutions were made. Solutions were freshly prepared on the day of experiment.

Preliminary Phytochemical Screening:

Ta.Cr was subjected to phytochemical screening for the detection of saponins, tannins, phenols, coumarins, alkaloids and anthraquinones as possible constituent of the plant (Harborne, 1984; Harborne, 1998). The saponin presence was detected by the froth formation on vigorous shaking of the aqueous extract solution. Development of blue green or dark green coloration on mixing of aqueous FeCl_3 with extract solution indicated presence of phenols and tannins. The coumarins as plant constituents were detected on emission of fluorescence in UV light from pieces of filter paper which were exposed to the vapors emerging from boiling aqueous solution of plant extract, subsequent to treatment with NaOH. The alkaloid presence was noted by the appearance of yellowish brown coloration on mixing of Dragendorff's reagent with HCl treated aqueous plant extract solution. The appearance of pink, violet or red coloration on exposure to NH_4OH of the mixture of benzene with aqueous solution of plant extract already acidified with 1% HCl was taken as presence of anthraquinones among the plant constituents.

2.4. Chemicals:

The chemicals, solvents, drugs and reagents used in these experiments were of highest purity and of reagent analytical grade. Acetylcholine chloride, carbachol (carbarylcholine), isoprenaline, propranolol, potassium chloride (KCl) and verapamil hydrochloride were purchased from Sigma Chemical Company, St. Louis, MO, USA, while calcium chloride (CaCl_2) was purchased from

Merck (Merck, Darmstadt, Germany). All the solutions were freshly prepared in distilled water on the day of experiment.

Animals and housing conditions

Rabbits, guinea-pigs and mice of either sex of local breed were used for the experiments and kept at the animal house of The Aga Khan University, Karachi, maintained at 23-25°C and were given standard diet and tap water. Food was withdrawn 24 hr prior to the experiments from animals but had free access to water. Guinea-pigs were sacrificed by cervical dislocation and rabbits sacrificed by a blow on back of the head. Mice were used for the *in vivo* anti-diarrhoeal study.

Isolated Rabbit Jejunum Preparation

The anti-spasmodic activity of plant material was studied using isolated jejunum as described by Gilani *et al.* (2005a; 2006).

Rabbit was killed through cervical dislocation, abdomen was incised; jejunum segment of 2 cm length was dissected out and was rendered free from adhering mesenteries. Each segment was mounted between two stainless steel hooks in 10 ml tissue bath containing normal Tyrode's solution (pH 7.4), maintained at 37°C and aerated with carbogen (5% CO₂ and 95% O₂). A pre-load of 1 g was applied and tissue was allowed to equilibrate for a period of 30 minutes, during which the tissue was washed with fresh fluid at an interval of every 10 minutes prior to the addition of any drug. The spontaneous contractions were recorded isotonicly through Powerlab Data Acquisition system (AD instruments, Sydney, Australia).

Subsequent to equilibration, the tissue preparation was repeatedly treated with acetylcholine (0.3 µM) at 3 min intervals to stabilize the preparation. When three successive responses to acetylcholine were found to be identical in magnitude, the preparation was considered stable and exposed to test materials for the evaluation of antispasmodic action in a cumulative fashion. The test material-induced relaxant effect was measured as percent change in spontaneous contractions of rabbit jejunum immediately before the addition of test materials.

Determination of Calcium Channel Blocking Activity

The possible mechanism of the relaxant action on the part of test material was determined by its effects on high K⁺(80 mM)-induced contractions in isolated rabbit jejunum preparations as described by Faree *et al.* (1999). The isolated rabbit jejunum preparations on exposure to high tissue bath concentration of K⁺(80 mM), exhibits a sustained contractile response. Test materials were added to the tissue bath in a cumulative fashion to achieve concentration-dependant relaxant responses in the isolated rabbit jejunum preparations (van-Rossum, 1963). The extent of relaxation of pre-contracted intestinal preparation was expressed as percent of the control responses mediated by K⁺(80 mM). The smooth muscle contraction on exposure to high K⁺(80 mM) is mediated through the influx of Ca⁺² from extra-cellular fluid and the test material capable to inhibit this contraction is likely to exert its effect through Ca⁺² channel blockade (Bolton, 1979).

The proposed calcium channel blocking mechanism of the test materials was confirmed further by the methods described by Gilani *et al.* (2005a). The isolated rabbit jejunum preparation was allowed to stabilize in normal Tyrode's solution, followed by 45 min exposure to a K⁺ normal,

Ca⁺² free and EDTA (0.1 mM) containing Tyrode's solution for removal of calcium from the tissues. The isolated tissue preparation was further incubated for 30 min in a Tyrode's solution (K⁺ rich, Ca⁺² free and containing EDTA) of the following composition (mM): KCl (50), NaCl (91.04), MgCl₂ (1.05), NaHCO₃ (11.90), NaH₂PO₄ (0.42), glucose (5.55) and EDTA (0.1). Subsequently, Ca⁺² was added to the tissue bath in a cumulative fashion to obtain the control calcium concentration-response curves (CRCs). The stepwise increase in contractile response of the tissue revealed that its contractions are dependant on K⁺-induced influx of extra-cellular Ca⁺².

On finding the control CRCs of Ca⁺² to be super imposable (usually after two cycles), the tissue preparation was then washed and equilibrated with the test material for 60 min. Followed by reconstruction of the concentration response curves for Ca⁺² and compared to the control curves. The concentration response curves of Ca⁺² were developed in the presence of various concentrations of test material to assess a possible Ca⁺² channel blocking effect. The calcium channel blocking activity on the part of test material was confirmed on shifting the concentration-response curves of calcium constructed in calcium-free medium towards right in a dose-dependant manner (Bolton, 1979).

Isolated rabbit tracheal preparations

Rabbits were starved for 24 hrs prior to experiment and were killed by a blow on the back of the head and tracheal tube was dissected out. The trachea was cut into rings about 3-4 mm in width, each containing about two cartilages. Each ring was opened by longitudinal cut on ventral side opposite to the smooth muscle layer, forming a tracheal strip with a central part of smooth muscle sandwiched between cartilaginous portions on the edges. The preparation was suspended in a 20 ml tissue bath containing Krebs physiological salt solution at 37°C aerated with carbogen. A tension of 1g was applied to each of tracheal strip and was kept constant throughout the experiment. The isolated rabbit tracheal preparation was equilibrated for 45 min before isometric tension of tracheal strips were recorded via force displacement transducers (FT-03) connected to Grass Polygraph Model 7.

The broncho-relaxant effect of the test material was studied on carbachol- and high K⁺(80 mM)-induced contractions in isolated tracheal preparation. Spasmodic contractions are produced in the isolated tissue preparations on exposure to high K⁺(80 mM) tissue bath concentrations. Afterward, cumulative addition of test material relaxed the isolated tracheal preparation. The process was repeated with carbachol as well.

The isolated rabbit paired atria preparations:

The paired rabbit atria from healthy rabbits were dissected out, cleaned from fatty tissues and mounted in tissue organ baths containing Krebs physiological salt solution. The isolated tissue preparation was aerated with carbogen gas at 37°C. The isolated paired rabbit atrial preparation used to exhibit spontaneous beating under the resting tension of 1g due to intact pacemaker cells. The tissue was allowed to be equilibrated for a period of 30 min before exposure to any test material. The isolated paired atrial preparation allows evaluation of the possible effect of test material on both rate and force of atrial contractions. The amplitude of the recorded tracings represented the force of atrial contractions, whereas the number of contractions on the recorded chart represented the rate of atrial contractions. Isoprenaline (1 µM) was used as control inotropic agent and its tracings were recorded in duplicate. The contractile response of the

isolated tissue preparation was recorded via a Grass force-displacement transducer (model FT-03) linked to the Grass Model 7 Polygraph.

2.14. Statistical analysis

All the data were expressed as mean \pm standard error of the mean (S.E.M) and the median effective concentrations (EC_{50} values) are given with 95% confidence intervals (CI). The statistical parameter applied was the χ^2 -test with $P < 0.05$ was considered as significantly different.

RESULTS

Preliminary phytochemical analysis:

Preliminary phytochemical analysis of the crude methanolic extract of *Terminalia arjuna* Roxb. (Ta.Cr) bark revealed the presence of saponins and tannins as methanol soluble extractable constituents of *Terminalia arjuna* Roxb.

Effect of *Terminalia arjuna* Roxb. on isolated rabbit jejunum preparations

The crude methanolic extract of barks of *Terminalia arjuna* Roxb. (Ta.Cr) was tested on spontaneously contracting isolated rabbit jejunum preparations for possible relaxant effect. The Ta.Cr exhibited concentration dependent relaxant effect in tissue bath concentration range of 0.01 to 5.0 mg/ml with EC_{50} value of 2.015 mg/ml (95% CI, 1.473-2.756, $n = 5$) (Figure 1), whereas in the presence of propranolol the relaxations were achieved at a concentration of 0.1 to 10.0 mg/ml, with EC_{50} value of 3.454 mg/ml (95% CI; 3.138-3.800, $n = 5$) (Figure 2). The Ta.Cr caused partial relaxation of low K^+ (25 mM; $n=5$), high K^+ (80 mM; $n=5$), and carbachol (1 μ M; $n=5$)-induced contractions in isolated rabbit jejunum preparations (Figure 3).

Effect of crude extract of *Terminalia arjuna* Roxb on isolated rabbit tracheal preparations

The Ta.Cr while testing on isolated rabbit tracheal preparations, exerted concentration dependent (0.1-10.0 mg/ml) relaxant effect on carbachol (1 μ M)-induced contractions with EC_{50} value of 3.478 mg/ml (95% CI; 2.413-5.011, $n = 5$), however, relaxation was found to be partial in presence of propranolol ($n = 5$). The Ta.Cr also partially relaxed the low K^+ (25 mM; $n = 5$) and high K^+ (80 mM; $n=5$)-induced contractions (Figure 4&5).

Effect of crude extract of *Terminalia arjuna* Roxb on isolated rabbit aorta preparations

The Ta.Cr on application to isolated rabbit aorta preparations, partially relaxed the phenylephrine (1 μ M)-induced contraction ($n=5$) (Figure 6).

Effect of the crude extract of *Terminalia arjuna* Roxb on isolated rabbit paired atria

The Ta.Cr while testing in isolated rabbit paired atria, exhibited a positive inotropic and positive chronotropic effects at low tissue bath concentrations (0.01-0.3 mg/ml), which were found to be blocked on adding propranolol. On further increase in tissue bath concentration of Ta.Cr, there was decrease in both in force of contractions as well as rate of hear beat (1.0-3.0; $n=5$), however,

addition of atropine to the tissue bath blunted the inhibitory influences and restored both the force of contractions as well as rate of heart beats (Figure 7).

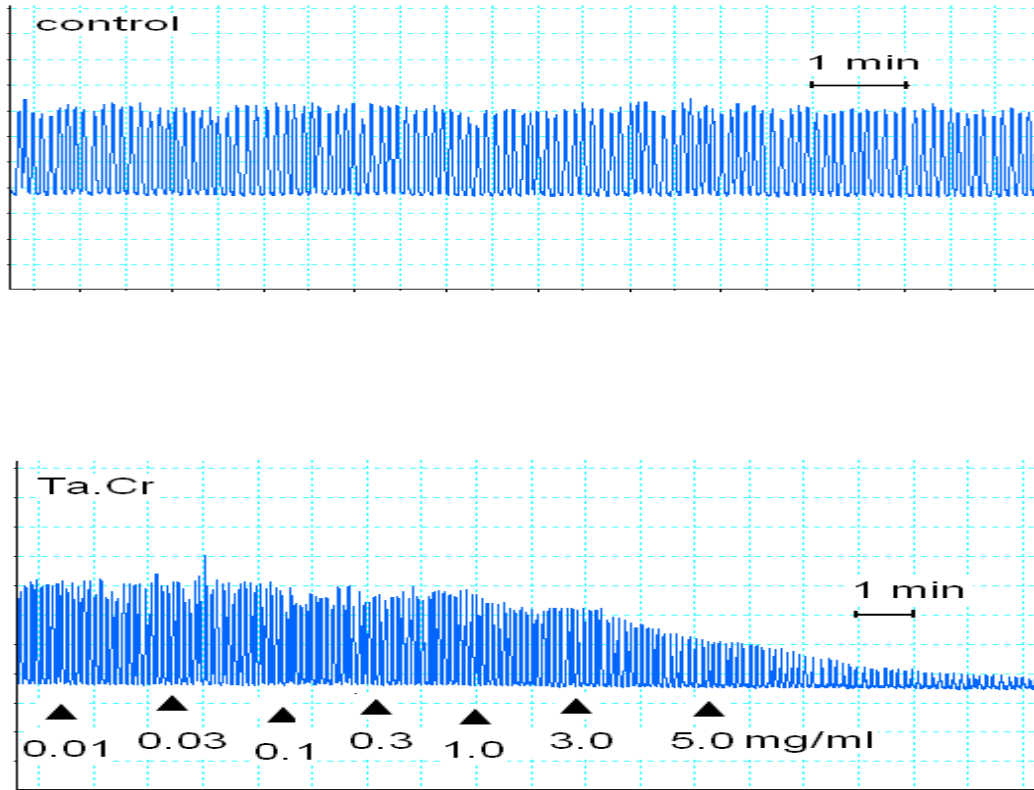
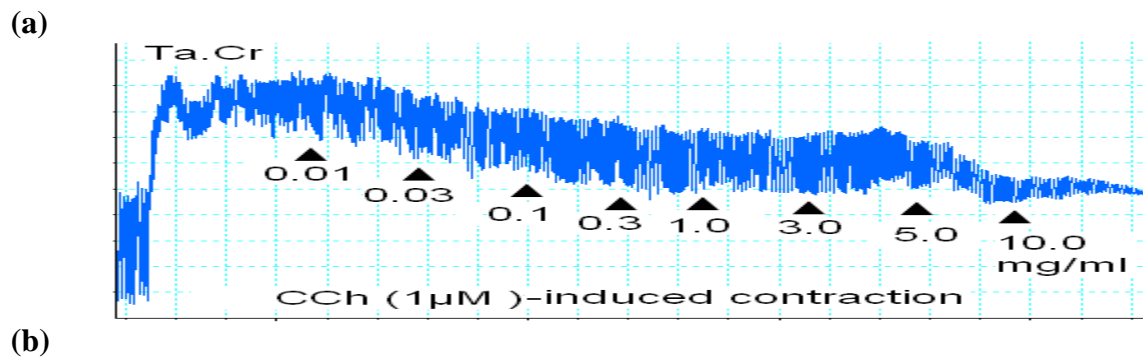
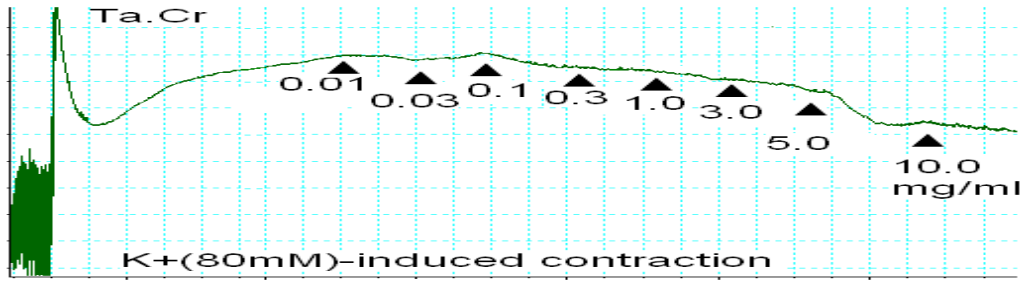


Figure 1: Tracing showing effect of crude methanolic extract of *Terminalia arjuna* Roxb (Ta.Cr) on the spontaneously contractions of isolated rabbit jejunum preparations.





(c)

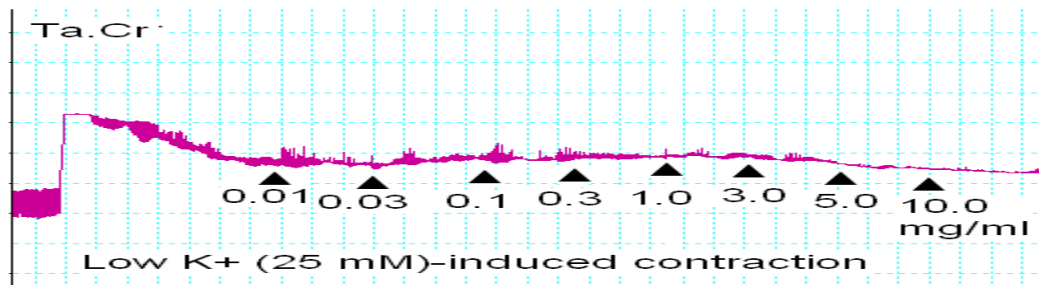


Figure 2: Tracing showing effect of crude methanolic extract of *Terminalia arjuna* Roxb (Ta.Cr) on (a) carbachol (1 μ M), (b) high K^+ (80 mM) and (c) low K^+ (25 mM)-induced contractions in isolated rabbit jejunum preparations.

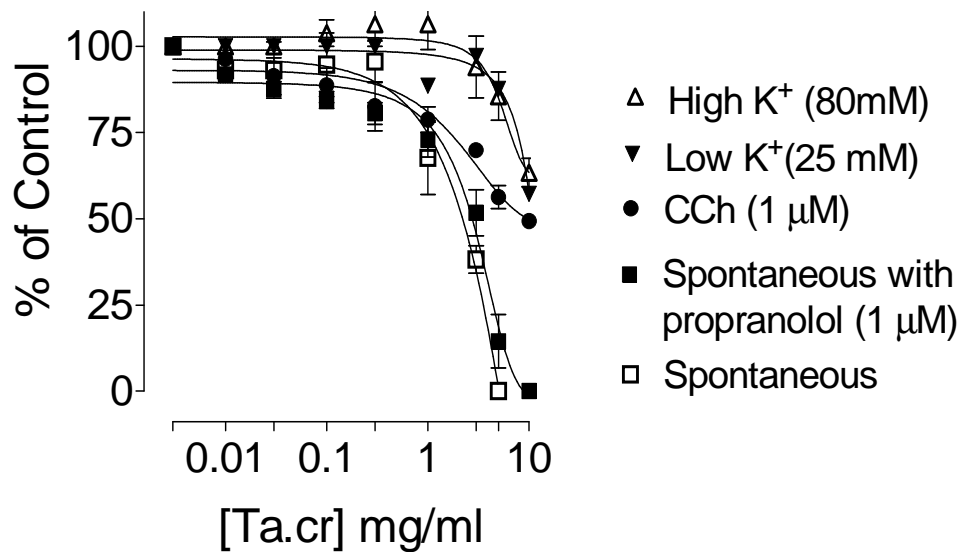


Figure 3: Effect of crude methanolic extract of *Terminalia arjuna* Roxb. (Ta.Cr) on the spontaneous contractions in the absence and presence of propranolol, high K^+ (80 mM), low K^+ (25 mM), carbachol (1 μ M)-induced contractions in isolated rabbit jejunum preparations. Values are shown as mean \pm S.E.M., n = 5-6

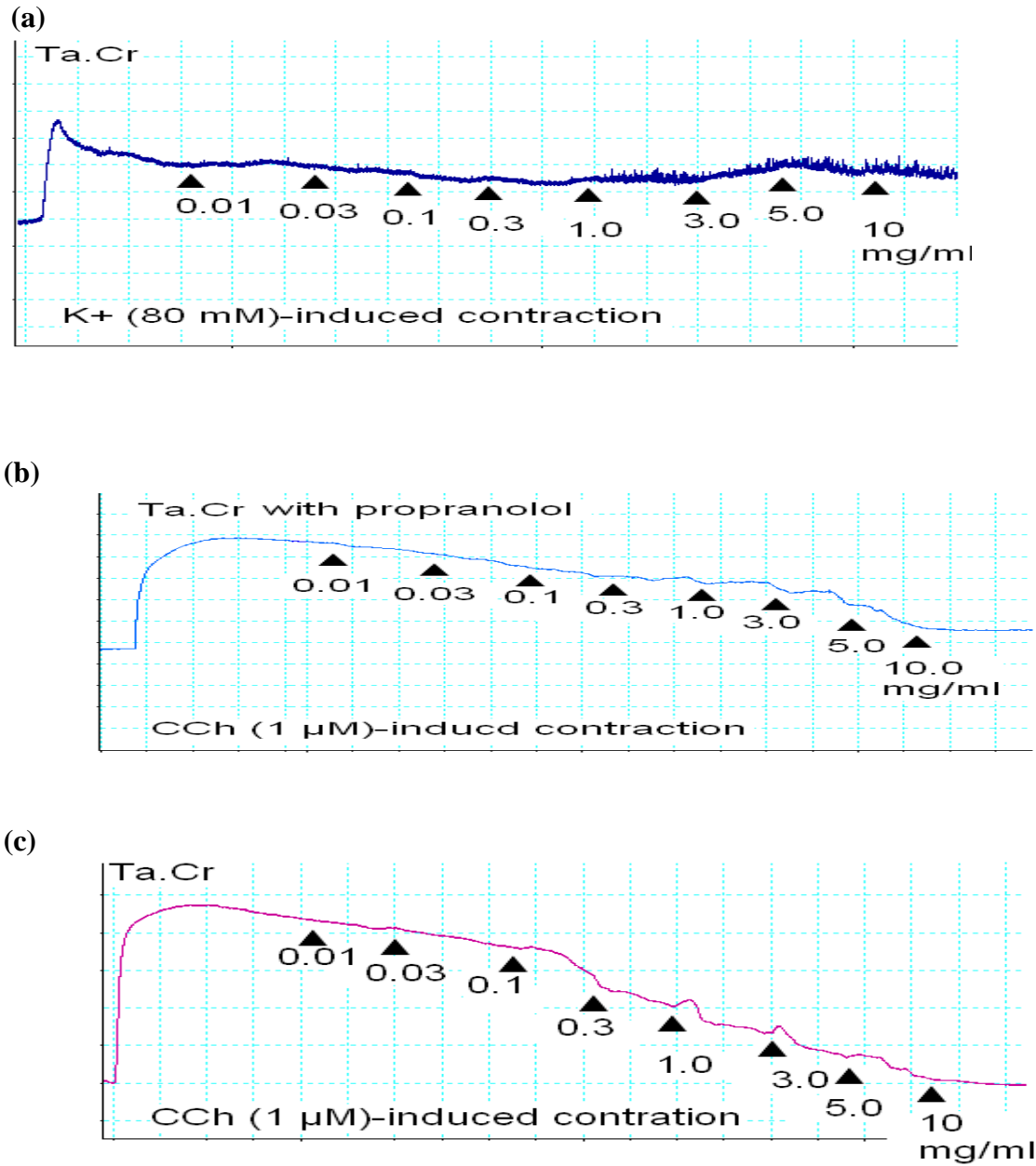


Figure 4: Tracing showing the effect of crude extract of *Terminalia arjuna* Roxb on (a) high K^+ (80 mM)-induced concentration and (b) carbachol (1 μ M)-induced contraction in the absence and presence of propranolol in isolated rabbit tracheal preparations.

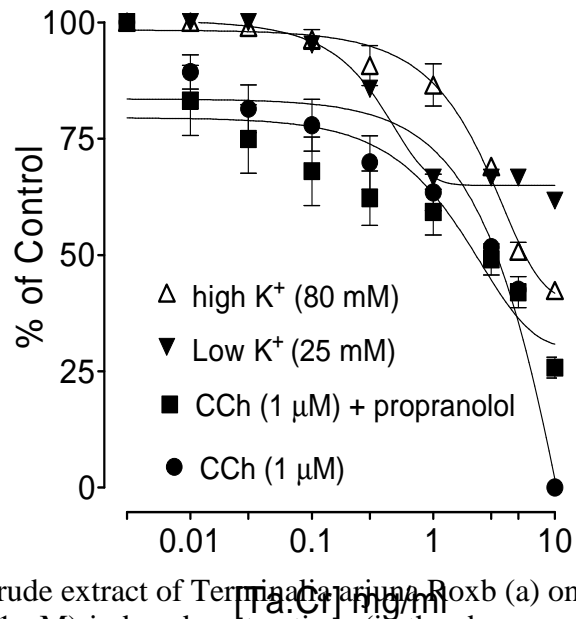


Figure 5: Effect of crude extract of *Terminalia arjuna* Roxb (a) on high K⁺ (80 mM), low K⁺ (25 mM) and carbachol (1 μM)-induced contractions (in the absence and presence of propranolol) on isolated rabbit tracheal preparations. Values are shown as mean ± S.E.M., n = 5-6.

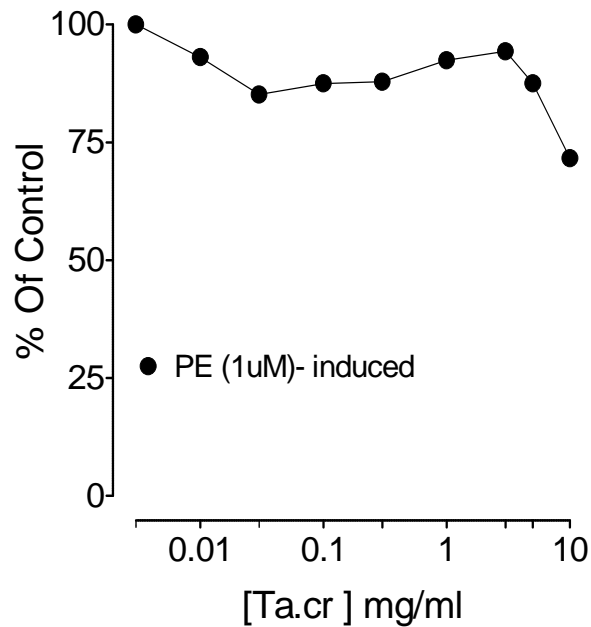
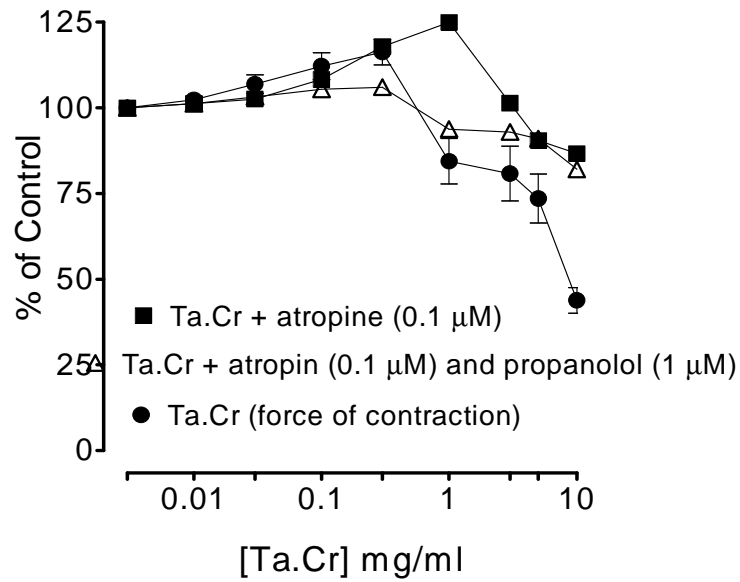


Figure 6: Effect of crude extract of *Terminalia arjuna* Roxb on phenylephrine (1uM)-induced contractions in isolated rabbit aorta preparations. Values are shown as mean ± S.E.M., n = 5.

(a)



(b)

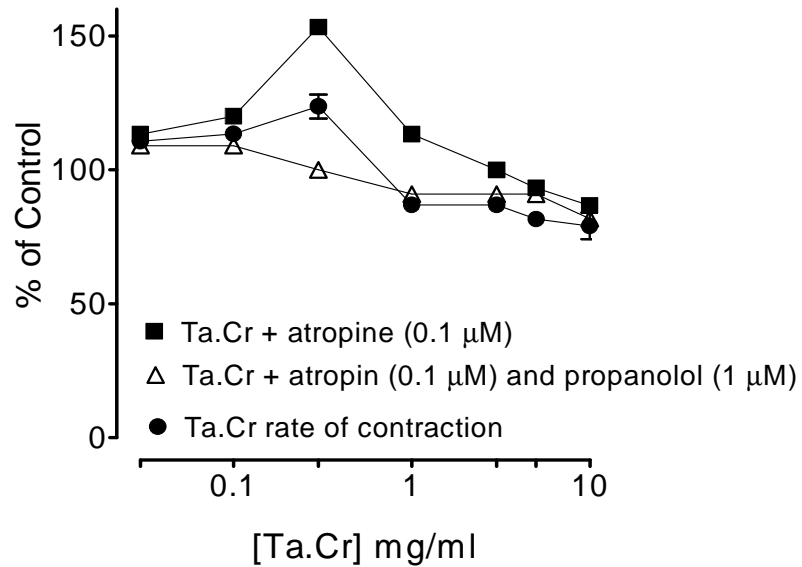


Figure 7: Effect of crude extract of *Terminalia arjuna* Roxb on (a) force and (b) rate of contraction in the absence and presence of atropine (0.1 μM) and propranolol (1 μM) in isolated rabbit paired atria preparations.

DISCUSSION

The preliminary phytochemical analysis of the crude methanolic extract of bark of *Terminalia arjuna* (Ta.Cr) revealed the presence of tannins and saponins as methanol soluble extractable constituents.

The *Terminalia arjuna*, Roxb possesses the folkloric repute of antispasmodic activity, hence, it was subjected to pharmacological investigations to explore its possible relaxant effect on spontaneously contracting isolated rabbit jejunum preparations. The Ta.Cr exhibited a concentration dependent (0.01 to 5.0 mg/ml) relaxant effect likely to be mediated either through Ca^{+2} channel blocking (CCB) mechanism, through K^{+} channel opening mechanism or muscarinic receptor antagonism (Gilani *et al.*, 2005 a, b & c). The Ca^{+2} channel blocking activity is used to be determined by depolarization of the isolated tissue preparations following exposure to high K^{+} (80 mM) as described by Farre *et al.*, (1991). Exposure to high tissue bath concentrations, K^{+} (> 30 mM) is reported to cause smooth muscle contraction through opening of voltage-dependent L-type Ca^{+2} channels, thus allowing influx of extra-cellular Ca^{+2} and hence, causing contractile effect (Bolton, 1979). The substances exerting inhibition of high K^{+} -induced contractions are considered to be blocker of Ca^{+2} influx (Godfraind *et al.*, 1986). The Ta.Cr was also tested on high K^{+} (80 mM)-induced contractions in isolated rabbit jejunum preparations to explore the mechanism of the observed relaxant effect. The Ta.Cr exerted weak inhibitory effect on high K^{+} -induced contractions, hence possibility of mediation through blockade of Ca^{+2} channels was ruled out and observed relaxation of spontaneously contracting isolated rabbit jejunum was presumed to be mediated via some alternative mechanism(s). The substances that selectively relax the low K^{+} (25 mM)-induced contractions in isolated rabbit jejunum preparations are considered to be potassium channel openers (Kishii *et al.*, 1992), but Ta.Cr did not exert relaxant effect in this case, hence, it was speculated that observed relaxant effect of Ta.Cr was likely to be due to the presence of some anti-muscarinic component(s) and idea was supported by the evidence that Ta.Cr caused partial blockade of the carbachol (1 μM)-induced contractions. The *Terminalia arjuna*, Roxb has traditionally been used in the management of respiratory diseases, hence Ta.Cr was subjected to further evaluation for possible bronchodilator activity in isolated rabbit tracheal preparations. The Ta.Cr demonstrated weak relaxation of the high K^{+} (80 mM) and low K^{+} (25 mM)-induced contractions, hence, involvement of calcium channel blocking or potassium channel opening activities was not supported (Hamilton *et al.* 1986; Gilani *et al.* 2005c).

The Ta.Cr exhibited partial relaxation of the carbachol (1 μM)-induced contractions in isolated rabbit jejunum preparations but exhibited complete relaxation of the carbachol (1 μM)-induced contractions in isolated rabbit tracheal preparations, which may possibly be mediated through muscarinic receptor blockade or due to some alternative mechanism(s) like β -adrenergic agonistic activity as well as phosphodiesterase (PDE) inhibitory activity (Brain and Hoffman, 2001). The mechanism of the observed relaxant effect of Ta.Cr on carbachol (1 μM)- induced-contractions in isolated rabbit tracheal preparations was evaluated further by testing Ta.Cr on carbachol (1 μM)-induced contractions in the presence of propranolol (1 μM); a β -adrenergic receptor antagonist (Westfal and Westfal, 2006). The relaxant effect of Ta.Cr on carbachol (1 μM)-induced contraction was partially blocked by propranolol, thus, indicating that Ta.Cr may possess some β -adrenergic agonistic activity.

The *Terminalia arjuna*, Roxb is also reputed for its folkloric use in the management of the cardiovascular diseases, hence it was evaluated for its possible effect on isolated rabbit aorta preparations. The Ta.Cr was unable to demonstrate any effect when applied to the isolated rabbit aorta preparations but it partially relaxed the phenylephrine (1 μ M)-induced contractions, which is likely to be mediated through β adrenergic agonist, α adrenergic antagonist or muscarinic agonist activities

The β -adrenergic receptors agonists via stimulation of adrenergic receptors may cause increase in intracellular level of cAMP, whereas the raised intracellular level of cAMP produces relaxant effect in smooth muscles, and increase in inotropic and chronotropic activities of the cardiac tissues (Orallo *et al.*, 2005). The possible presence of β -adrenergic agonistic activity on the part of Ta.Cr, as detected in case of isolated rabbit tracheal preparation was investigated further on isolated rabbit paired atria preparations. The Ta.Cr demonstrated positive inotropic and positive chronotropic effects at lower tissue bath concentrations, which was blocked on addition of propranolol to the tissue bath and was suggestive of the presence of β -adrenergic agonistic activity. However, the observed decrease in heart rate and force of contraction on elevating the tissue bath concentration of TaCr, which was blocked on addition of atropine to the tissue bath, reflected the presence of cholinergic muscarinic activity. Thus, indicating that there might be presence of multiple components likely to exert β -adrenergic agonistic, cholinergic muscarinic agonist and antagonist activities in crude methanolic extract of *Terminalia arjuna*, Roxb.

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