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Evaluation of cardiac activity of some traditionally used backyard Indian medicinal plants

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ABSTRACT

To evaluate the cardiac effects and anti diabetic activity of extracts derived from the leaves of *Ocimum tenuiflorum*, *Ocimum sanctum* (also Sri tulsi, krishna tulsi), stem of *Cissus quadrangularis* (Nalleru), leaves of *Trachyspermum ammi* (Vamu), *Azadirachta indica* A. (Neem flowers) and flowers of *Alangium salvifolium*. The leaves of *Ocimum tenuiflorum*, *Ocimum sanctum* (also Sri tulsi, krishna tulsi), stem of *Cissus quadrangularis* (Nalleru), leaves of *Trachyspermum ammi* (Vamu), *Azadirachta indica* A. (Neem flowers) and flowers of *Alangium salvifolium* were extracted with Frog ringer's solution.. The extracts were screened for their effects on frog-heart *in situ* preparation. The extracts produced significant positive inotropic and negative chronotropic actions on frog heart. The leaves of *Ocimum tenuiflorum* exhibited more cardiotoxic and cardiac stimulant effect than other plants. The extraction of flowers of *Alangium salvifolium* exhibited anti diabetic principles.

Keywords: cardiac activity, cardiotoxic, medicinal plants

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INTRODUCTION

These species may have promised as therapeutic agents, but additional preclinical pharmacological and toxicological studies are needed to establish their therapeutic effectiveness and safety in human disease. These species possess glycosides, large quantities of flavonoids, tannins, alkaloids and minerals. Flavonoids have been detected to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides have been detected for cardiogenic effect.

Ocimum (Ocimum tenuiflorum Linn, Ocimum sanctum Linn) is an aromatic plant in the family Lamiaceae. It is an erect, much branched subshrub 30-60 cm tall with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed. Flowers are purplish in elongate racemes in close world [7]. There are three varieties of tulasi plants used by Hindus. There is a dark variety called Syama or Krishna tulasi, a white variety called Rama tulasi, and an extremely hardy variety sometimes called Fujian or Thai tulasi. The activities of compounds cirsilineol, cirsimaritin, isothymusin, isothymonin, apigenin, rosmarinic acid were comparable to ibuprofen, naproxen, and aspirin at 10-, 10-, and 1000-microM concentrations, respectively. These results support traditional uses of *O. sanctum* and identify the compounds responsible [5].

Cissus quadrangularis Linn plant grows throughout India, especially, in gnotter regions and in Sri Lanka; the tendril climber has quadrangular stems with ridges. The stems are often leafless, when old. Few aerial roots arise from the joined nodes and grow towards the soil; the leaves are small, simple, heart – shaped and toothed. The flowers are small, greenish white, in short cymes. The fruits are globose or ovoid berries, red when ripe. The author of Nighantu Ratnakara mentions that there are two varieties of this climber, namely, of four – sided and three – sided stems. But only one variety of four – sided stem is reported. Phytochemical analyses of *Cissus quadrangularis* revealed high contents of ascorbic acid, carotene, anabolic steroidal substances, and calcium. The stem contains two asymmetric tetracyclic triterpenoids, and two steroidal principles. The presence of β -sitosterol, δ -amyrin, δ -amyrone, and flavanoids (quercetin) has also been reported [4,8] all these components having potentially different metabolic and physiologic effects.

Trachyspermum ammi Linn is erect, glabrous or minutely pubescent, branched annual lower leaves 2-3 pinnatisect, withered by flange time, long petiolate upper leaves smaller, similar, or simply pinnatisect shortly petiolate, short sheathing base. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. The plant prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It requires moist soil. The principal constituents of the essential oil from the fruits are the phenols, mainly thymol and some carvacrol. The Indian Pharmacopoeia requires ajowan oil to contain not less than 40 per cent thymol. The remainder of the oil is called 'thymene'. Thymene, which constitutes .45 per cent of the oil, has the following composition: p-cymene, 50-55%; g-terpinene, 30-35%; α - and β -pinenes, 4-5%; and

dipentene, 4-6%. Presence of minute amounts of camphene, myrcene and D3-carene are also reported [3].

Azadirachta indica Linn plants have increasingly become an integral part of the human society in combating various diseases, ranging from skin infection to gastrointestinal problems, since the dawn of civilization. The *Neem* tree (*Azadirachta indica* A. Juss) is one such medicinal plant, and symbolizes all that is wondrous in nature: for every part of the tree has been used as traditional medicine for household remedy against various human ailments from antiquity. In fact, it is considered to be the “village pharmacy” in many parts of India and has played a key role in Ayurvedic medicine and agriculture since time immemorial. In traditional Ayurveda medicine a decoction made from the bark, leaf, root, fruits and flowers is used in the treatment of blood morbidity, biliary afflictions, itching, skin and peptic ulcers [9].

Alangium salvifolium Linn is tall thorny tree. It grows to a height of about 3 to 10 meters. The bark is ash colored, rough and faintly fissured. The leaves are elliptic oblong, elliptic lanceolate or oblong lanceolate. The flowers are greenish white, fascicled, axillary or on old wood. The berries are ovoid, ellipsoid or nearly globose, glabrous, smooth and violet to purple. The flowering season is February to June. a- and b- Alangine, Alangicin, Marckindine, Tubulosin and emitine are obtained from this plant.

MATERIAL AND METHODS

Material

The plant materials were collected from the herbal garden of Shri Vishnu college of pharmacy, Bhimavaram. The authentication was done by prof.M.C.Prabhakara, Department of pharmacology, Shri Vishnu College of pharmacy. The fresh leaves of 35gm of *Ocimum tenuiflorum* (syn. *O. sanctum*, Holy Basil, Sri tulsi), 35gm of *Ocimum sancium* (Krishna Tulsi) ,70gm of *Trachyspermum ammi* leaves and 50gms of fresh stems of *Cissus quadrangularis* were collected from herbal garden. 30gm of *Neem* flowers and 30gm of *Alangium salvifolium* flowers were collected in the month of March (2009). After authentication fresh plant material was collected in bulk, washed, dried under shade and made juice with frog Ringers solution with the help of mechanical grinder.

Finally we collected extracts of 25ml of *Ocimum tenuiflorum*, 25ml of *Ocimum sancium*, and 60 ml of *Trachyspermum ammi*, 25 ml of *Cissus quadrangularis*, and 20ml of *Neem* flower juice and 20 ml of *Alangium salvifolium* flower juice.

Methods

Experimental method

The pharmacological screening of various traditionally used Indian medicinal plants was done using the following models.

- Frog's isolated perfused heart [2]
- Frog's hypodynamic heart **frog heart perfusion using syme's cannula** [2]

Purpose

To study the effect of plants juice on isolated frog heart by Syme's technique.

Requirements:

Frog Ringers solution (physiological solution), Syme's Cannula
Starling's heart lever etc.

Method

The frog was killed with a head-blow using a steel rod. The skin and abdomen were cut and opened. The pectoral girdle was cut using a bone cutter and pericardium was removed. Syme's cannula was connected to the reservoir of frog Ringers solution and immediately into the Sinus venous of the heart through posterior vena cava. The connecting blood vessels were cut and the heart was taken out from the animal and mounted on a stand. The heart was covered with a thin layer of cotton wool to prevent drying. The heart was wet frequently with the physiological solution to prevent drying. The heart was connected to the Starling lever, which in turn is connected to the smoked drum for recording the responses of the heart.

The level of frog Ringers solution in the Syme's cannula was maintained by a fixing a glass tube into the cork fixing to the reservoir (Marriott's bottle) tightly. The heart was allowed to stabilize and when the heart rate and cardiac output were taken, the recordings were made on slow rotating drum, to which a sooted kymograph paper was affixed. The effects of plants juice were studied on isolated perfused frog hearts. The parameters studied include the force of contraction, heart rate and cardiac output. A minimum 5 min time was allowed between the addition of samples per se (in frog Ringer Solution) and its fractions. When a blocker was used, it was diluted with known amount of the frog Ringers solution in the syringe itself and added slowly. Solutions of these plants were prepared in the frogs Ringer solutions. No suspending agents were used. The heart was constantly moistened with frog Ringers solutions from time to time. The study protocol was approved by Institutional Animal Ethical Committee, Shri Vishnu College of pharmacy, Andhra University.

Frog's Hypodynamic Heart

Purpose

To study the effect of various plant juices on isolated hypo dynamic frog heart.

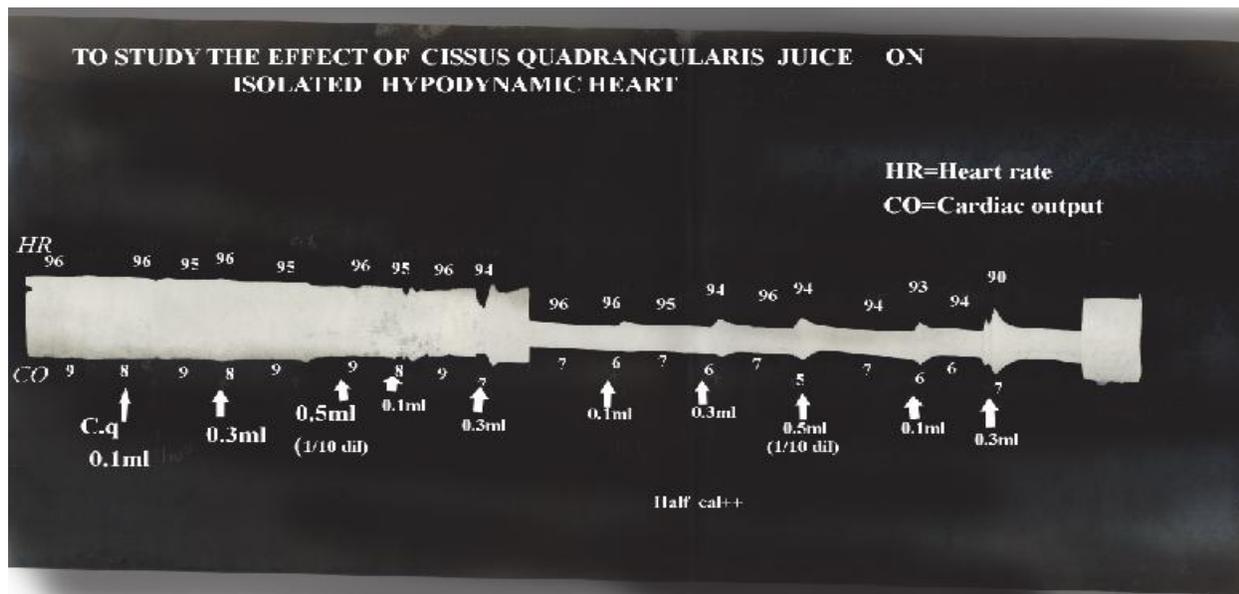
Method: An isolated from heart preparations described under Syme's technique was set up. Instead of one reservoir, two reservoirs each for $\frac{1}{2}$ calcium and full calcium were used. The levels in the reservoirs were maintained constant, which was tested by connecting each of the reservoirs to the Syme's cannula.

Experiments were conducted by rendering the frog heart hypodynamic by letting into the heart; frog Ringers solution containing half-calcium from another reservoir through Syme's cannula. Force of contraction was monitored to give approximately half the magnitude of normal force of contraction.

Effect on Normal Heart and Hypodynamic Heart

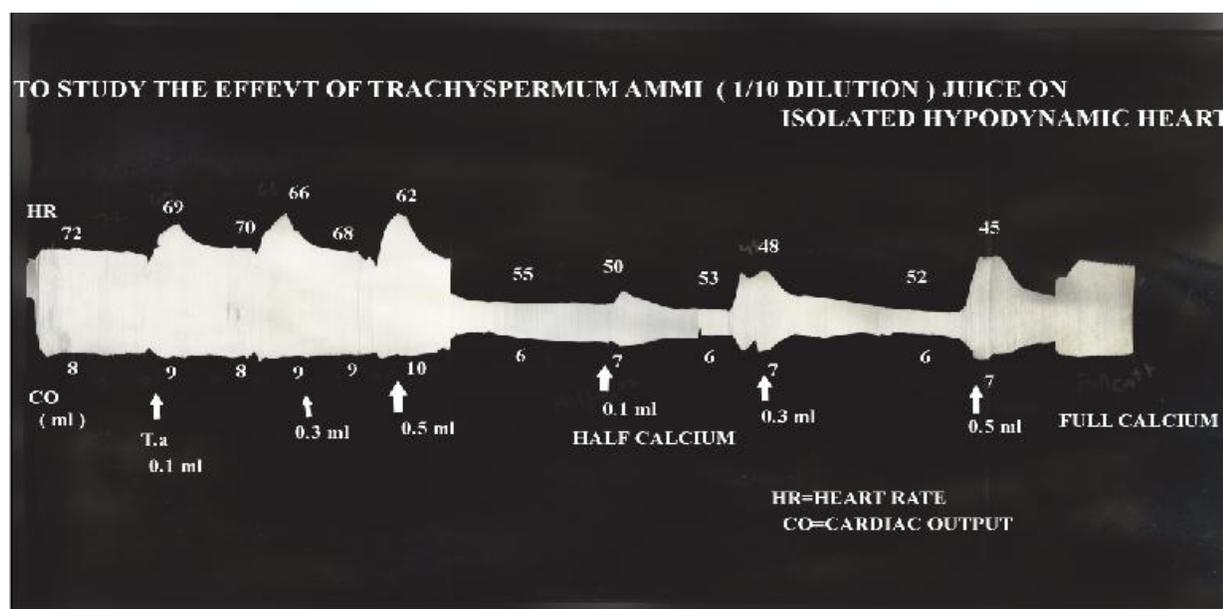
Effect of plants juice on isolated frog heart (normal and hypodynamic heart) preparation and their action was studied at the dose range of 0.1ml, 0.3ml, 0.5 ml per se and diluted 10 times. The parameters studied include force of contraction, heart rate and cardiac output.

FIG: 1



Effect of juice of *Cissus quadrangularis* stem on isolated heart (normal and hypodynamic heart) preparation and study of the action of plant stem juice. At the dose range of 0.1ml, 0.3ml, 0.5 ml and diluted 10 times and per se., the parameters studied include force of contraction, heart rate and cardiac output. Plants juice effect on isolated heart does not elicit dose dependent response. In 10 time diluted doses (0.1, 0.3, 0.5ml) shown dose dependent increased force of contraction that is positive ionotropic effect and negative chronotropic effect. It indicates the presence of cardiac stimulant activity, whereas on hypodynamic heart the dose of plant stem juice per se (0.1ml, 0.3ml, 0.5ml) elicited positive ionotropic effect, no change in cardiac output and heart rate indicates the presence of cardio tonic activity in the juice of plant stem .The dose range (0.1ml, 0.3ml, 0.5ml) without dilution initially showed depressant effect again stimulant effect, and there was no significant difference in cardiac output. Plant stem juice has both cardiac stimulant and mild cardio tonic effect.

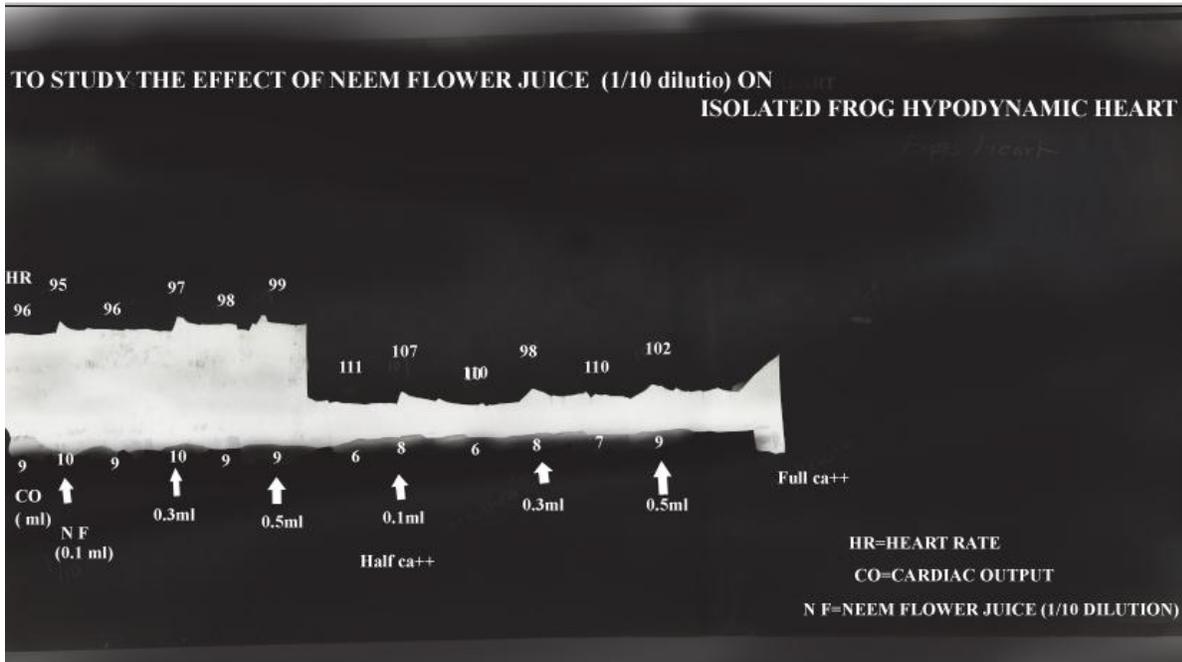
FIG: 2



Effect of juice of *Trachyspermum ammi* leaves on isolated heart (normal and hypodynamic heart) preparation and study of the action of juice of plant leaves. At the dose range of 0.1ml, 0.3ml, 0.5 ml and diluted 10 times and per se, the parameters studied include force of contraction, heart rate and cardiac output. Juice of *Trachyspermum ammi* leaves effect on isolated heart does not elicit dose dependent response. In 10 times diluted doses (0.1, 0.3, 0.5ml) there is dose dependent increase in force of contraction that is positive ionotropic effect and negative chronotropic effect. It indicates the presence of cardiac stimulant activity, whereas on hypodynamic heart the dose of plant leaves juice per se (0.1ml, 0.3ml, 0.5ml) elicited positive ionotropic effect, no change in cardiac output and heart rate indicates the presence of cardio tonic activity in juice of the plant leaves .The dose range (0.1ml, 0.3ml,

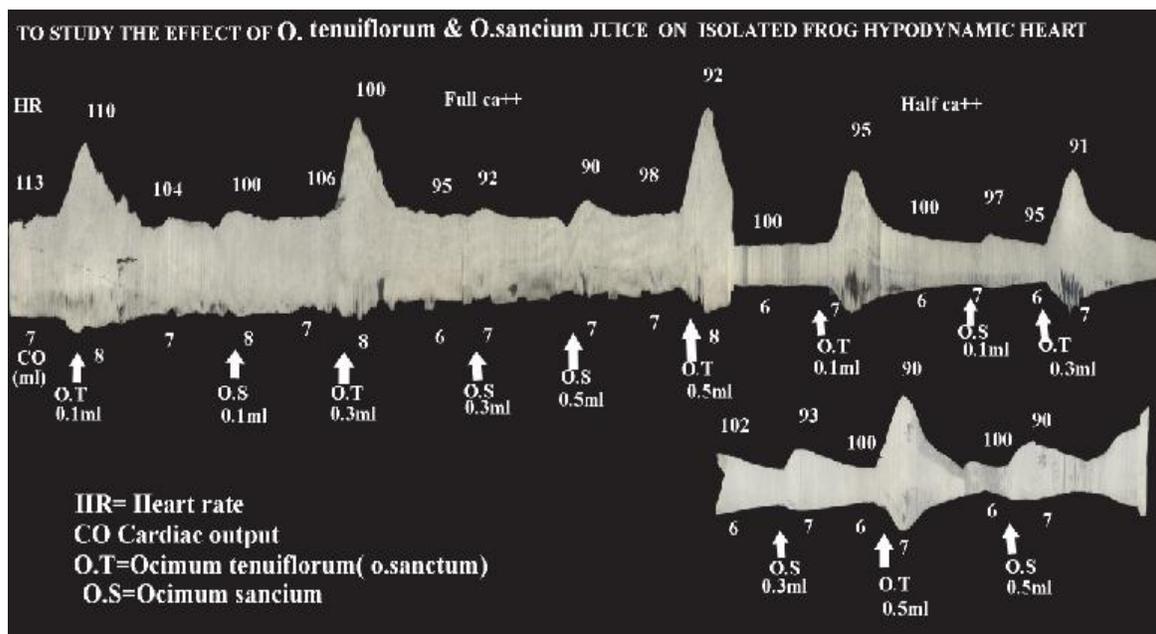
0.5ml) without dilution initially showed depressant effect again stimulant effect, and there was no significant difference in cardiac output. Juice of Plant leaves has both cardiac stimulant and cardio tonic effect.

FIG: 3



Effect of juice of Neem flowers on isolated frog heart (normal and hypodynamic heart) preparation and studied on the action of juice of Neem flowers. At the dose range of 0.1ml, 0.3ml, 0.5 ml and diluted 10 times and per se. the parameters studied include force of contraction, heart rate and cardiac output. Juice of Neem flowers effect on isolated heart does not elicit dose dependent response. In 10 times diluted doses (0, 1, 0.3, 0.5ml) there is dose dependent increasing force of contraction that is positive inotropic effect and negative chronotropic effect. It indicates the presence of cardiac stimulant activity, whereas on hypodynamic heart, the dose of juice of Neem flower per se (0.1ml 0.3ml 0.5ml) elicited positive inotropic effect, no change in cardiac output and heart rate indicating the presence of mild cardiotonic activity in the juice of flowers. The dose range (0.1ml, 0.3ml, 0.5ml) without dilution initially showed depressant effect again stimulant effect, and there was no significant difference in cardiac output. Juice of flowers has both cardiac stimulant and mild cardiac tonic effect.

FIG: 4



Effect of juice of *Ocimum tenuiflorum* (syn.*O.sanctum*, Holy Basil, Sri tulsi) and *Ocimum sancium* (Krishna Tulsi) leaves on isolated frog heart (normal and hypodynamic heart) preparation and the parameters studied includes force of contraction, heart rate and cardiac output. Juice of *Ocimum tenuiflorum* and *Ocimum sancium* leaves does not elicit dose dependent response on isolated heart preparation. In 10 times diluted doses (0.1, 0.3, 0.5ml) there is dose dependent increase force of contraction that is positive inotropic effect and negative chronotropic effect. It indicates the presence of cardiac stimulant activity, whereas on hypodynamic heart the dose of juice of *O. tenuiflorum* and *O. sancium* effect per se (0.1ml 0.3ml0.5ml) elicited positive inotropic effect, no change in cardiac output and heart rate indicating the presence of cardiotoxic activity in the juice of leaves.The dose range (0.1ml, 0.3ml, 0.5ml) without dilution initially showed depressant effect again stimulant effect, and there was no significant difference in cardiac output. Juice of leaves has both cardiac stimulant and cardio tonic effect.

RESULTS

The Effect of the flowers of *Alangium salvifolium* on Isolated Frog's Heart (FIG: 6, 7):
 The tracing shows that epinephrine and nor-epinephrine produced an increase in the heart rate and force of contraction. Epinephrine is a hormone, secreted by the adrenal medulla, released predominantly in response to hypoglycemia.The test sample had exhibited the hypoglycemic activity that indicates the release and potentiation of the action of epinephrine. The test sample drastically increased the oxygen demand and lead to tachycardia. The cardiotoxic activity exhibited has positive inotropic and chronotropic effect on an isolated frog's heart probably due to the presence of alkaloids. The characterization of the isolated compound based

on structural studies is under progress; moreover, it promises a lot of scope for further envisage on its cardiac activity [10].

FIG 6

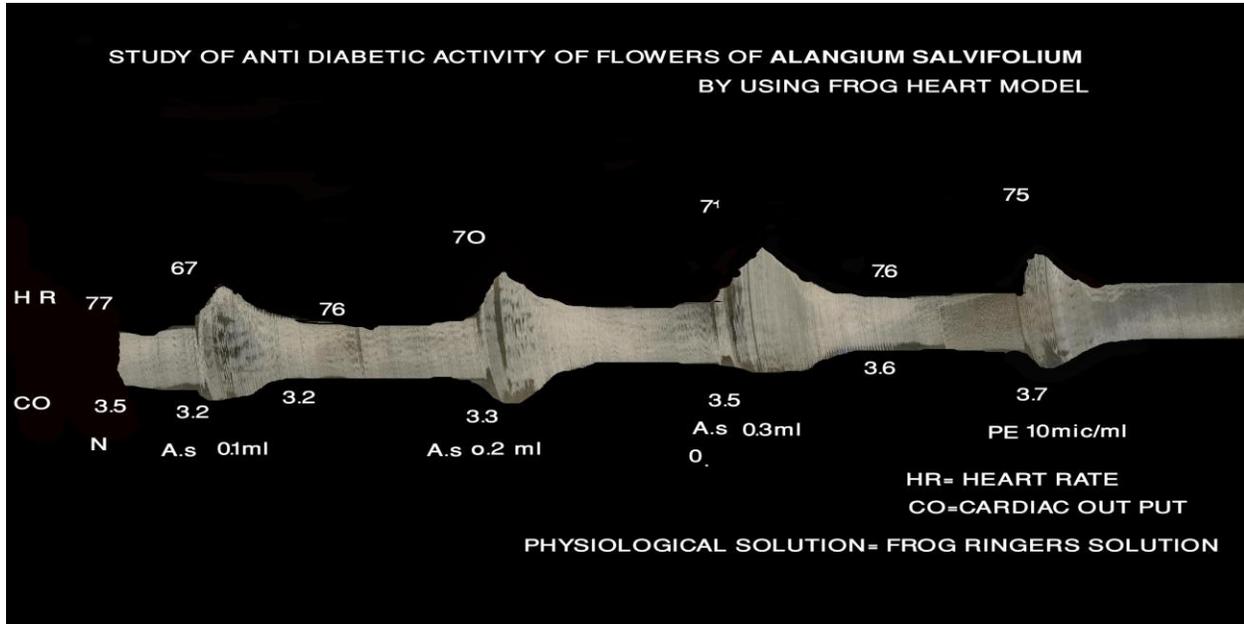
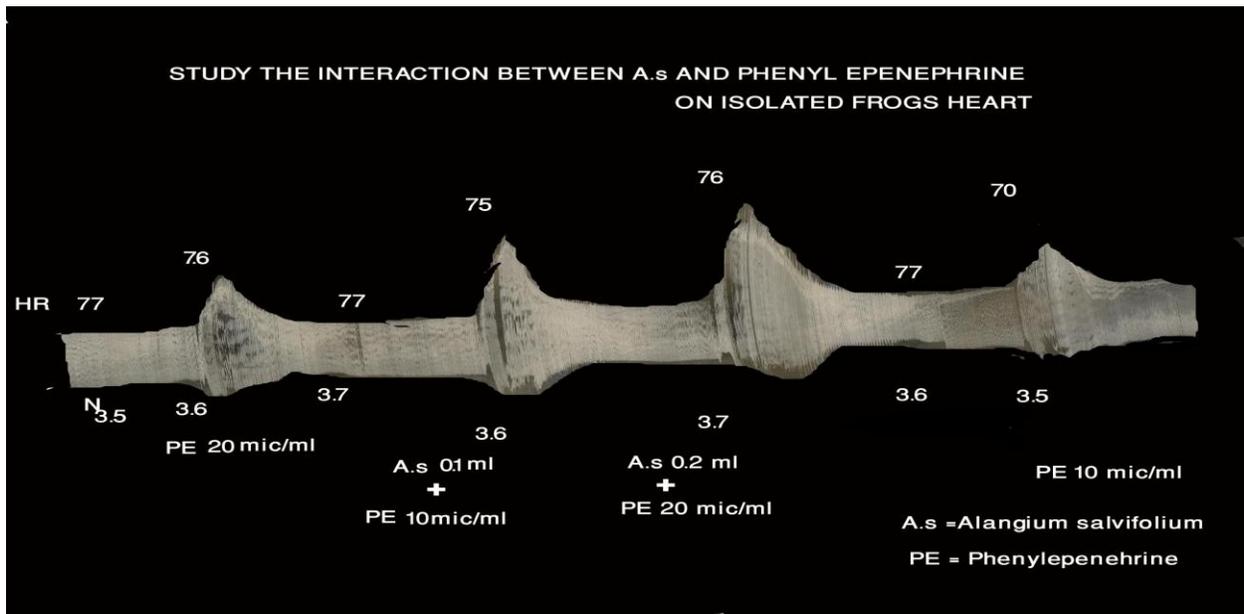
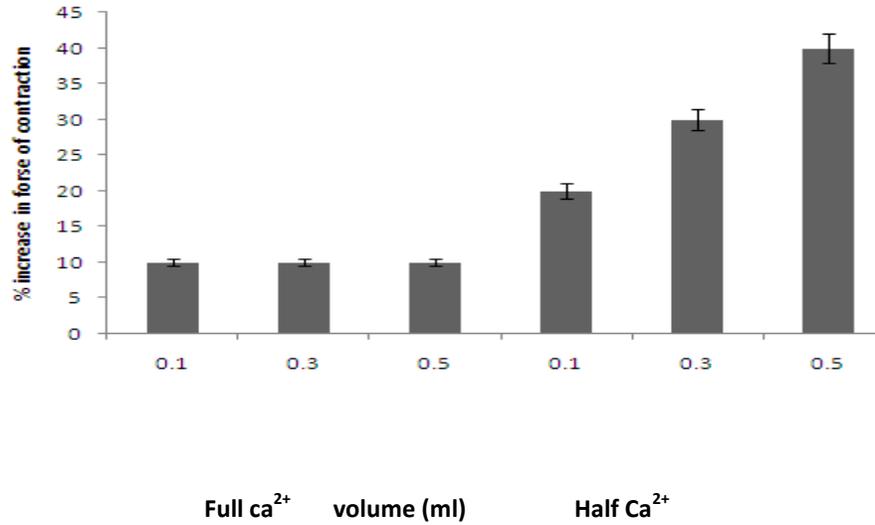


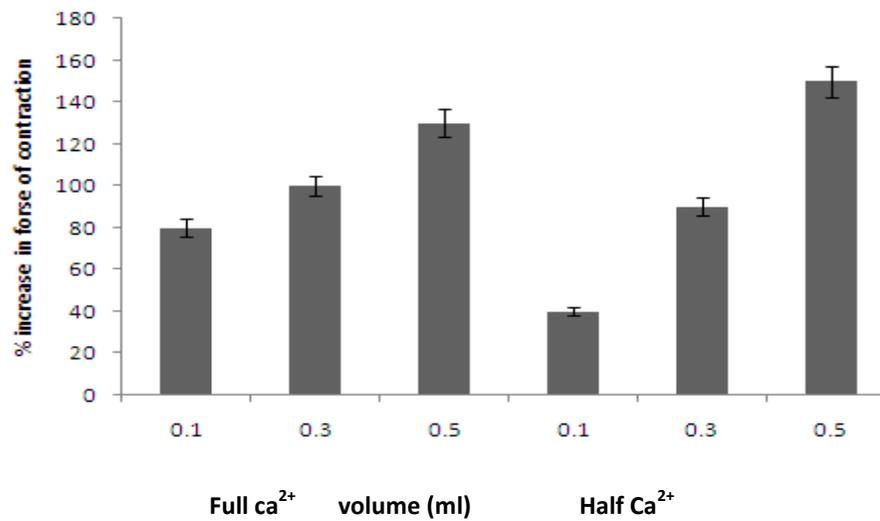
FIG: 7



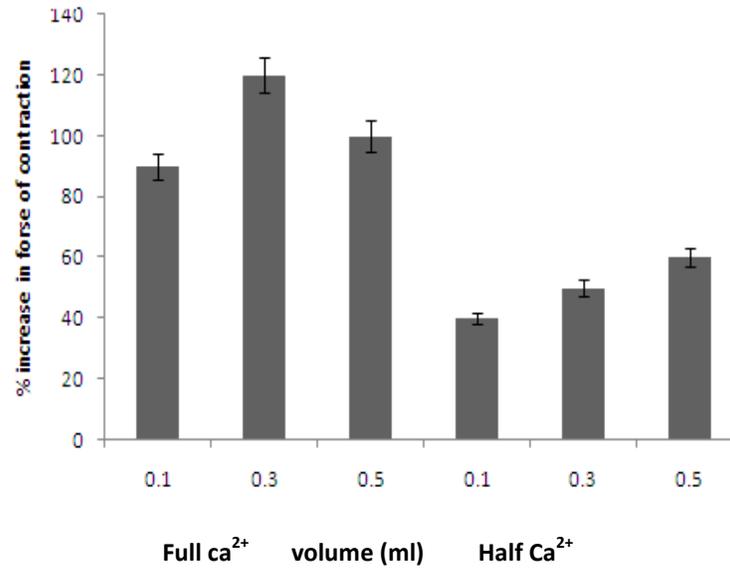
To study the cardiotoxic activity of juice of stem of *Cissus Quadrangularis* on frogs heart



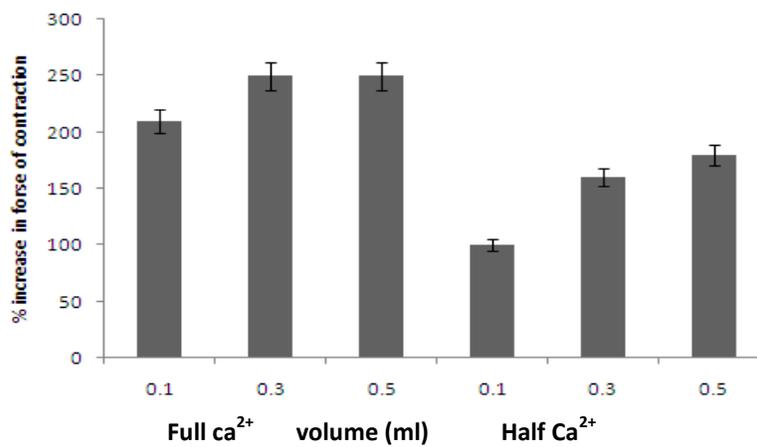
To Study The Cardiotoxic Activity Of Juice Of Leaves Of *Trachyspermum Ammi* On Frogs Heart



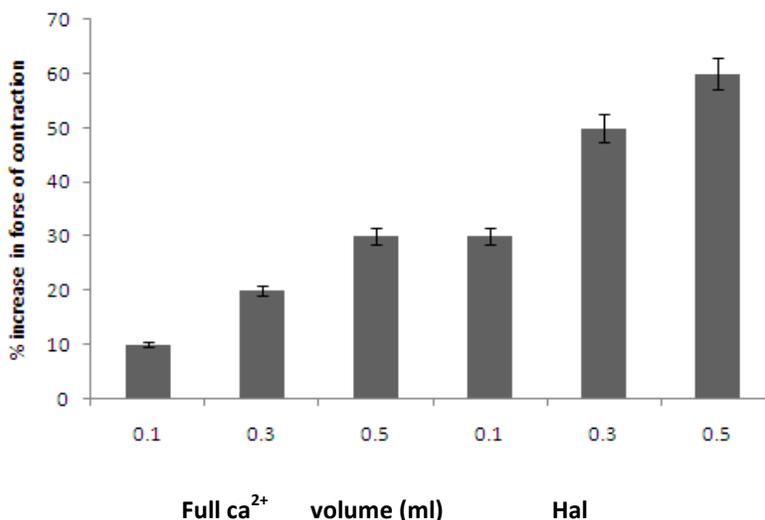
To Study the Cardiotonic Activity of Juice of Neem Flowers On Frogs Heart



To Study The Cardiotonic Activity Of Juice Of Leaves Of Ocimum Tenuiflorum On Frogs Heart



To Study The Cardiotonic Activity Of Juice Of Leaves Of Ocimum Sancium On Frogs Heart



DISCUSSION

Cardiac glycosides and catecholamine have been used as the main therapeutic drugs in the treatment of congestive cardiac failure [6] However, the dangers of cardiac glycosides in toxication are well documented [1] and doubts have been expressed about their long term effectiveness. The use of catecholamine is limited by their insufficient differentiation between positive inotropic and chronotropic action, their potential arrhythmogenic properties and tachyphylaxis due to receptor down regulation [6].

From the above observation the infusion of juices of leaves of *Ocimum tenuiflorum* and *Ocimum sancium* were found to restore cardiac activity of the hypodynamic frog heart, which is characterized by positive inotropic negative chronotropic action on the myocardial muscles of the perfusion frog heart. The juices of plant leaves did not elicit dose dependent cardiac stimulant and cardiac tonic activity. *Ocimum tenuiflorum* showed greater cardiac stimulant and cardiac tonic activity. Nifedepine did not block the cardiotonic activity of leaves juice. It indicates that activity does not mediate through the Ca^{2+} ions.

From the above observation the infusion of juice of *Trachyspermum ammi* leaves and *Alangium salvifolium* flowers was found to restore cardiac activity of the hypo dynamic frog heart, which was characterized by positive inotropic negative chronotropic action on the myocardial muscles of the perfused frog heart. The juice of plant leaves did not elicit dose dependent cardiac stimulant and cardiac tonic activity; it had initially showed depressant effect

again stimulant effect. In 10 time diluted dose (0.1, 0.3, 0.5 ml) elicited dose dependent cardiac stimulant and cardiac tonic activity. This effect was significantly blocked by Timolol.

From the above observation the infusion of juice of *Cissus quadrangularis* stem was found to restore cardiac activity of the hypodynamic frog heart, which was characterized by positive inotropic negative chronotropic action on the myocardial muscles of the perfused frog heart. The juice of plant leaves does not elicit dose dependent cardiac stimulant and cardiaotonic activity. The stimulant effect was significantly blocked by Timolol. It indicates that the cardiac stimulant is mediated through β -1 receptors.

Neem flower juice was found to restore cardiac activity of the hypodynamic frog heart, which was characterized by positive inotropic negative chronotropic action on the myocardial muscles of the perfusion frog heart. The juice of flower did not elicit dose dependent cardiac stimulant and cardiaotonic activity. This cardiac stimulant effect was significantly blocked by Timolol. It indicates that the cardiac stimulant is mediated through β -1 receptors.

CONCLUSION

Isolated Perfused Frog Heart: It can be inferred that different plants juices like (*O.tenuiflorum*, *O.sancium*, *T.ammi*, *C.quadrangularis*, *Alangium salvifolium* flowers and Neem flower) per se (in frog Ringers solution) did not elicit dose dependent positive inotropic effect on normal and hypodynamic heart. Timolol was able to block the above (except *O.tenuiflorum* and *O.sanctum* juice) responses indicating that its action is mediated through β_1 -adrenergic receptor.

Juice of *O.tenuiflorum* leaves has greater cardiac stimulant and cardiac tonic effect. Timolol did not block the stimulant effect juice of leaves. It indicates the stimulant activity does not mediate through the β_1 adreno receptors. Nifedepine did not block the cardiotoxic activity juice of leaves. Ca^{++} channels are not influenced in their action on the frog's heart.

The order of cardiac stimulant activity of plants juice is as follows:

O.tenuiflorum > *T.ammi* > *Alangium salvifolium* flowers > *Neem flower* > *O.sancium* > *C.quadrangularis*

The order of cardiotoxic activity of plants juice is as follows:

O.tenuiflorum > *T.ammi* > *Alangium salvifolium* flowers > *Neem flower* > *O.sancium* > *C.quadrangularis*

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