ORIGINAL ARTICLE

Evaluation of cardiotonic activity of leaves of *Vitex negundo* Linn

P. T. Pai, R. S. Adnaik, S. N. Mule, N. S. Naikwade, C. S. Magdum

Department of Pharmacology, Appasaheb Birnale College of Pharmacy, South Shivaji Nagar, Sangli - 416 416, India

The present study was undertaken to evaluate the cardiotonic activity of the aqueous extract of leaves of *Vitex negundo* Linn. The leaves are believed to contain some antioxidants and hence pose it to be used in the prevention of cardiovascular diseases. The cardiotonic effect of aqueous extract of leaves of *V. negundo* Linn. was studied by using isolated frog heart perfusion technique (IFHP). Ringer solution without calcium was used as a vehicle for administration of aqueous extract as test and digoxin as standard. A significant increase in the height of force of contraction (positive ionotropic effect) and decrease in heart rate (negative chronotropic effect) was observed at smaller doses (0.4 mg). The effect increased as dose was increased. The test extract had not produced cardiac arrest even at a dose of 2 mg, a higher concentration as compared to standard, digoxin that showed cardiac arrest at dose of 0.2 mg. Hence, as compared to standard, test drug showed wide therapeutic index.

Key words: Digoxin, isolated frog heart perfusion technique, therapeutic index, *Vitex negundo* Linn

INTRODUCTION

Numbers of deaths in industrial world are increasing due to cardiac disease. Cardiac diseases are emerging as single largest contributors for morbidity in India. Cardiac glycosides and catecholamines are agents of choice in treatment of congestive cardiac failure (CCF).[1] But cardiac glycosides (e.g. digoxin) have narrow therapeutic index and hence cause many a times intoxication. Despite of the advancement of knowledge in understanding the basic pharmacology of cardioactive drugs glycosides still have its adverse effects in terms of toxication.[2] Hence, there is a need for new drug research with wide therapeutic index and good cardiac activity, and by this aim, we have chosen *Vitex negundo* Linn. plant and evaluated its cardioactive potential.

Indian people still in large number depend upon the traditional system of medicine i.e. Ayurveda. One such plant is *V. negundo* Linn., belonging to family verbenaceae, which is an important medicinal plant that is distributed throughout India. The leaf extract is used in Ayurvedic, Siddha and Unani system of medicine. *V. negundo* Linn. is a large shrub or sometime a small slender tree with thin grey bark and branchlet glandular, whitish with a fine tomentum. Leaves 3-5 foliolate, acute, the terminal leaflet 5-10 by 1.6-3.2 cm long, the lateral leaflet smaller with a very short petiole, all nearly glabrous above, covered with a fine white tomentum beneath, acute base; common petilate 2.5-3.8 cm long.[3,4] A few laboratory studies have shown that *V. negundo* materials did exhibit a wide range of biological and pharmacological activities, which may substantiate the therapeutic use of this plant in traditional medicine.

The merit of the traditional use of *V. negundo* has also been supported by the isolation and identification of several possible active chemical constituents, mainly flavonoids, iridoids, terpenoids; fatty acids have been isolated from different parts leaves and twigs, bark, seeds and roots. Among the chemical constituent, flavonoids are the major. Leave and twig were reported to contain the known flavonoids such as casticin, orientin, isoorientin, luteolin, lutecin-7-O-glucoside, corymbosin, gardenins A and B, 3-O-desmethylartemetin, 5-O-desmethylnobiletin and 30, 40, 5, 50, 6,7,8-heptamethyoxy flavone. Besides, many glycosidic iridoids, alkaloids and terpenoids have also been isolated.[5]

The leaves are aromatic, tonic and vermifuge. A decoction of nirgundi leaves is given with the addition of long pepper in catarrhal fever with heaviness of head and dullness of hearing. A pillow stuffed with the leaves of nirgundi is placed under the head for relief of headache. The juice of leave is said to have the property of removing foetide discharges and worms from ulcers.[3,4]

Address for correspondence: Dr. P. T. Pai, Flat No. 201, Jai Apartment, Trade Center, Station Road, Shahupuri, Kolhapur-416 003, Maharashtra, India. E-mail: pundlikpai@gmail.com

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MATERIALS AND METHODS

- Drug: The fresh leaves of *V. negundo* Linn. were collected from the local area of Kolhapur district and authenticated by Dr. U. S. Yadav, Department of Botany, Willindon College, Sangli.
- Animals: Frogs of *Rana tagrina* species were obtained from animal house of Sangli. Animals were feed with food and water *ad libitum*. They were maintained as per the norms of CPCSEA, and the experiment was conducted as per CPCSEA norms.
- Drug extract: The aqueous extract of dried leaves of *V. negundo* Linn. was prepared in 250 ml distilled water by the Soxhlet extraction technique and filtered over Buckner funnel. The extract was dried by spray drying and stored in refrigerator.
- Experimental methodology: Isolated Frog Heart Perfusion technique was used for the study of cardiotonic activity.[6] Here, the activity of test extract was compared with digoxin. The methodology was divided into four sections.

Effect of Different Concentrations of Calcium

Here the effect of different concentrations of calcium (as in ringer solution) was tested on frog heart. And ringer solution without calcium was used for testing the activity as it represented failing heart very well.

Recording Cardiotonic Activity

Basal cardiac contraction was recorded on a kymograph with calcium-free ringer solution that was 42 beats/min and contraction amplitude (HFC) was 9 mm. Then responses showed by digoxin and test extract were recorded at different concentrations. Effects were converted to respective percentages. In between the results, the heart was washed with calcium-free ringer solution.

Effect of Drug on Blocked Heart

The heart when blocked with 0.2 mg of digoxin, it was not washed with ringer, rather was perfused with drug extract. The effect is shown in Table 1.

Effect of Drug on Failed Heart

Here the effect of drug on failed heart was analysed. The washing with ringer in between different concentrations of drug extract was not provided and the effect of drug was recorded [Table 2].

RESULTS

With decreasing concentrations of calcium it was seen that there was decrease in height of force of contraction while increase in heart rate i.e. positive chronotropic and negative ionotropic effect [Table 3]. The incremental dosage of test extract (*V. negundo* Linn. leaves) produced positive ionotropic and negative chronotropic effects. The cardiotonic effect shown by test extract at various concentrations is given in Table 4. The effect of digoxin is shown in Table 4. Similarly, the blocked heart started its normal rhythm when perfused with drug extract. The drug also showed its promising effect on failed heart with a successive increase in the height of force of contraction without any ringer washings.

DISCUSSION

Kymograph obtained indicates that even lower doses of test extract give a significant increase in height of contraction. The dose at which digoxin showed cardiac arrest was 0.2 mg and test extract showed a therapeutic effect in the range of 0.25-2 mg without any cardiac arrest. Hence, as compared to digoxin, test extract showed wide therapeutic index [Figure 1]. Also, drug extract showed its promising effect on the blocked and failed heart without

Table 1: Effect of drug on blocked heart

Drug	Concentration (mg/ml)	Dose (ml)	Conc. at different doses (mg)	HR (heart rate beats/min)	HFC in mm (height of force of contraction)	Cardiac output (HR × HFC)
Digoxin	0.5 mg/ml	0.4	0.2	-	Heart blocked	-
Test extract	5 mg/ml	0.05	0.25	28	5	140
		0.1	0.5	34	6	204
		0.2	1	36	8	288

Table 2: Effect of drug on failed heart

Drug	Concentration (mg/ml)	Dose (ml)	Conc. at different doses (mg)	HR (heart rate beats/min)	HFC in mm (height of force of contraction)	Cardiac output (HR × HFC)
Failed heart	-	-	-	-	-	-
Drug extract	5 mg/ml	0.2	1	35	11	385
		0.3	1.5	31	13	403

Table 3: Effect of different concentrations of calcium

Drug	Conc. of calcium with respect to its conc. in normal ringer	HR (Heart rate beats/ min)	HFC in mm (height of force of contraction)	Change in HF (%)	Cardiac output (HR × HFC)
Ringer	Normal	39	12	100	468
	1/4th	44	10	83.33	440
	1/2	41	14	116.66	574
	3/4th	36	16	133.33	576
	Full	38	21	175	798

Table 4: Cardiotonic activity

Drug	Concentration (mg/ml)	Dose (ml)	Conc. at different doses (mg)	HR (Heart rate beats/min)	HFC in mm (height of force of contraction)	Change in HFC (%)	Cardiac output (HR × HFC)
Test extract	5 mg/ml	Control	-	42	9	-	-
		0.05	0.25	40	14	55.55	560
		0.1	0.5	36	16	77.77	576
		0.2	1	33	18	100	594
		0.3	1.5	28	19	111.11	532
		0.4	2	24	22	144.44	528
Digoxin	0.5 mg/ml	0.1	0.05	38	13	44.44	494
		0.2	0.1	36	16	77.77	576
		0.3	0.15	35	21	133.33	735
		0.4	0.2	-	Heart blocked	-	-

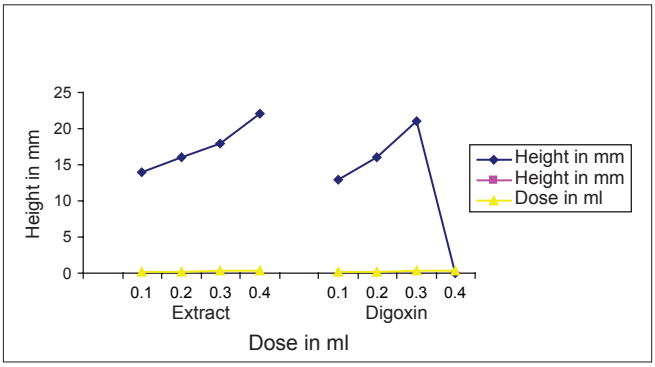

Figure 1: Cardiotonic activity of *Vitex negundo* Linn

ringer washings. We all know the adverse effects shown by digoxin and difficulty in its dose adjustments. Also, in the market, there is still no safer alternative for digoxin and it is considered as a sole drug for the treatment of congestive cardiac failure. From the above-shown observations, the limitation of using digoxin can be overcome by using the aqueous extract of *V. negundo* Linn. leaves which has been found to have excellent cardiotonic activity with the wide therapeutic index as compared to digoxin. Hence, test extract can be a safe alternative to digoxin in congestive cardiac failure. Free radicals play a main role in the prognosis of cardiovascular diseases, e.g. Free radicals cause endothelial dysfunction and activation of macrophages leading to atherosclerosis. In myocardial infraction, free radicals cause ischemic reperfusion injury and myocyte necrosis.[7] *V. negundo* leaves contain some poly-phenolic constituents which have proven antioxidant potential[8] and hence, the plant poses itself as a substance for the prevention of cardiovascular diseases. Further investigation is necessary for evaluation of traditional uses and phytochemical nature of the constituents that are responsible for cardiotonic activity. This is the preliminary study and if proper constituents responsible for the effect are isolated, and in turn, if they can be synthesized, then the drug can add its value in the market.

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