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Evaluation of cardiovascular effects and cardiotonic activity of *Phyllanthus amarus* and *Phyllanthus fraternus*

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Abstract

A great number of preclinical and clinical studies have not only confirmed but have also extended the medicinal uses of species of the genus *Phyllanthus* mentioned in traditional medicine. While effects of the extracts of *Phyllanthus amarus* & *Phyllanthus fraternus* on cardiovascular system using their different concentrations has not been previously reported. The cardiovascular activity was evaluated by using isolated frog heart perfusion technique. The extracts of *P. amarus* & *P. fraternus* at a dose of (50-200 µg/ml) had no effect on the cardiovascular system of normal frog heart whereas, when given to a hypodynamic heart the extracts improved its function. The current study reveals the cardiotonic activity of extracts of *P. amarus* & *P. fraternus* by improving the function of a hypodynamic heart. Digoxin was used as a positive control.

Keywords: *Phyllanthus amarus, Phyllanthus fraternus,* Cardiotonic activity *and* Digoxin

INTRODUCTION

Cardiac disease is an important cause of premature death in industrialized countries. It is estimated that cardiac disease will emerge as single largest contributor to morbidity in India accounting for nearly one third of total deaths in near future [1]. Cardiac glycosides and catecholamines have been used as main therapeutic agents in the treatment of congestive cardiac failure [1]. However, the danger of cardiac glycosides intoxication is well documented and doubts have been expressed about their effectiveness. Despite continuing advancement in understanding the basic pharmacology of cardio active drugs and cardiac glycosides, intoxication with digitalis, a narrow therapeutic index drug, remains a common clinical problem. Synthetic catecholamine has been reported to cause a severe oxidative stress in the myocardium research free radical formation [1] .

The plants belonging to the genus *Phyllanthus* (*Euphorbiaceae*) are widely distributed throughout tropical and subtropical countries [2]. These plants are used in folk medicine for treatment of several diseases, such as disturbances of kidney and bladder calculi, intestinal infections, diabetes and hepatitis B virus [2-4]**.** Also the review of the literature reveals that, good number of preclinical and clinical studies have confirmed the medicinal use of various *Phyllantus* species that have been mentioned in the traditional medicine [2-4]. In the present study we have evaluated the effects of extracts of *Phyllanthus amarus* and *Phyllanthus fraternus* on cardiovascular system and their possible cardiotonic activity.

MATERIALS AND METHODS

Plant material:

Phyllanthus amarus Schum and Thonn and *Phyllanthus fraternus* Webster family-*Euphorbiaceae* were obtained from different places in Karad western Maharashtra. The plant species were identified and authenticated by Botanical survey of India, Pune [Reference No: BSI/WC/Tech./2012/644].

Preparation of the *P. fraternus* extract:

The dried leaves, stems and roots of *P. fraternus* was minced and extracted with 70% ethanol-water in the proportion of 70:30, being stirred and macerated at room temperature (22-28°C) for 15 days. The ethanol was evaporated and the extract (yield 5-7%) was

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concentrated to the desired level and stored in a refrigerator.

Extracts of *Phyllanthus amarus:*

The standardized extract of *Phyllanthus amarus* whole plant (water extract) Reference No: SR/KN/CL/1/2012-L12030241, was procured as a gift sample from Chemiloids Ltd., Vijaywada.Standardized methanolic extract of *Phyllanthus amarus* leaf (Methanol extract contains >2.5% of Phyllanthin and Hypophyllanthin) Report No: FP1112042- PA/11LOT05 and the standardized hydro-methanolic extract of *Phyllanthus amarus leaf* (60% Methanol Hydroalcoholic extract contains >5% of Corilagen) - Report No: FP1102034 - PA/11LOT/02 were procured as a gift sample from Natural Remedies Pvt. Ltd., Bangalore.

Experimental Animals:

Frogs of *Rana tigrina* species from the animal house froggery of Govt. College of Pharmacy Karad were used for the study and were maintained as per CPCSEA guidelines. A prior approval [Approval number-GCOPK/2011-12/ CPCSEA/616] was obtained from the Animal Ethics Committee of GCOP, Karad.

Instruments used:

Sherington Rotating Drum for tracing and recording resposes, Sterling's heart lever and Symes venous cannulae.

Drugs:

The marketed Digoxin, Atenolol and Amlodipine (Sun Pharma Ltd.) were obtained from the local market.

Preparation of *Phyllanthus* infusion:

The standardized aqueous extract of *P. amarus* whole plant (PAAE), standardized methanolic extract of *P. amarus* leaf (PAME), standardized hydro methanolic extract of *P. amarus* leaf (PAHME) and the standardized hydro ethanolic extract *P. fraternus* (PFHEE) at dose of 0.5 mg/ml were dissolved in the ringer solution or distilled water just before use.

Preparation of digoxin solution:

Various different concentrations were made with distilled water. The prepared samples were evaluated for their cardiotonic activity and were treated as reference standard.

Evaluation of cardiovascular effects:

Isolated frog heart perfusion model was used to evaluate the activity of various concentrations of *phyllanthus* extracts. The frog was pithed and pinned to the frog board. A midline incision was given on the abdomen, the pectoral girdle was removed and the heart was exposed. The pericardium was carefully removed and few drops of frog ringer were poured over the exposed heart. The inferior vena cava was traced, a thread was tied around it and a small cut was given in order to insert the venous cannula. The cannula was inserted in the vein and the thread was tied to assure that the cannula was in place which was in turn was connected to a saline bottle containing frog ringer solution. A small cut was given to the aorta for the ringer to come out. Heart was isolated and attached to the stand with moderate flow of ringer. A thin pin hook was passed through the tip of the ventricle and with the help of a fine thread attached to the hook; it was tied to the free limb of the Sterling's heart lever which was fixed to a stand. A proper tension was adjusted by altering the height of the lever. The normal heart rate was noted. All test samples were administered in different doses viz. 0.1ml, 0.2ml, 0.4ml. The rate and force of heart contraction were noted [5-6].

Evaluation of cardiotonic activity:

Above model was used to evaluate the cardiotonic activity of various concentrations of *phyllanthus* extracts. Except, for this experiment was carried out by using Ca^{++} free ringer solution/modified ringer (Hypodynamic ringer solution) instead of frog ringer solution was used. The Hypodynamic ringer solution was prepared by using standard method (Table-1) [1, 7].

Table 1: Composition of hypodynamic ringer solution

Ingredients	Quantity
Sodium chloride (NaCl)	6.5 gm
Potassium chloride (KCl)	0.14 gm
Calcium Chloride (CaCl2)	0.03 gm
Sodium bicarbonate (NaHCO3)	0.2 gm
Glucose	2 gm
Distilled Water	Up to 1000 ml

The basal cardiac contraction was recorded on a kymograph after the administration of calcium free ringer solution. The average basal heart rate and the

contraction amplitude were noted as beats/min and in mm respectively. The responses of digoxin and *phyllanthus* extract at various concentrations were recorded on kymograph and their cardiac activity in terms of heart rate and height of force of contraction was noted and compared. The frog heart was washed with ringer solution after every administration of test extract and reference drug till it was brought to normal state.

Statistical analysis:

Results are presented as means + SEM (standard error of mean), except the Cardiac outputs that are presented as geometric means accompanied by their respective 95% confidence limits. Statistical significance between groups was calculated by means of analysis of variance followed by students T- test. $P < 0.05$ was considered significant.

RESULTS

Effect of *phyllanthus* extracts on normal isolated frog heart preparation:

The results revealed that there was no significant effect observed on the height of force of contraction (no inotropic effect) nor the heart rate (no chronotropic effect) when the dose of *phyllanthus* extracts was increased. (Table-2 and Figure-1)

Table 2 Effect of different concentrations of Phyllanthus extracts on normal heart of frog

Drug	Concentration	Dose in ml	Concentration at different doses (µg/ml)	Heart rate [HR]	Height of force of contraction [HFC]	Cardiac output CO = HR x HFC
PAAE	-	Normal	0	36.33 ± 0.330	14.66 ± 0.33	532.59
	0.5 mg/ml	0.1 ml	50	36.66 ± 0.330	15.0 ± 0.577	549.90
		0.2 ml	100	38.33 ±0.330	15.0 ± 0.000	574.95
		0.4 ml	200	36.66 ± 0.881	15.66 ± 0.33	574.09
PAME	-	Normal	0	36.33 ± 0.330	18.0 ± 0.577	653.94
	0.5 mg/ml	0.1 ml	50	35.33 ± 0.330	18.33 ± 0.33	647.59
		0.2 ml	100	35.33 ± 0.330	17.66 ± 0.33	623.92
		0.4 ml	200	36.0 ± 0.577	17.0 ± 0.570	612.00
PAHME	-	Normal	0	37.0 ± 0.577	13.66 ± 0.88	505.42
	0.5 mg/ml	0.1 ml	50	37.0 ± 0.577	15.0 ± 0.577	555.00
		0.2 ml	100	38.0 ± 0.577	15.66 ± 0.33	595.08
		0.4 ml	200	36.33 ± 0.330	14.66 ± 0.33	532.59
PFHEE	-	Normal	0	36.33 ± 0.570	12.0 ± 0.577	435.96
	0.5 mg/ml	0.1 ml	50	37.33 ± 0.570	14.0 ± 0.577	522.62
		0.2 ml	100	36.66 ± 0.881	13.66 ± 0.881	500.77
		0.4 ml	200	36.33 ± 1.660	14.33 ± 0.33	520.60

Effect of *phyllanthus* extracts on hypodynamic isolated frog heart preparation:

Calcium-free Ringer solution was used as vehicle for administration of *phyllanthus* extracts as a test extract and digoxin as a standard. The results indicated that a significant increase in height of force of contraction (positive inotropic effect) and in heart rate (positive chronotropic effect) at a very low concentration (50µg/ml) was observed with *phyllanthus* extracts as compared to the same dose of a standard digoxin (Table-3, Figure-2).

These preliminary studies confirm the cardiotonic activity of *phyllanthus* extracts. All the concentrations of phyllanthus extracts restore the cardiac activity of Hypodynamic frog heart i.e. it increases rapidity and force of contraction. It was found that higher concentration of *phyllanthus* extracts showed better response as compared to the lower ones. It is interesting to know that *phyllanthus* extracts has rapid onset of action compared to Digoxin.

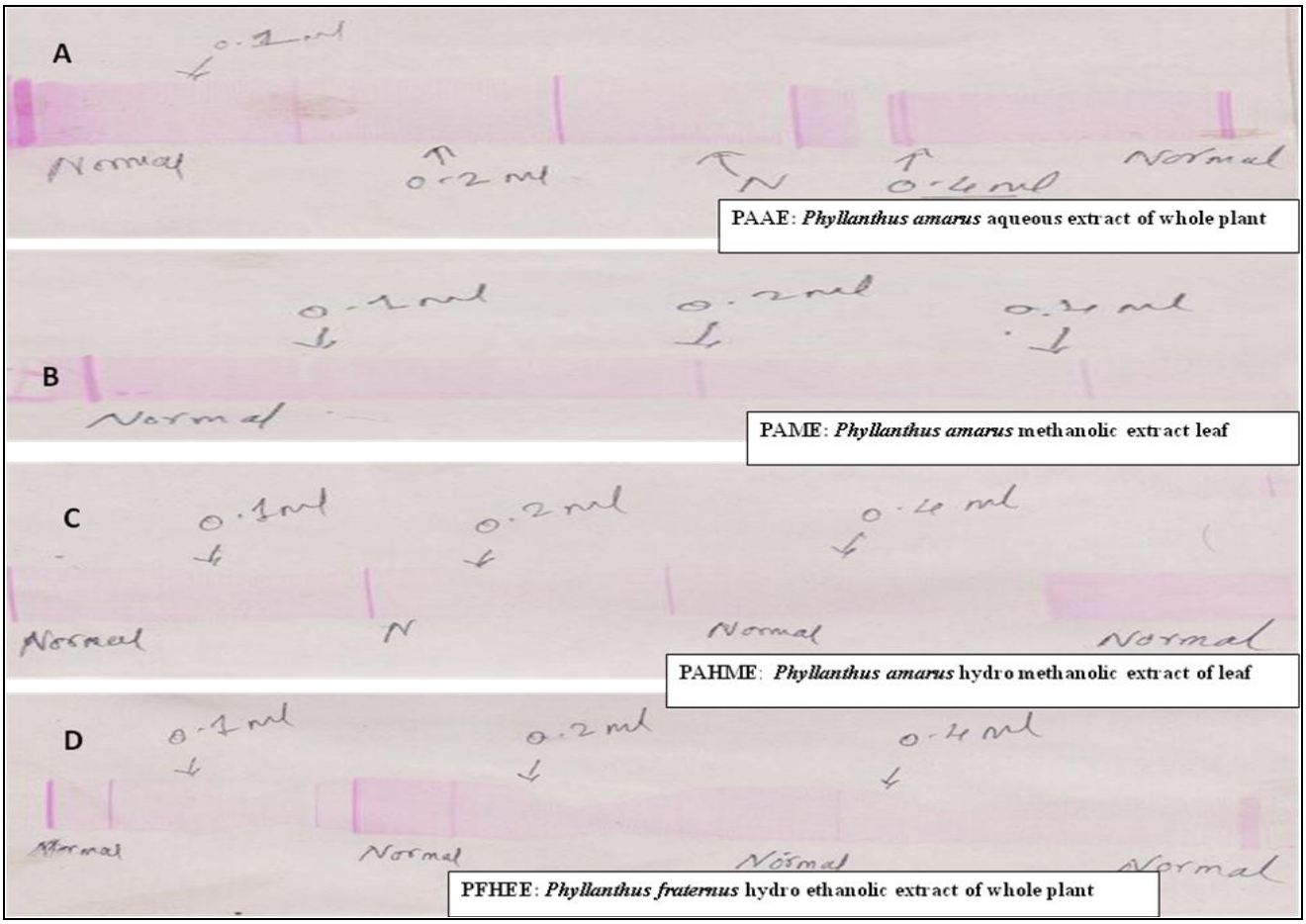

Figure 1. Kymographs showing effect of the Phyllanthus extracts on normal frog heart

Table 3 Effect of different concentrations of Digoxin and Phyllanthus extracts on hypodynamic heart of frog

Drug	Concentration	Dose in ml	Concentration at different doses (µg/ml)	Heart rate [HR]	Height of force of contraction [HFC]	Cardiac output CO = HR x HFC
DIGOXIN	0.5 mg/ml	0.1 ml	50	37.25 ± 0.853	16.25 ± 0.629	605.312
		0.2 ml	100	39.0 ± 0.707	18.75 ± 0.250	731.25
		0.4 ml	200	41.0 ± 0.707	22.0 ± 0.707	902
PAAE	0.5 mg/ml	0.1 ml	50	38.0 ± 0.408	16.25 ± 0.487	617.50
		0.2 ml	100	39.25 ± 0.250	17.25 ± 0.478	677.062
		0.4 ml	200	40.75 ± 0.478	18.50 ± 0.645	753.875
PAME	0.5 mg/ml	0.1 ml	50	37.75 ± 0.629	20.25 ± 0.853	764.437
		0.2 ml	100	40.25 ± 0.478	24.25 ± 0.478	976.062
		0.4 ml	200	41.75 ± 0.4787	26.25 ± 0.478	1095.937
PAHME	0.5 mg/ml	0.1 ml	50	37.75 ± 0.478	15.25 ± 0.750	575.687
		0.2 ml	100	40.75 ± 0.478	19.25 ± 0.478	784.437
		0.4 ml	200	42.75 ± 0.478	22.75 ± 0.478	972.562
PFHEE	0.5 mg/ml	0.1 ml	50	36.25 ± 1.109	14.0 ± 0.408	507.50
		0.2 ml	100	38.75 ± 0.250	16.50 ± 0.645	639.375
		0.4 ml	200	40.75 ± 0.478	19.75 ± 0.750	804.812

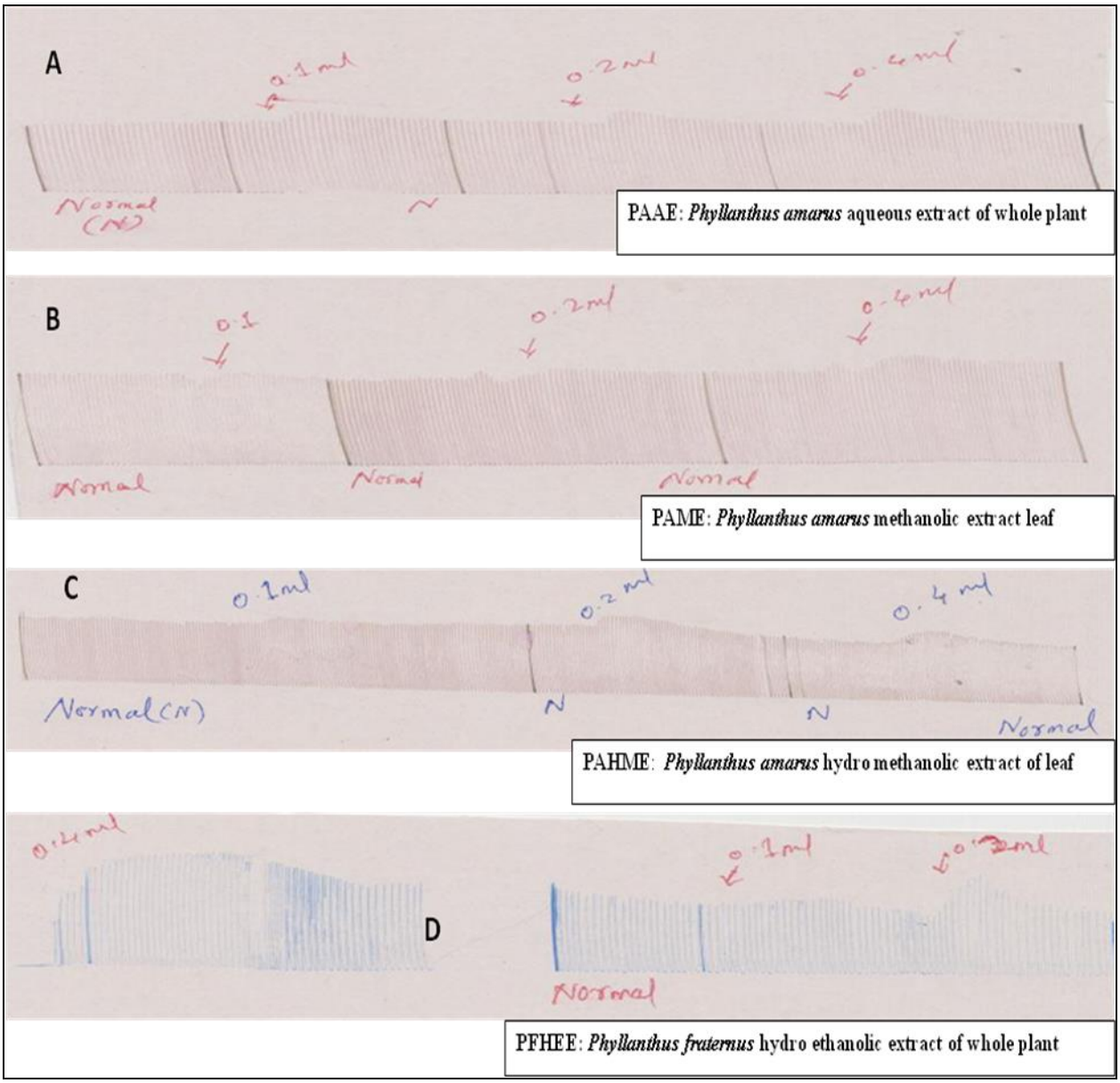

Figure 2. Kymographs showing effect of the Phyllanthus extracts on hypodynamic frog heart

Evaluation of mechanism of action:

In order to test the involvement of the adrenergic system or the role calcium channel involved in the cardiotonic effect of the *phyllanthus* extracts, the isolated perfused heart was treated with the β- blocker Atenolol 5mg /ml and calcium channel blocker Amlodipine 1mg/ml. But they failed to inhibit the positive chronotropic and ionotropic effect of the *phyllanthus* extracts. (Figure-3) It indicates that cardiotonic activity is neither mediated through β receptors nor through the Ca^{++} ions.

DISCUSSION

The plants belonging to the genus *Phyllanthus* (family *Euphorbiaceae*) comprises more than 600 species which are widely distributed throughout tropical and subtropical countries and has long been used in traditional medicine to treat several diseases.[2-4] Although the extracts or active principles of these plants have been investigated in several biological models as far as is known, the antinociceptive properties of the four species have not yet been reported.

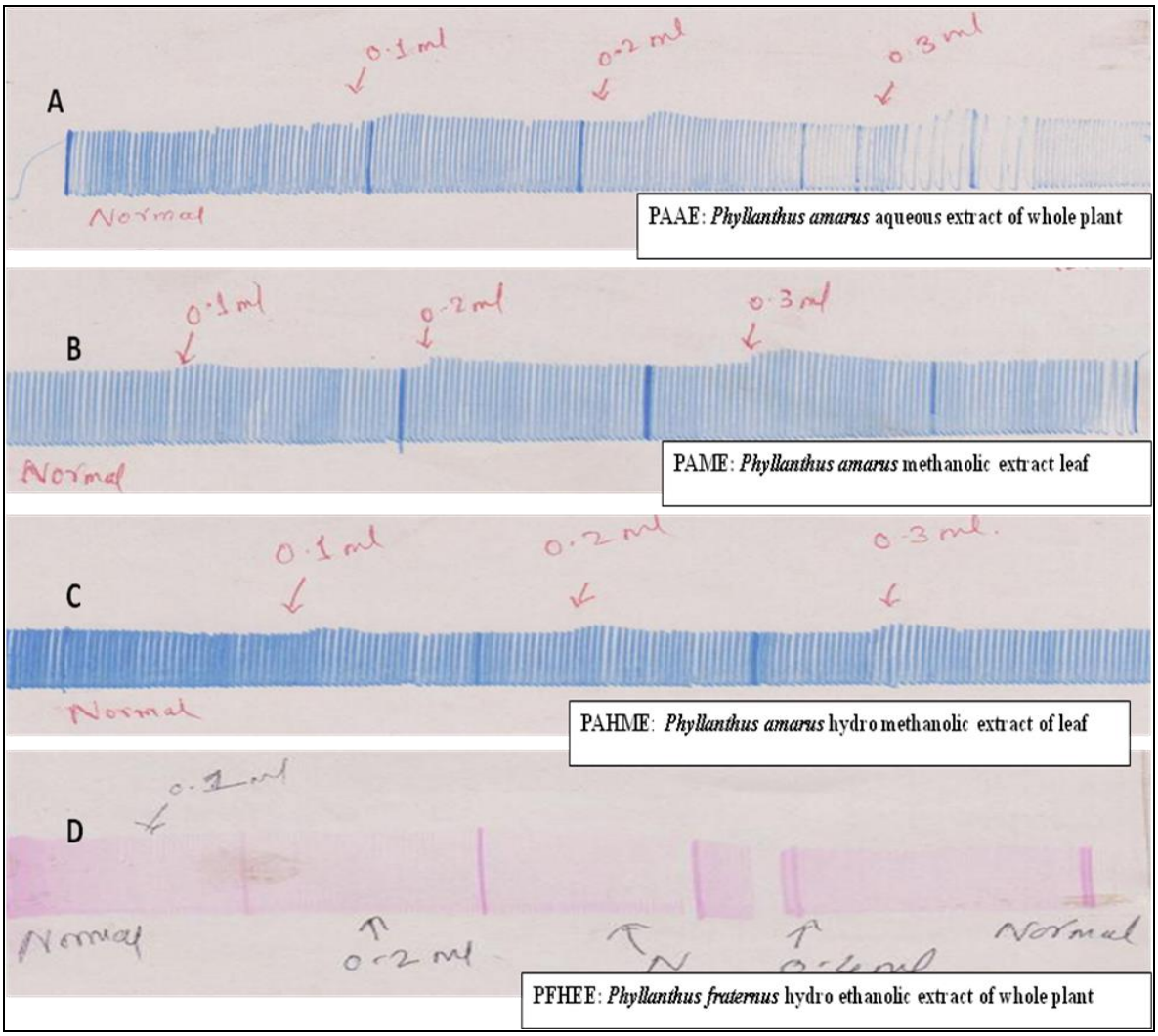

Figure 3. Kymographs showing effect of the Phyllanthus extracts on Adrenergic antagonists and calcium channel blocker in hypodynamic frog heart

Different classes of organic compounds of medicinal importance have been isolated in *phyllanthus* extracts including alkaloids, flavonoids, hydrolysable, tannins (*Ellagitannins*), major lignans, polyphenols, triterpenes, sterols and volatile oil [2-4].

It has been previously reported that flavonoids and phenolic compounds are potent antioxidants and are believed to prevent cardiovascular diseases and exhibit a wide range of cardiovascular effects [8-9].

The cardiotonic activity was exhibited in the form of positive ionotropic and chronotropic effect probably due to the presence of lignans and flavanoids. Lignans isolated from *phyllanthus* extracts are phyllanthin, hypophyllanthin, niranthin, phyltetralin, nirtetralin, isonirtetralin, hinokinin, lintetralin, isolintetralin, demethylenedioxy-niranthin, 5-demethoxyniranthin, etc. The imporatant flavonoids isolated from *phyllanthus* extracts are gallocatechin, rutin, quercetin, phyllanthusiin, kaempferol etc. [2-4].

The characterization of the isolated compound based on structural studies is under progress; moreover, it promises a lot of scope for further research on its cardiac activity. It will be interesting to isolate the active chemical constituents which are responsible for the cardiotonic activity as well as to determine the possible mechanism of action.

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