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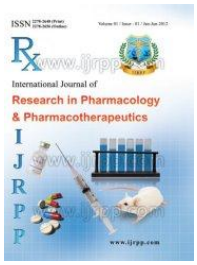

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Evaluation of Cardiotonic and Cardioprotective Effects of *Paederia foetida*.

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ABSTRACT

Aim of the study

The present study is undertaken to study the cardiotonic activity and its protective role on heart by *Paederia foetida* belonging to the family Rubiaceae.

Materials and Methods

Paederia foetida leaf extracts with ethanol and hydro alcoholic (aq. Methanol) as solvents is prepared by maceration for 72h. Both the extracts have shown presence of cardiac glycosides, iridoids glycosides, alkaloids, flavonoids, tannins, phytosterols and aminoacids on phytochemical screening. These extracts PFEE and PFHE were screened for cardio tonic activity by "Isolated Frog Heart Perfusion Technique" and protective role studies by using Isoproterenol induced cardio toxicity in Wistar albino rats by administering PFEE and PFHE (200mg/kg) for a period of 14 days and Isoproterenol on 15^{th} day (5.25mg/kg) and 16^{th} day (8.5mg/kg) subcutaneously. Cardio protection was assessed by estimating the cardiac marker enzymes Aspartate dehydrogenase, Alanine dehydrogenase, Creatine Kinase MB, Lactate dehydrogenase and Total Protein levels in serum and heart tissues. Biochemical studies of assay of $Na^{+}K^{+}$ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase were performed by administering the extracts 200mg/kg orally for a period of 7 days.

Results

Paederia foetida extracts has produced significant positive inotropic and negative chronotropic effect similar to that of standard drug digoxin. These effects were antagonized in presence of nifedipine but not with propranolol. A significant ($p<0.05$) decrease in membrane $Na^{+}K^{+}$ATPase and Mg^{2+}ATPase and an increase in Ca^{2+}ATPase on comparison to normal control, further confirmed its cardiotonic activity. The levels altered by isoproterenol were restored significantly ($p<0.05$) by the administration of the extracts both in serum and heart tissue levels. This activity can be attributed due to presence of particular cardiac glycosides and iridoids glycosides which can further processed.

Conclusion

Our work clearly shows that *Paederia foetida* possesses cardio tonic activity without altering the normal physiology of heart.

KEY WORDS: Cardiotonic, digoxin, *Paederia foetida* and membrane bound enzymes.

INTRODUCTION

Cardiovascular diseases do constritate the principal cause of human mortality. Congestive heart failure (CHF) is a major contributor to morbidity and mortality worldwide. Epidemiological studies indicate that coronary heart diseases, especially heart failure, will constitute the major disease burden worldwide. Heart failure at a young age is considered the most leading cause of death worldwide even with the huge improvement in clinical care, and the wide use of various drugs. Cardiac glycosides and catecholamines have been used as main therapeutic agent in the treatment of congestive heart failure. While effective in relieving symptoms and in stabilizing patients with hemodynamic decomposition, such therapies have not improved survival. [1] There is no evidence that digitalis prolongs survival of coronary heart disease patients, major limitation in use of cardiac glycoside are low margin of safety, inability to retard the process which caused the heart to fail and its intoxication are well documented [2]. Synthetic catecholamine has been reported to cause a severe oxidative stress in the myocardium through free radical formation. Thus, there are continuous searches for new cardiotonic drugs especially from natural origins. There are several herbs which are having proven cardio tonic activity. Whereas many herbs which are claimed to have cardio tonic activity, remain without scientific and experimental evidences to justify the claims. It necessitates research for new drug and with this aim we have chose *Paederia foetida* to conduct cardio tonic activity and its cardio protective role in heart. *Paederia foetida*, locally known as "Gandhaprasanni" belongs to the family of Rubiaceae (English name "skunk vine"). The name derives from the distinct odor of carbon bisulphide when the leaves are crushed or bruised. It is used in the preparation of Dasmularishta. It contains various phytochemical compounds such as cardiac glycosides, saponin, tannin, phenol, flavonoids, terpenoid, alkaloid and reducing sugar. The major classes of chemical constituent present in this plant are iridoids glycosides, alkaloids, carbohydrates, protein, sitosterol, stigmasterol, amino acid and volatile oil. Traditionally, *P.foetida* has shown to be anti oxidant, anti diabetic, anti tussive, hepatoprotective and analgesic. [3] The phytochemical investigation of *Paederia foetida* has shown presence of cardiac glycosides and iridoid glycosides which are known to give cardiotonic activity and cardioprotective role. Hence, this plant is considered to evaluate cardio activity due to the presence of above constituents in this plant.

MATERIALS AND METHODS

Preparation of Extracts

The leaves of *Paederia foetida* plant is collected from Nadia district of West Bengal of India. The plant is authenticated by Dr. Mustafa, Taxonomist Department of Botany Kakatiya University Warangal Telangana**.** Around 5kg leaves were collected and air dried under shade. The finely powdered leaves (weight) were extracted with ethanol and aqueous methanol by maceration for 72hrs in equal amounts. The yield is calculated and was found to be 26.6% for ethanol product designated as PFEE and 28% for hydro alcoholic product designated as PFHE. This crude leaf extracts are used in the study.

Phytochemical Screening

Both the extracts PFHE and PHEE had shown positive for cardiac glycosides, iridoid glycosides, alkaloids, phytosterols, aminoacids, proteins and flavonoids on respective confirmatory phytochemical tests [3].

Evaluation of Cardiotonic activity

Animals

Frogs of *Rana tigrina* species maintained in the animal house are used for the studies. Albino rats of both the sex weighing between 160-200g were procured from Sainath agencies, Hyderabad. The study was approved by the Institutional Animal Ethics Committee (IAEC), St.Peter's Institute of Pharmaceutical Sciences, Warangal (Protocol No: 04/SPIPS/IAEC/14. The animals were maintained in accordance with the CPCSEA guidelines.

Isolated frog heart perfusion technique

The frog of species *Rana tigrina* was pithed and the heart was exposed. The inferior venacava was traced and frog ringer solution was perfused into heart. A proper tension was adjusted by altering the height of the lever. The basic composition of the frog ringer solution in mill moles: NaCl-110; KCl-1.9; $CaCl_2$-1.1; $NaHCO_3$-2.4; Glucose-11.1; NaH_2PO_4-0.06. For hypodynamic ringer solution Half Ca^{+2} is used to make a hypodynamic heart to

study the effect of plant extracts. Firstly the basal contractions of the heart are recorded on a smoked kymogaphic drum with normal and hypodynamic ringer solution [4]. The plant extracts was made into a solution by dissolving in ringer solution and the final volume was adjusted to obtain various concentrations of the extracts. The drugs digoxin and adrenaline are taken as standard to compare and study. The average basal heart rate and the contraction amplitude were 48 beats/min and 15 mm respectively. The effects of the drugs and extracts were transposed to the respective percentage of the basal values. Graded dose-response was recorded for each extract (0.5, 1 and 1.5 mg) and the dose which caused the maximum effect was chosen as the experimental dose. The frog heart was washed with the Ringer solution after every administration of extracts and drugs till it was brought back to the normal state.

The frog heart was perfused with propranolol, at 30 µM concentration in frog ringer solution for 60 sec followed by the administration of extracts and the recording were noted.

Nifedipine, at 28.8 µM concentration in frog ringer solution was administered for 60 sec followed by extracts and the recordings were noted.

Biochemical studies

Both male and female Wistar albino rats weighing 160-200g are selected for this study. The rats were randomly divided into 3 groups of six animals each. They are as follows:

Group I

Was treated with 5%tween 80 suspension i.p. for 7 days which served as control.

Group II

Was treated with hydro alcoholic (Aq.Methanol) extract 200mg/kg (approximately 1/10 of the LD50) body weight i.p. for 7days.

Group III

Was treated with ethanolic extract 200 mg/kg (approximately 1/10 of the LD50) body weight i.p. for 7days.

On 8th day, all the animals from 3 groups were sacrificed and the blood was collected by cardiac puncture and heart tissue was collected and serum was separated from the blood. The heart was washed in ice-cold saline and 100 mg of tissue was weighed and homogenized in chilled 0.1 M Tris-HCl Buffer (Ph 7) and the homogenate was used for the assay of Na^+/K^+ ATPase(Bonting *et al.,* 1970)[5], Ca^{2+}ATPase (Hjerten and Pan, 1983)[6] and Mg^{2+}ATPase (Ohinishi *et al.,* 1982)[7].

Cardioprotective role

Both male and female Wistar albino rats weighing 160-200g are selected for this study. The rats were randomly divided into 4 groups of six animals each. They are as follows:

Group I

Normal control – treated with vehicle (5% Tween 20)

Group II

Disease control - standard diet and water *ad libitum* for initial 14 days and administered isoproterenol (5.25 mg/kg and 8.5 mg/kg) subcutaneously on 15th and 16th day respectively.

Group III

Ethanol extract for 14 days+ Isoproterenol on 15th and 16th day subcutaneously.

Group IV

Aqueous methanol extract for 14 days i.p + Isoproterenol on 15th and 16th day subcutaneously.

The day after isoproterenol treatment all animals are anesthetized with CO_2 for collection of blood samples. Blood samples were collected by retro-orbital plexus and heart tissues were collected by cardiac puncture [8]. The heart was washed in ice-cold saline and 100 mg of tissue was weighed and homogenized in chilled 0.1 M Tris-HCl Buffer (Ph 7). The serum and homogenate samples were used for the estimation of parameters [9].

Statistical Analysis

The intergroup variation between various groups was analyzed statistically using one-way analysis of variance (ANOVA) using the Graph Pad Prism version 5.0, followed by Dunnett's multiple comparison tests. Statistical significance was evaluated at $p< 0.05$.The experimental results expressed as the Mean± S.E.M or as percent activity compared to control animals.

RESULTS

The leaf extract of *Paederia foetida* has shown significant cardio tonic activity as assessed by the isolated frog heart perfusion technique and also exhibited by the in vivo models which have proven

mechanism of action. Cardiac markers have also shown up to the normal levels in pathological conditions showing the normal physiology of heart in rats. Preliminary phytochemical screening revealed the presence of cardiac glycosides, iridoids glycosides, flavonoids, alkaloids and triterpeniods is shown in table 1.

Table 1: Phytochemical screening of *Paederia foetida* leaf extracts.

Chemical tests	**Specific Tests**	**Ethanol extract (PFEE)**	**Hydro alcoholic extract (PFHE)**
Carbohydrates	Molisch's test	-	+
	Benedict's test	-	+
Alkaloids	Dragendroff's test	+	+
	Mayer's test	+	+
Cardiac glycosides	Keller-Killiani test	+	+
	Baljet's test	++	++
Flavonoids	Shinoda test	+	+
	Lead acetate test	+	+
Iridoids glycosides	Trim-Hill reagent test	+++	+++
Amino acids	Ninhydrin test	+	+
Proteins	Xanthoprotein test	+	+
	Millon's test	+	+
Tannins	Ferric chloride solution test	++	+
	Lead acetate test	+	+
	Bromine water test	-	-
Phytosterols	Salkowski reaction	+	+
	Libermann Burchard reaction	+	+

Both the extracts have shown positive inotropic and negative chronotropic effect which is similar to that of digoxin with increase in force of contraction and decrease in heart rate as seen in Figures- 1, 2, 3. The heart rate, force of contraction and cardiac output are collected and presented as percentage response in Table-2, 3,

Table 2: Effect of *Paederia foetida* leaf extracts on force of contraction on isolated frog heart perfusion technique.

Treatment	Frog Ringer		Frog Ringer + Nifedipine ($3x10^5$)		Frog Ringer + Propranolol ($3x10^5$)	
	Force of contraction (mm)	FOC (%)	Force of contraction (mm)	FOC (%)	Force of contraction (mm)	FOC (%)
Digoxin	$27.80\pm0.771^{***3}$	184.84± 3.179	$13.58 \pm 0.909^{***3}$	90.29± 0.393	-	-
Adrenaline	36.40 ± 0.464^{c3}	242.02± 2.424	-	-	24.87 ± 1.46^{a3}	165.35± 2.961
Ethanol extract (PFEE)	$21.30\pm0.341^{c***3}$	141.62± 2.586	$10.17 \pm 0.266^{a***3}$	67.62±1.802	$20.91 \pm 0.378^{c*2}$	139.03± 1.839
Hydro alcoholic extract (PFHE)	$20.07 \pm 0.268^{c***}$	133.44± 1.832	$9.11 \pm 1.103^{b***3}$	60.57± 0.096	$19.69 \pm 0.860^{c**2}$	130.91± 1.188

Basal values: Force of contraction = 15.04 ± 0.993 mm; FOC (%) = 100%.Values are expressed as mean ± SEM. One way ANOVA ($p< 0.05$) followed by Dunnett's Multiple Comparison Test revealed significant difference between experiments.
Superscripts a, b, c -values are statistically different when compared to Digoxin treatment at $P<0.05$, $P< 0.01$ and $P<0.001$ respectively;
Superscripts ∗, ∗∗, ∗∗∗ - values are statistically different when compared to Adrenaline treatment at $P<0.05$, $P< 0.01$ and $P<0.001$ respectively;
Superscripts 1, 2, 3 - values are statistically different when compared to basal values at $P<0.05$, $P< 0.01$ and $P<0.001$ respectively.

Table 3: Effect of *Paederia foetida* extracts on heart rate on isolated frog heart perfusion technique.

Treatment	Frog Ringer		Frog Ringer + Nifedipine ($3x10^5$)		Frog Ringer + Propranolol ($3x10^5$)	
	Heat rate (HR)	**HR %**	**Heat rate (HR)**	**HR %**	**Heat rate (HR)**	**HR %**
Digoxin	$36.20 \pm 0.800^{***3}$	74.11 ±1.41	$26.00 \pm 0.548^{***3}$	53.49±0.34	-	-
Adrenaline	54.60 ± 0.980^{c3}	112.3 ± 0.02	-	-	37.40 ± 0.872^{c3}	76.95±0.92
Ethanol extract (PFEE)	$44.00 \pm 0.632^{c***3}$	90.53 ± 1.13	$18.77 \pm 0.624^{c***3}$	38.62±0.58	$43.47 \pm 0.533^{c***3}$	89.45 ± 1.96
Hydro alcoholic extract (PFHE)	$44.60 \pm 0.748^{c***}$	91.77 ± 1.18	$18.68 \pm 0.577^{c***3}$	38.44±0.70	$43.00 \pm 0.447^{c***3}$	88.48 ± 1.78

Basal values: Heart rate = 48.6 ± 0.245 (per min); HR (%) = 100%.Values are expressed as mean ± SEM. One way ANOVA (P< 0.05) followed by Dunnett's Multiple Comparison Test revealed significant difference between experiments.
Superscripts a, b, c -values are statistically different when compared to Digoxin treatment at P<0.05, P< 0.01 and P<0.001 respectively;
Superscripts ∗, ∗∗, ∗∗∗ - values are statistically different when compared to Adrenaline treatment at P<0.05, P< 0.01 and P<0.001 respectively;
Superscripts 1, 2, 3 - values are statistically different when compared to basal values at P<0.05, P< 0.01 and P<0.001 respectively.

Table 4: Effect of *Paederia foetida* extracts on cardiac output on isolated frog heart perfusion technique.

Treatment	Frog Ringer		Frog Ringer + Nifedipine ($3x10^5$)		Frog Ringer + Propranolol ($3x10^5$)	
	Cardiac output (per min)	**CO (%)**	**Cardiac output (per min)**	**CO (%)**	**Cardiac output (per min)**	**CO (%)**
Digoxin	$11.16 \pm 0.186^{3***}$	74.42 ± 0.21	$8.04 \pm 0.169^{***3}$	51.54 ± 1.34		
Adrenaline	17.68 ± 0.286^{c3}	113.34 ± 0.02	-	-	8.95 ± 0.751^{c3}	57.37 ± 1.92
Ethanol extract (PFEE)	$14.25 \pm 0.094^{c***1}$	91.35 ± 1.13	$5.94 \pm 0.259^{c***3}$	38.07 ± 2.58	$11.93 \pm 0.718^{c***3}$	76.47 ± 1.96
Hydro alcoholic extract (PFHE)	$14.19 \pm 0.262^{c***1}$	90.96 ± 0.18	$5.06 \pm 0.761^{c***3}$	32.44± 1.70	$11.68 \pm 0.369^{c***3}$	74.87 ± 0.78

Basal values: Cardiac output = 15.6± 0.214; CO (%) = 100%.Values are expressed as mean ± SEM. One way ANOVA (P< 0.05) followed by Dunnett's Multiple Comparison Test revealed significant difference between experiments. a-P<0.05 compared with digoxin. P<0.05 compared to Adrenaline.1 P<0.05 compared to basal valve.

Superscripts a, b, c -values are statistically indicates when compared to Digoxin treatment at P<0.05, P< 0.01 and P<0.001 respectively;

Superscripts ∗, ∗∗, ∗∗∗ - values are statistically different when compared to Adrenaline treatment at P<0.05, P< 0.01 and P<0.001 respectively;

Superscripts 1, 2, 3 - values are statistically different when compared to basal values at P<0.05, P< 0.01 and P<0.001 respectively.

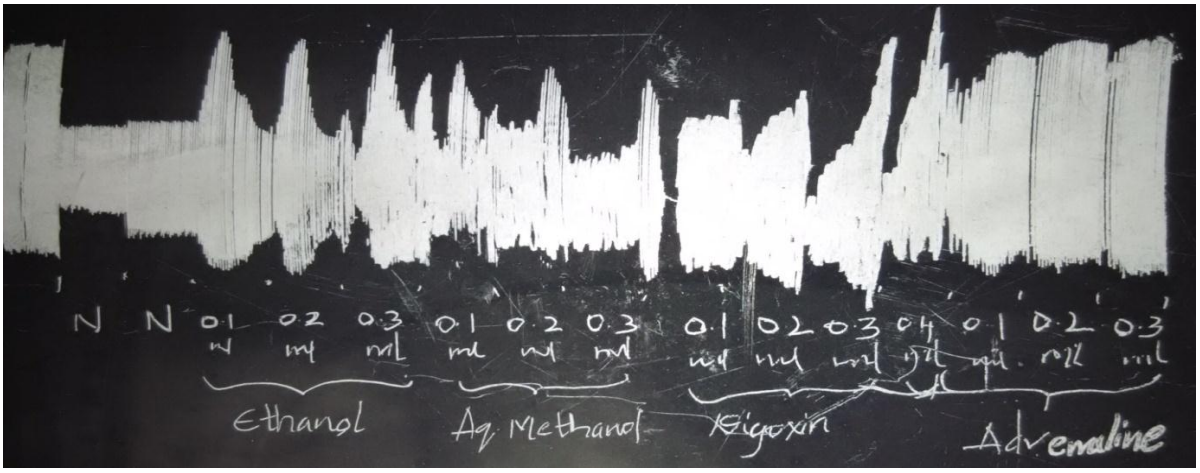


Figure 1: Effect of adrenaline, digoxin, PFEE and PFHE of *Paederia foetida* leaf on isolated frog heart perfusion technique.

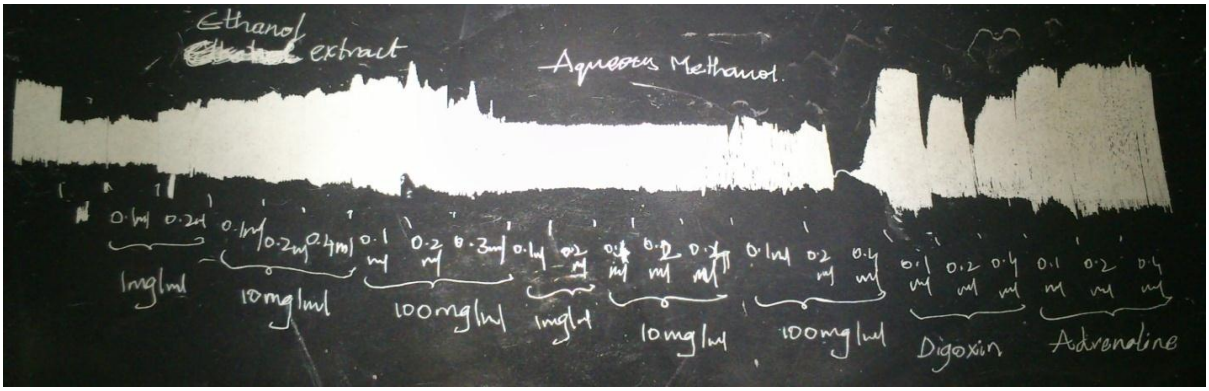


Figure 2: Effect of adrenaline, digoxin, PFEE and PFHE of *Paederia foetida* leaf on isolated frog heart perfusion technique.

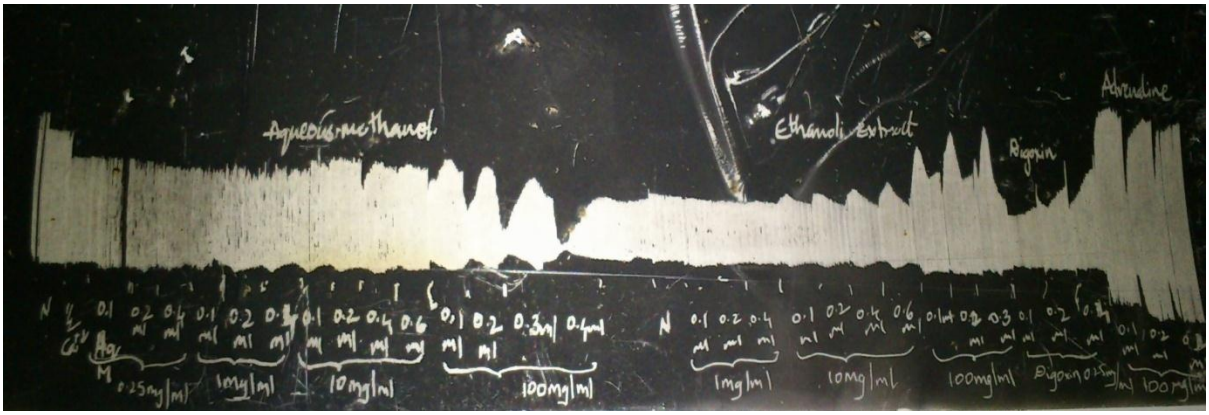


Figure 3: Effect of adrenaline, digoxin, PFEE and PFHE of *Paederia foetida* leaf on isolated frog heart perfusion technique.

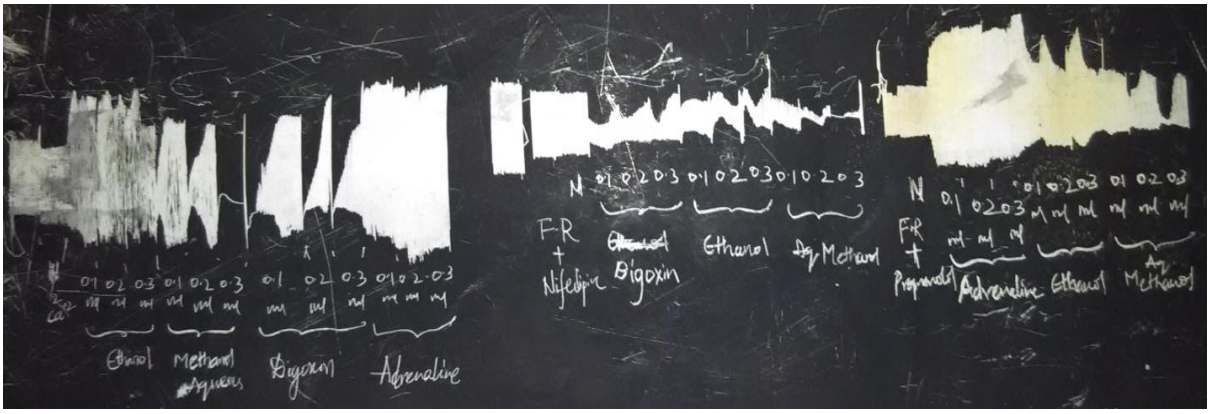


Figure4: Effect of *Paederia foetida* leaf extracts in presence of nifedipine and propranolol.

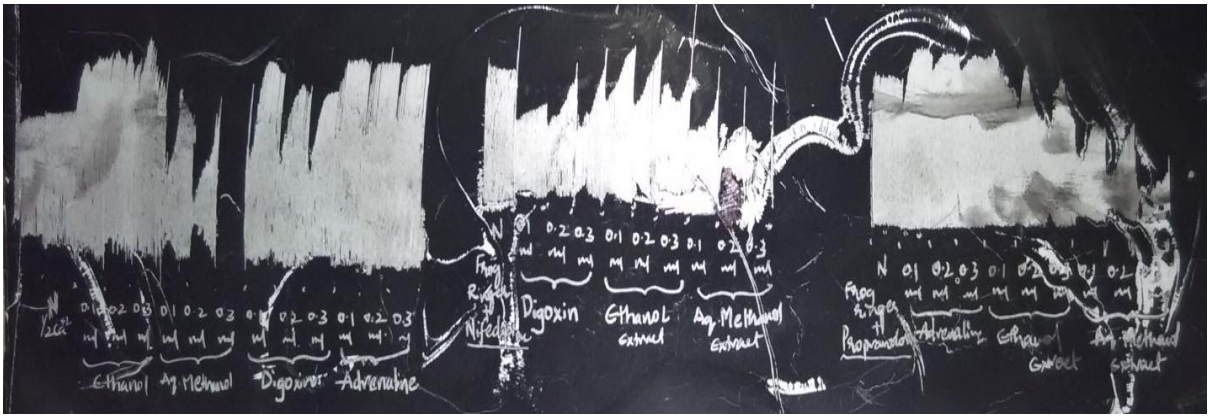


Figure 5: Effect of *Paederia foetida* leaf extracts in presence of nifedipine and propranolol.

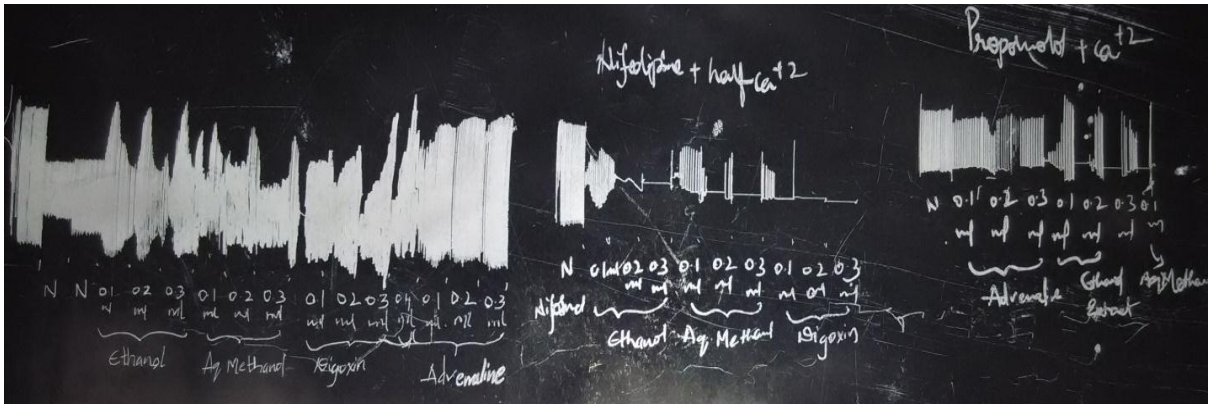


Figure 6: Effect of *Paederia foetida* leaf extracts in presence of nifedipine and propranolol.

Biochemical Studies

The similarity in mechanism of action of extracts with digoxin is shown by the blocking action with nifedipine, calcium channel blocker but not with propranolol, an adrenaline blocker. The blocking effect can be seen in kymograph sheets as in Figures- 4, 5, 6. Significant ($p<0.05$) decrease in membranous Na^+K^+ATPase and Mg^{2+}ATPase and increase in Ca^{2+}ATPase is seen when compared to normal control rats on seven days treatment with both the extracts PFEE and PFHE which further confirmed the cardiotonic activity which is shown in Table 5.

Table 5: Effect of *Paederia foetida* leaf extract on membrane bound enzymes in hearts of rats.

Groups	Na^+K^+ATPase (µmol of phosphorus liberated/min/mg protein)	Ca^{2+}ATPase (µmol of phosphorus liberated/min/mg protein)	Mg^{2+}ATPase (µmol of phosphorus liberated/min/mg protein)
Control (NC)	1.382 ± 0.035	0.876 ± 0.044	4.005 ± 0.187
Ethanol extract (PFEE)	1.004 ± 0.086***	1.109 ± 0.024*	3.255 ± 0.180*
Aq.Methanol extract (PFHE)	1.109 ± 0.032**	1.477 ± 0.092***	3.142 ± 0.233*

Values are expressed as Mean ± SEM. n=6. *P< 0.05 compared with normal control. One way ANOVA followed by Dunnett's multiple comparison tests.

6.2.3. Cardio protective role

Cardioprotective role of *Paederia foetida* in isoproterenol induced cardiotoxicity has shown significant (p<0.05) decrease of cardiac marker enzymes such as AST, ALT, CK-MB, LDH and total protein in serum and significant increase in heart tissue levels when compared to isoproterenol treated group and no significant change when compared to normal control Tables-6 and Figures-7, 8, 9, 10, 11.

Table 6: Effect of *Paederia foetida* leaf extracts on clinical marker enzymes in serum and heart of rats.

Marker Enzymes	Normal Control (NC)		Isoproterenol Treated (ISO)		Ethanol Extract (PFEE) + ISO		Hydro alcoholic Extract (PFHE) + ISO	
	Heart IU/L	Serum IU/L	Heart IU/L	Serum IU/L	Heart IU/L	Serum IU/L	Heart IU/L	Serum IU/L
Creatine Kinase MB	94.02 ± 20.34**	31.40 ± 11.31**	21.60 ± 9.563##	129.5± 27.89##	77.22± 9.572*	48.80± 15.66**	75.35± 13.76*	44.31± 4.636**
Lactate Dehydrogenase (LDH)	64.83± 9.9***	6.320 ± 1.311**	421.7 ± 57.8###	148.3 ± 60.06##	102.7± 16.1***	27.88 ± 4.44*	163.4±49.6***	26.47± 6.789*
Aspartate Transaminase (SGOT)	31.44 ± 3.88***	24.50 ± 4.40***	3.308 ± 1.06###	133.0 ± 23.1###	14.86 ± 2.28*###	42.66 ± 10.5***	14.43± 2.2*###	40.36 ± 13.62***
Alanine Transaminase (SGPT)	17.82 ± 2.70***	20.56 ± 4.51***	2.84 ± 0.73###	93.05 ± 19.9###	9.74 ± 1.43*##	30.26 ± 4.321**	8.89 ± 1.02*##	36.79± 5.102**
Total Protein (TP)	1.608 ± 0.195**	10.42 ± 0.554*	0.688 ± 0.173##	12.88 ± 0.696#	1.358 ± 0.109*	9.05 ± 0.67***	1.495± 0.14**	9.55± 0.492**

Values are expressed as Mean ± SEM; n=6. Values are significant at p<0.05 when compared to Normal control (NC) and are significant at p<0.05 when compared to disease control (ISO). One way ANOVA followed by Dunnett's multiple comparison tests

Figure 7: Effect of *Paederia foetida* leaf extracts on Creatine Kinase MB (CK-MB)

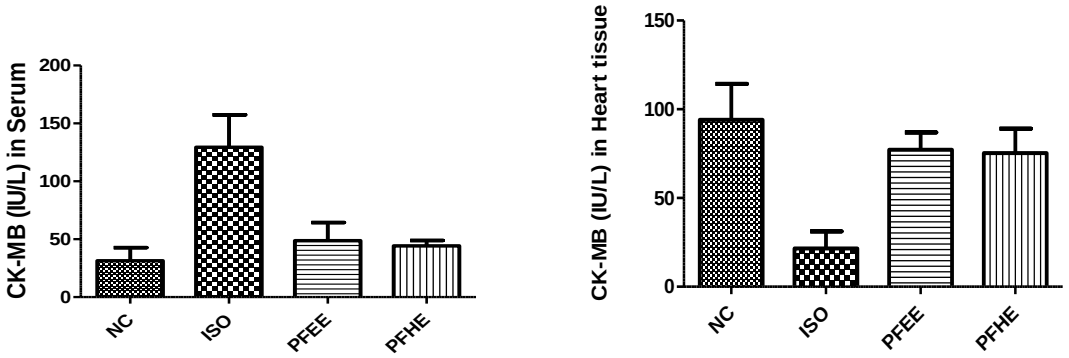


Figure 8: Effect of *Paederia foetida* leaf extracts on Lactate dehydrogenase (LDH)

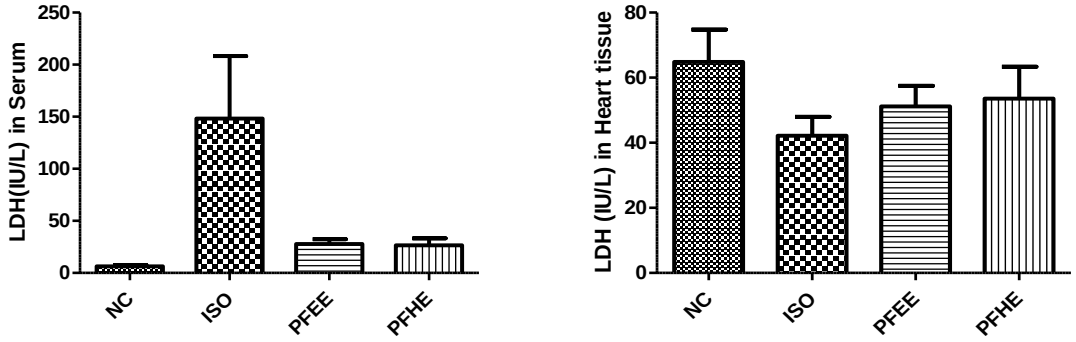


Figure 9: Effect of *Paederia foetida* leaf extracts on Aspartate Transaminase (AST)

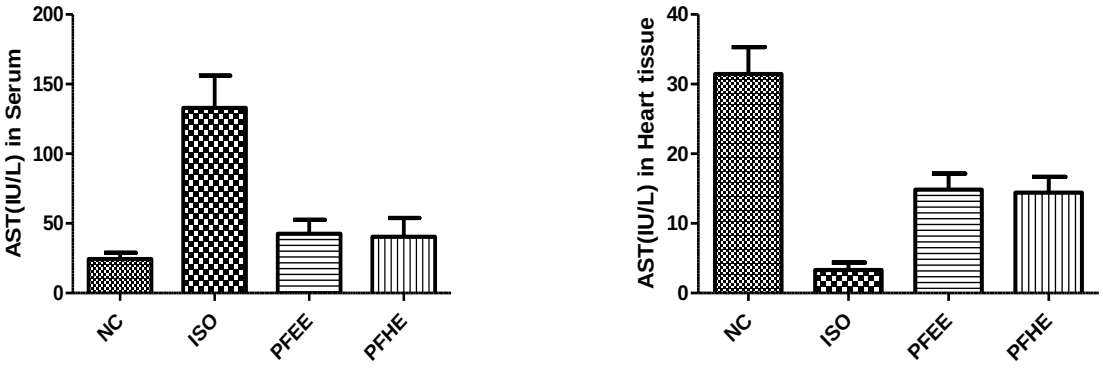


Figure 10: Effect of *Paederia foetida* leaf extracts on Alanine Transaminase levels (ALT)

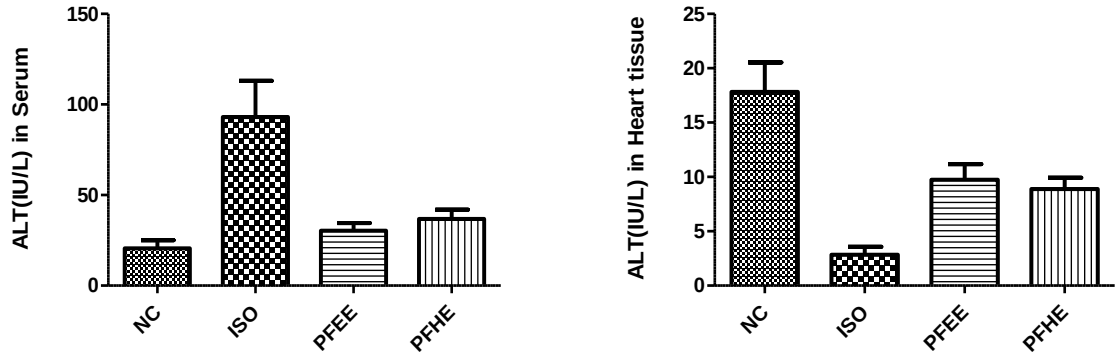
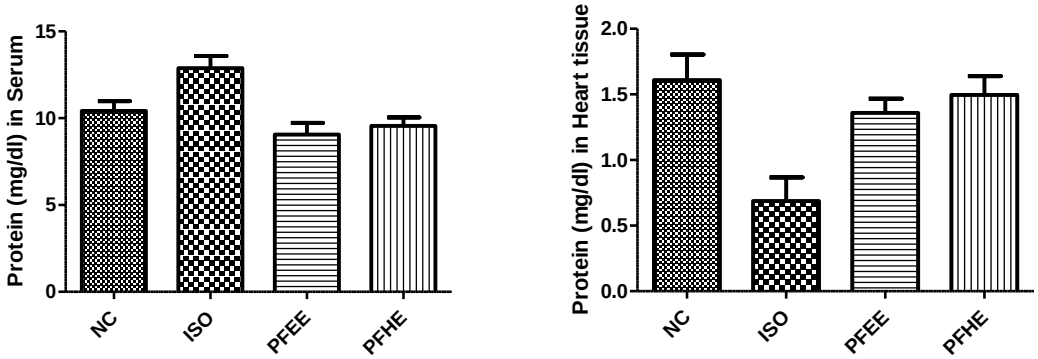


Figure 11: Effect of *Paederia foetida* leaf extracts on Total Protein levels.



DISCUSSION

Cardiac glycosides and catecholamine have been used as the main therapeutic drugs in the treatment of coronary heart disease [10]. Catecholamines are used in limited since they have insufficient differentiation between positive inotropic and chronotropic actions, potential arrythmogenic properties, tachyphylaxis due to receptor down-regulation and cause a severe oxidative stress in the myocardium through free radical formation [2]. Cardiac glycosides are the drugs having cardiac inotropic property. They increase contractions in myocardium and output in hypodynamic heart without a proportionate increase in O_2 consumption. Thus efficiency of failing heart is increased [11]. Both the ethanol (PFEE) and hydro alcoholic extracts (PFHE) of *Paederia foetida* elicited the cardiotonic activity by showing positive inotropic (Force of Contraction) and negative chronotropic (Heart Rate) actions as shown in Fig.1, Fig.2 and Fig.3. This effect was significantly antagonized by the calcium channel blocker, nifedipine but not significantly blocked by the β-adrenoreceptor antagonist, propranolol as shown in Fig.3, Fig.4 and Fig.5. The effect can be adequately found from Tables 2, 3, 4. Thus, *Paederia foetida* is showing the positive result of cardio tonic activity like that of standard drug digoxin since it possesses cardiac glycosides and iridoids glycosides. Further it has be evaluated by reducing levels of Na^+K^+ATPase, Mg^{2+}ATPase and significant increase in Ca^{2+}ATPase in biochemical studies performed in both the extracts which is shown in Table 5. This inhibition of Na^+K^+ATPase is similar to the action of cardiac glycosides like digoxin [12]. Cardiac glycosides are specific and unique inhibitors of Na^+K^+ATPase at normal concentrations (10^{-8} to 10^{-9}M) [13]. Na^+K^+ATPase inhibition by cardiac glycosides leads ultimately increased intracellular Ca^{+2} concentrations through Na^+/Ca^{+2} exchange and an associated increase in slow inward Ca^{+2} current as well as transient Ca^{+2} current [14]. Ca^{+2} induced Ca^{+2} releases is a general mechanism that most cells use to amplify Ca^{+2} signals [15]. In heart cells, this mechanism is operated between Voltage-gated L-type calcium channels (Lcc) in the plasma membrane and calcium release channel in the sarcoplasmic reticulum [16]. Nifedipine is an Lcc antagonist.

Since nifedipine blocks the cardiotonic action of ethanolic extract significantly, the extract might have produced its action by opening the voltage sensitive slow Ca^{2+} channel. In connection with the cardiotonic effects observed their exists relationship between the inhibitory levels of the activities of Mg^{2+} ATPase and $Na^{+}K^{+}$ ATPase [17]. The significant rise in the level of activity of Ca^{2+} ATPase might be due to the rise of cytosolic Ca^{2+}.[1] Hence the significant increase in Ca^{2+}ATPase and decrease in $Na^{+}K^{+}$ATPase, Mg^{2+}ATPase when compared to normal control may be due to similar actions like that of cardiac glycosides.

Creatine phosphokinase-MB (CPK-MB) test is a cardiac marker used to assist diagnosis the cardiac injury. Damage to the myocardium, as will occur in acute myocardial infarction (AMI), will result in an increased circulating level of the CK-MB isoform. The enzymes LDH is concentrated in heart, kidney, liver muscle and other body tissues. LDH levels increases in cases of myocardial infarction. The concentration of Lactate Dehydrogenase in tissues is several hundred folds higher than in serum or plasma and even a small amount of tissue damage can lead to an elevation in LDH activity. This makes LDH useful in the diagnosis and monitoring of disease states where tissue turnover is accelerated such as the liver, cardiac muscle, skeletal muscle, kidneys and erythrocytes. Level of AST (SGOT) rises markedly in conditions of extensive damage to muscle especially cardiac muscles. Estimation of this enzyme is widely sought for, to confirm diagnosis of myocardial infarction.

In pathological conditions, the enzymes such as CK, LPH, AST and ALT leak from the necrotic heart cells to the serum and these are important measures of cardiac injury. These enzymes are not specific for myocardial injury individually; however, evaluation of these enzymes together may be an indicator of myocardial injury.

The cardio protective role of *Paederia foetida* on isoproterenol induced cardio toxicity is shown in Tables: 6 and Fig. 7-11. From the above results the plant extract treated groups has shown significant decrease of cardiac marker enzymes such as AST, ALT, CK-MB, LDH and total protein in serum and significant increase in heart tissue levels. Therefore, these results confirm that both the extracts of *Paederia foetida* do not alter the physiological conditions of the heart and shows cardiotonic activity.

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