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EVALUTION OF CARDIOTONIC ACTIVITY OF *PELTOPHORUM PTEROCARPUM*

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ABSTRACT:
Extracts of *Peltophorum pterocarpum* we attempted to evaluate the cardiotonic activity. Petroleum ether extract produced significant positive inotropic and positive chronotropic actions similar to that of adrenaline on frog heart and it is indicated by an increase in the force of contraction and the heart rate. Ethanol extract produced significant positive inotropic and a slight negative chronotropic effect similar to that of digoxin on frog heart and it is indicated by an increase in the force of contraction and a decrease in the heart rate. This cardiatonic effect of the ethanol extract was not antagonized by propronolol, whereas nifedipine treatment significantly reduced the cardiotonic effect as with digoxin. There was a significant decrease in membranous Na^+K^+ ATPase and Mg^{2+} ATPase ($P<0.05$) and an increase in Ca^{2+} ATP ase, when compared with that of control on seven days treatment with both petroleum ether extract and ethanolic extract in rats and this further confirmed its cardiotonic effect. These activities may be due to the presence of mixture of steroidal glycosides (campesterol-3-0-beta-D-glucopyranoside, stigmasterol-3-0-beta-D-glucopyranoside and β-sistosterol-3-0-beta-glucopyranoside)

KEYWORDS: *Peltophorum pterocarpum,* cardiotonic activity

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. Cardiac glycosides and catecholamines have been used as main therapeutic agents in the treatment of congestive cardiac failure. 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 cardioactive drugs, 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. It necessitates research for new drug and with this aim we have chose *Peltophorum pterocarpum* and evaluated its cardiotonic activity (Mohire NC *et al*., 2007).

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Peltophorum pterocarpum is a handsome tree found in coastal forests of the Andaman Islands and grown in many parts of India for its ornamental value. A mixture of steroidal glycosides (campesterol-3-0-beta-D-glucopyranoside, stigmasterol-3-0-beta-D-glucopyranoside and β-sistosterol-3-0-beta-glucopyranoside) was isolated from the yellow fragrant flowers of *Peltophorum pterocarpum*. Bergenin, an isocoumarin was also found to be present in the flowers.

The phytochemical investigation on *Peltophorum pterocarpum* leaves resulted in the isolation of eight flavonoids among which a unique flavone was able to inhibit acetylcholinesterase.[21]Based on the reports of flavonoids having cardiotonic activity (Bhemachari J *et al.,* 2005) and the presence of flavonoids in *Peltophorum pterocarpum* we attempted to evaluate the cardiotonic activity of flowers of *Peltophorum pterocarpum* extracts.

MATERIALS AND METHODS

II. Flower collection and authentication

The flowers of *Peltophorum pterocarpum* were collected from the agricultural college Madurai in July 2008. The flowers were authenticated by Dr. D. Stephen, Lecturer, American College, and Madurai. The flowers were dried under the shade for a few days and were powdered.

II. Extraction of flowers of *Peltophorum pterocarpum*

The flower powder was packed in the soxhlet apparatus. Then it was extracted with petroleum ether at about 50^0C by hot percolation method for about 12 hours. After 12 hours, the extract was filtered and the filtrate was concentrated to a dry mass by heating on a water bath for a few hours. The extract was kept in sealed containers and then stored in a desicator for further study.

The marc left after the petroleum ether extract was taken and subsequently extracted with 95% ethanol for about 20 hours at about 70^0C in soxhlet apparatus. Then it was filtered and the filtrate was concentrated to a dry mass by heating on a water bath for a few hours. The extract was kept in sealed containers and then stored in a desiccator for further study.

III. Phytochemical evaluation (Khandelwal K. *et al.,* 2000).

1. Tests for carbohydrates:

Molish's test: To 2 – 3 ml extract few drops of molish's reagent (alpha naphthol solution in alcohol) was added. The test tube was shaken well and conc. sulphuric acid was added along the sides of the test tube. Formation of violet ring at the junction of two liquids was observed. This inferred the presence of carbohydrates.

2. Test for reducing sugars

Fehling's test: In a test tube 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added. These mixed solutions were boiled for a minute. Then equal amount (2 ml) of test solution was added. Brick red precipitate was observed which confirmed the presence of carbohydrates.

3. Tests for proteins

A) Xanthoprotein test: 3ml of test solution was taken in a test tube. To this 1ml of conc. Sulphuric acid was added along the sides of the test tube. Yellow precipitate has to be observed but was not formed. This inferred the absence of proteins.

B) Millon's test: 1ml of test solution was taken in a fresh test tube followed by the addition of 3 ml of million's reagent. The solution was boiled. No brick red colour was observed. This confirmed the absence of protein.

4. Test for Amino acid

Ninhydrin test: About 1 ml of test solution was taken in a test tube. To this solution 3 drops of Ninhydrin reagent was added and boiled. Purple (or) bluish colour has to be seen which not appeared. This inferred the absence of the amino acids.

5. Tests for sterols

A) Salkowski reaction: 2ml of extract was taken in a test tube. To this 2 ml of chloroform was added. Then 2 ml of conc. sulphuric acid was added along the sides of the test tube slowly and shaken well. Greenish yellow fluorescence appeared. This confirmed as the presence of sterols.

B) Liebermann's reaction: About 1 ml of extract was taken in a fresh clean test tube. To this 1 ml of acetic acid was added. This solution was heated and cooled. Then few drops of conc. Sulphuric acid are added along the sides of the test tube. Blue colour was observed. This confirmed the presence of sterols.

C) Libermann – Burchard reaction: In a test tube, 2 ml of test solution was taken followed by the addition of choloroform. To this 2 ml of acetic anhydride was added and heated. Solution was allowed to cool for few seconds then conc. sulphuric acid was added slowly along the sides of the test tube. Blue colour appeared which confirmed the presence of sterols.

6. Tests for Alkaloids

Little quantity of extract was taken in a test tube. To this, 2 ml dil. HCl was added. The solution was shaken well and filtered. This filtrate was used to perform the following tests:

A) Dragendorff's reaction: 2 to 3 ml of filtrate was taken in a fresh test tube. To this few drops of Dragendroff's reagent was added. Orange brown precipitate was not observed. This inferred the absence of alkaloids.

B) Mayer's test: 2 to 3 ml of filtrate was taken in a test tube followed by the addition of mayer's reagent. A white precipitate not formed which confirmed the absence of alkaloids.

7. Tests for Tannins:

A) Ferric chloride solution test: Little quantity of extract was taken in a test tube. To this, 2 ml ethanol was added and mixed well followed by the addition of 1ml of 5 % ferric chloride reagent. Deep blue colour was observed which inferred the presence of tannins.

B) Lead acetate test: 2 m1 of extract was taken in a test tube followed by the addition of alcohol and shaken well. To this 2 ml lead acetate was added. White precipitate formed which inferred the presence of tannins.

C) Bromine water test: 2 ml of extract was taken in a test tube followed by the addition of bromine water. Decolouration of solution was observed which inferred the presence of tannins.

8. Tests for Glycosides

A) Keller – Killiani test: 2 ml of extract was taken in a test tube. To this, 1m1 glacial acetic acid and 1 ml 5 % ferric chloride solution were added followed by the addition of 2 ml conc. sulphuric acid along the sides of the test tube. Reddish brown colour appeared at the junction of the two liquid layers. Appearance of this colour confirmed the presence of glycosides.

B) Baljet's test: 2 ml of test solution was taken in a test tube followed by the addition of picric acid. Appearance of orange colour confirmed the presence of glycosides.

C) Legal test: The extract is dissolved in pyridine; sodium nitro prusside solution is added to it and made alkaline. Appearance of red colour confirmed the presence of glycosides.

9. Tests for Flavonoids

A) Shinoda test: Little quantity of extract was taken in a test tube. To this, 5 ml 95 % ethanol was added followed by the addition of 2 ml conc. HCl along the sides of the test tube slowly. Then 0.5 g magnesium turnings were added. Appearance of pink colour confirmed the presence of flavonoids.

B) Lead acetate test: Small quantity of residue was taken in a tube to which lead acetate solution was added.Yellow colour precipitate formed which inferred the presence of flavonoids.

EVALUATION OF CARDIOTONIC ACTIVITY

Animals

Frogs of Rana hexadectyla species maintained in the animal house and male Wistar albino rats (150 – 200 g) housed in cages at 27 $\pm$ 2^{o}C on a 12 hour light/dark cycle were used for the studies. The animals were fed with food and water *ad libitum*. The animals were maintained as per the norms of CPCSEA approved IAEC guidelines and the experiments were cleared by IAEC.

Isolated Frog heart perfusion technique

Frogs were pithed and the hearts were exposed. The inferior venacava was traced and cannulated for perfusing the heart with the frog ringer solution (The composition of the frog ringer solution in millimoles: NaCl-110; KCl-1.9; $CaCl_2$,-1.1; $NaHCO_3$,-2.4; NaH_2 PO_4-0.06; Glucose-11.1). The basal cardiac contraction was recorded on a smoked kymographic drum after the administration of frog ringer's solution and Tween 80 (1%). The administration of Tween 80 was done to see that it did not contribute to the effects of extracts. The drugs and extracts were administered through the cannula. The average basal heart rate and the contraction amplitude were 54 beats/min and 8 mm respectively. The effects obtained with the drugs and extracts were transposed to the respective percentage of the basal values. Graded dose-response was recorded for each extract (0.25, 0.5 and 1 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, a β - adrenergic blocker at 3 x 10^{-5} M concentration in frog Ringer solution for 60 seconds followed by the administration of extracts and the recording were noted. Nifedipine, a calcium-channel blacker at 2.88X10^{-5} M concentration in frog Ringer solution was administered for 60 seconds followed by extracts and the recordings were noted.

Biochemical studies in Wistar albino rats

Wistar albino rats were divided into 3 groups of 6 animals each.

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

Group II: Was treated with petroleum ether extract 50 mg/kg (approximately 1/10 of the LD_{50}) body weight i.p. for 7days.

Group III: Was treated with ethanolic extract 50 mg/kg (approximately 1/10 of the LD_{50}) 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 tissue was washed in ice – cold saline and 100 mg of tissue was weighed homogenized in chilled 0.1 M Tris – HCl buffer (pH7) and the homogenate was used for the assay of Na^+ K^+ ATP ase, Ca^{2+} ATP ase and Mg^{2+} ATP ase. Serum and homogenized samples were assayed for the clinical marker enzymes like CK, LDH, AST and ALT.

Statistical analysis:

The data are expressed as mean $\pm$ SEM. Statistical comparison were performed by Tukey Kramer multiple comparison test in (Graphpad-Instat software version) $P < 0.05$ was considered as significant, $P<0.01$ was considered very significant and $P<0.001$ was considered extremely significant.

RESULTS

The results of the phytochemical evaluation are given in Table-1. Petroleum ether extract showed positive reaction for sterols and flavonoids and ethanolic extract showed positive reaction for carbohydrates, reducing sugars, sterols, tannins, glycosides and flavonoids. Graded dose response study showed that 0.5 mg (0.2 ml of stock solution) was the effective dose. So heart rate and force of contraction recorded with 0.2 ml are given in Table 2, 3.

Petroleum ether extract produced significant positive inotropic and positive chronotropic actions similar to that of adrenaline on frog heart and it is indicated by an increase in the force of contraction and the heart rate. (Tables-2,3). The similarity of mechanism of action of petroleum ether extract with that of adrenaline on isolated heart is further confirmed by the blocking of inotropic and chronotropic activity by propranolol as on adrenaline which was shown by the same force of contraction and heart rate as that of adrenaline (Tables-2, 3).

Ethanol extract produced significant positive inotropic and a slight negative chronotropic effect similar to that of digoxin on frog heart and it is indicated by an increase in the force of contraction and a decrease in the heart rate (Tables-2,3). This cardiatonic effect of the

ethanol extract was not antagonized by propronolol, whereas nifedipine treatment significantly reduced the cardiotonic effect as with digoxin (Tables-2, 3). There was a significant decrease in membranous $Na^{+}K^{+}$ ATPase and Mg^{2+} ATPase ($P<0.05$) and an increase in Ca^{2+} ATP ase, when compared with that of control on seven days treatment with both petroleum ether extract and ethanolic extract in rats and this further confirmed its cardiotonic effect (Table-4). Both the extracts do not produce any significant changes in the levels of CK, LDH, AST and ALT in heart and in serum samples when compared with that of control (Table-5).

Table – 1: Evaluation of Phytochemicals

S.No.	Plant constituent	Test	Petroleum ether extract	Ethanolic extract
1	Carbohydrates	Molish's reagent	-	+
2	Reducing sugar	Fehling's solution	-	+
3	Proteins	Xanthoprotein test	-	-
		Millon's test	-	-
4	Amino acids	Ninhydrin test	-	-
5	Sterols	Salkowski reaction	+	+
		Libermann's reaction	+	+
		Libermann – Burchard reaction	+	+
6	Alkaloids	Dragendroff's reagent	-	-
		Mayer's reagent	-	-
7	Tannis	Ferric chloride solution test	-	+
		Lead acetate test	-	+
		Bromine water	-	+
8	Glycosides	Keller – Killiani test	-	+
		Baljet's test	-	+
		Legal test	-	+
9	Flavnoids	Shinoda test	+	+
		Lead acetate test	+	+

Table 2: Effect of *Peltophorum pterocarpum* extracts on force of contraction on isolated frog heart perfusion technique

Treatment	Frog Ringer		Frog Ringer + propranolol ($3x10^{5}$M)		Frog Ringer + nifedipine ($3x10^{5}$M)	
	Force of contraction (mm)	FOC (%)	Force of contraction (mm)	FOC (%)	Force of contraction (mm)	FOC (%)
Digoxin	14.5 ± 0.182^{3}	181.25 ± 2.286	-	-	8.83 ± 0.330	110.41 ± 4.167
Adrenaline	8.75 ± 0.214^{1}	110.41 ± 3.090	4.75 ± 0.214	59.375 ± 2.676	-	-
Petroleum Ether Extract	$9.33 \pm 0.247^{C,**,3}$	116.66 ± 3.090	$2.58 \pm 0.153^{C,***,3}$	32.29 ± 1.921	$10.0 \pm 0.288^{a,3}$	125.0 ± 3.652
Ethanolic Extract	$11.33 \pm 0.278^{C,***,3}$	141.6 ± 3.486	$6.75 \pm 0.214^{C,***,3}$	84.37 ± 2.676	$6.58 \pm 0.153^{C,3}$	82.29 ± 1.921

Basal values: Force of contraction = 8.0 ± 0.156 mm; Values are expressed as mean ± SEM; 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 *Peltophorum pterocarpum* extracts on heart rate on isolated frog heart perfusion technique

Treatment	Frog Ringer		Frog Ringer + propranolol ($3x10^{5}$M)		Frog Ringer + nifedipine ($3x10^{5}$M)	
	Heart rate (per min)	Heart rate (%)	Heart rate (per min)	Heart rate (%)	Heart rate (per min)	Heart rate (%)
Digoxin	41.16 ± 0.477^{3}	85.41 ± 0.882	--	--	31.33 ± 0.330	65.20 ± 0.616
Adrenaline	55.50 ± 0.885^{3}	115.62 ± 1.630	46.83 ± 0.477	97.50 ± 0.885	--	--
Petroleum Ether Extract	$54.66 \pm 0.881^{C,3}$	113.87 ± 1.633	$41.66 \pm 0.421^{3,***}$	86.66 ± 0.701	$49.33 \pm 0.614^{C,3}$	102.70 ± 1.139
Ethanolic Extract	$45.88 \pm 0.872^{b,***,3}$	95.58 ± 1.612	45.66 ± 0.760^{3}	95.00 ± 1.407	$25.5 \pm 0.763^{C,3}$	53.12 ± 1.415

Basal values: Heart rate = 48.0 ± 0.012/min; (remaining same as above)

Table 4: Effect of flowers of *Peltophorum pterocarpum* extracts on membrane bound enzymes in heart of rats

Marker enzymes	Control		Petroleum Ether Extract		Ethanolic Extract	
	Heart	Serum	Heart	Serum	Heart	Serum
Creatine Kinase (IU/L)	0.56 ± 0.013	214.08 ± 0.680	0.517 ± 0.017	212.44 ± 0.518	0.522 ± 0.035	212.04 ± 0.524
Lactate dehydrogenase (IU/L)	2.85 ± 0.036	103.16 ± 0.209	2.931 ± 0.019	102.7 ± 0.363	2.845 ± 0.028	102.25 ± 0.306
Alanine transaminase (IU/L)	0.143 ± 0.012	22.77 ± 0.405	0.12 ± 0.012	21.94 ± 0.012	0.13 ± 0.013	21.93 ± 0.495
Aspartate transaminase (IU/L)	0.187 ± 0.003	35.75 ± 0.745	0.176 ± 0.005	34.22 ± 0.407	0.18 ± 0.007	34.01 ± 0.608

n = 6 values are mean ± SEM.

Table 5: Effect of *Peltophorum pterocarpum* extracts on clinical marker enzymes in rats

Groups	Na^+K^+ATPase (μmol of phosphorous liberated /min/mg protein)	Ca^{2+} ATPase (μmol of phosphorous liberated /min/mg protein)	Mg^{2+} ATPase (μmol of phosphorous liberated /min/mg protein)
Control	0.473 ± 0.002	1.73 ± 0.0120	5.478 ± 0.102
Petroleum Ether Extract	0.432 ± 0.011**	1.813 ± 0.03*	4.641 ± 0.169**
Ethanolic Extract	0.446 ± 0.004***	1.925 ± 0.030***	4.63 ± 0.192**

n = 6. Values are expressed mean ± SEM. Enzyme units for heart creatine phosphokinase: μmoles of phosphorus liberated/min/mg protein, for serum creatine phosphokinase: μ moles x 10^{-3} of phosphorus liberated/min/mg protein, Lactate dehydrogenase: μmoles x 10^{-3} of pyruvate liberated/min/mg protein. Aminotransferases: μmoles x 10^{-2} of pyruvate liberated/min/mg protein.

DISCUSSION

Cardiac glycosides and catecholamines have been used as the main therapeutic drugs in the treatment of congestive cardiac failure. However, the dangers of cardiac glycoside intoxication are well documented and doubts have been expressed about their long term effectiveness. The use of catecholamines is limited by their sufficient differentiation between positive inotropic (Muralidharan A et al., 2004) and chronotropic actions, their potential arrhythmogenic properties and tachyphylaxis due to receptor down -regulation (Muralidharan P et al., 2008).

Ethanolic extract elicited cardiotnic effect, which was characterized by positive inotropic and negative chronotropic actions. This effect was not significantly blocked by propronolol, whereas nifedipine the calcium channel blocker antagonized the effect significantly (Figs.3(c), 4(a), 4(c) and Tables-2, 3). The cardiac enzyme profile indicates that ethanolic extract exhibited cardiotonic activity which manifested as a result of general decrease in the activity of Na^+K^+ ATPase and Mg^{2+} ATPase and an increase in Ca^{2+} ATPase (Table-4).

This inhibition of Na^+K^+ ATPase is similar to the action of cardiac glycosides. Cardiacglycosides are specific and unique inhibitors of Na^+K^+ ATPase at normal concentrations ($10^{-8}$to 10^{-9}M). Na^+K^+ ATPase inhibition by cardiacglycosides leads ultimately to increase intra cellular Ca^{2+} concentrations through Na^+/Ca^{2+} exchange and an associated increase in slow inward Ca^{2+} current as well as in transient Ca^{2+} current. Ca^{2+} induced Ca^{2+} release is a general mechanism that most cells use to amplify Ca^{2+} signals.

In heart cells, this mechanism is operated between Voltage-gated L-type calcium channels (Lccs) in the plasma membrane and calcium release channel, commonly known as ryanodine receptors in the sarcoplasmic reticulum. 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 one could see a relationship that exists between the inhibitory levels of the activities of Mg^{2+} ATPase and Na^+K^+ ATPase. The significant rise in the level of activity of Ca^{2+} ATPase might be due to the rise of cytosolic Ca^{2+}.

Petroleum ether extract produced significant positive inotropic and positive chronotropic actions similar to that of adrenaline. Proprnolol antagonized the effect of petroleum ether extract but nifedipine could not antagonize the effect because of involvement of β-adrenoceptors (Figs. 3(c), 4(a) and Table-2,3). There was a significant decrease in membranous Na^+K^+ATPase and Mg^{2+} ATPase and an increase in Ca^{2+}ATPase when compared to control due to involvement of flavonoids (Table-4). Numerous reports of a high standard have appeared on the inhibition by flavonoids of a perplexing number and variety of enzymes e.g. hydrolases, hyaluronidase, alkaline phosphatase, aryl sulphatase, H^+ -ATPase of lysosomal and granular membranes, Na^+/ K^+-ATPase of the phospho

diesterase, lipases, lyases, hydroxylases and kiriases.(Raj Narayana K et al., 2001)

Apigenin inhibits phosphodiesterase (PDE) and the effect was greater on cAMP-PDE than cGMP-PDE levels by 40 percent and cGMP level remained unchanged. Thus the cardiotonic action is due to the inhibition of cardiac cAMP-PDE. Querectin showed the most potent intrinsic activity and produced thestrongest inotropic responses among the different flavonoids. CK is found in high concentration in skcletal muscle, myocardium and brain but not found in liver and kidney, small amounts are found in lungs not found in RB cells and its levels is not affected by hemolysis. It appears to be a sensitive measure of myocardial infarction. LDH has gained much clinical interest recently and measurement of its activity in blood is considered useful in the diagnosis certain cardiovascular disease conditions. AST level increase 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, which 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.

Levels of CK, LDH, AST and ALT on treatment with ethanol and petroleum ether extracts were not changed when compared to that of the control both in the serum and also in the heart (Table-5). Therefore, these results confirm that both the extracts do not alter the physiological conditions of the heart.

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