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Phytochemical Investigation and Cardiotonic Activity of *Rosa Centifolia* (Linn.) Flowers

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Abstract

Rosa centifolia Linn belong to the family Rosaceae is an erect shrub 1 to 2 meter in height. Flowers of the plant are large, showy and colorful. This plant is not explored still now, but traditionally it is used in treatment of diabetes, conjunctivitis. The present investigation was carried out to evaluate the cardiotonic activities of *Rosa centifolia* flowers. Dried flower extracts of *Rosa centifolia* was extracted with petroleum ether, chloroform, ethanol, methanol and water using soxhlet apparatus. Present study was carried out to determine the cardiotonic activity by using infusion of heartwood with different dilutions & compared with cardiotonic activity of digoxin-the lifesaving cardiotonic. The activity was tested by using isolated frog heart assembly. The preliminary phytochemical investigation of dried flowers showed presence of terpenoids, flavnoids, glycosides, steroids and phenolic compounds. From the above extracts, ethanolic extract showed significant cardiotonic activity. So, it was further subjected to thin-layer and column chromatographic studies. Later the residue was analysed by using U.V. and I.R. spectroscopy for characterization. The results of phytochemical investigation had led to the conclusion that the compound may be terpenoid derivative. The present preliminary studies confirm the better cardiotonic activity of *Rosa centifolia* than digoxin. Further studies can confirm the reduced toxicity & this will be the advantage of *Rosa centifolia* over digitalis. Thus, in future it will be interesting to isolate the active chemical constituents which were responsible for the cardiotonic activity.
Keywords: Cardiotonic activity, Digoxin, *Rosa centifolia*, Isolated frog heart.

Contents

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1. Introduction

There is no gainsaying that plants remain the greatest source of bioactive compounds which can be developed and used as drugs. A lot of them are still being used raw in herbal medicine for disease prevention and treatment. One of such plants is *Peperomia pellucida* which prompted research on its chemical constituents as well as its antibacterial

efficacy. *Peperomia pellucida* L. HBK belongs to the family *Piperaceae* comprising about 5 genra and 1400 species. The plant is found in Nigeria, all over Asia and in many South American countries growing in clumps, thriving in loose, humid soils under the shade of trees and a tropical to subtropical climate. *P. pellucida* is an annual, shallow rooted herbaceous plant that grows to a height of 15 to 45 cm. It is characterized by succulent stems, shiny light-green, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. The plant flowers year-round [1,2,3,4,5].

The leaves of *P. pellucida* are used topically for treatment of athlete foot and skin infections caused by bacteria and fungi in Igbo tribe of South Eastern Nigeria. The plant has been reported to be used ethno-medicinally for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders and rheumatic joint pain [6] and to treat breast cancer, impotence, measles, mental disorders and smallpox [7]. The roots are used to treat fevers and the aerial parts are used as dressing for wounds [8] while the whole plant has been reported to be used in stopping haemorrhage when crushed, mixed with water, heated and then orally administered [8]. The chloroform extracts of *P. pellucida* have been reported to exhibit antifungal activity against *Trichophyton mentagrophytes* in vitro [9] while the whole plant showed anti-malarial activity [8, 10]. In North Eastern Brazil, the plant has been used as a diuretic and to treat proteinuria; and in the Amazon region, it has been used as a cough suppressant, diuretic and emollient and to treat cardiac arrhythmia [2,3,4]. It has also been reported that the extracts from *P. pellucida* exhibited cytotoxicity against the cancer cell lines HL-60, MCF-7 and Hela [11]. The antiinflamatory, chemotherapeutic and analgesic properties of the crude extracts of *P. pellucida* have been reported [2,7]. The analgesic properties of the plant seemed to be related to its effect on prostaglandin synthesis [7].

P. pellucida has been used as a food item as well as a medicinal herb. Although mostly grown for its ornamental foliage, the entire plant is edible, both cooked and raw [7]. As aforementioned, the plant is abundantly used in herbal medicine in South Eastern Nigeria for the treatment of athlete foot, eczema, ringworm, boils and other skin infections caused by fungi and bacteria. This research is predicated on exploring the bioactive compounds present in the leaves of this plant and their bio-effect. Herein is therefore reported the chemical investigation and antibacterial activity of *Peperomia pellucida* leaves.

2. Materials and methods

Plant and extracts:

Rosa centifolia were collected from local areas of Tirupathi, Andhra Pradesh during the month of June the plant material were identified and authenticated by Prof. Madhav chetty, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh. The flower extract was macerated 50 gm of chopped; dried flower was macerated with 500ml of solvents. The solvent was then removed under reduced pressure until the extract volumes reached 20ml. The concentration in the final extract was 10%w/v.

Extraction procedure: The flowers of *Rosa centifolia* Linn were dried in shade. Then the shade dried flowers were powdered to get coarse powder. About 50 gm of dry powder was extracted first with various solvents like petroleum ether, chloroform, ethanol, methanol and water by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 18 hrs hors. All the ether extracts were filtered and concentrated to a dry mass by using vaccum distillation. The extract was kept in sealed containers and then stored in a desiccator for further study.

Phytochemical evaluation[15-17]

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: Wistar albino rats weighing between (150-200 g) were housed in isolated cages under standardized condition. The animals were provided with regular rat chow (Lipton India Ltd., Mumbai) and distilled water *ad libitum.* The animal care and experimental protocols were in accordance with CPCSEA / IAEC. Number of groups required for the study was as follows, each group contained six animals. Each group received the dose as follows.

Group I - Control (normal saline)
Group II - Reference drug (Digoxin) mg/kg body weight (i.p)
Group III - Petroleum ether extract (p.o.) 400mg/kg
Group IV - Chloroform extract (p.o) 400mg/kg
Group V - Methanol extract 400mg/kg
Group VI - Total alcoholic extract 400mg/kg
Group VII - Total aqueous extract 400mg/kg

Langendroff method by using Biopac data aquisation mp35: [18]

Before beginning the experiment**,** set the Langendroff's apparatus and ensure that the perfusion system is in good condition. Six wistar male rats weighing 250-280 g were injected with heparin (300 IU, i.p.) and after 20 minutes, anesthetized the rat with pentobarbitone sodium (45 mg/kg, given i.p.).The rats were sacrificed and Open thorax immediately, expose and remove the heart along with aorta as rapidly as possible and plunge the tissue into ice-cold Kreb's Hanselet solution. The composition of the Kreb's Hanselet solution in (mM/L): Nacl 118, KCl 4.7, $Mgso_4$1.2, KH_2PO_4 1.2, $CaCl_2$ 1.8, $NaHCO_3$ 25 and $C_6H_{12}0_6$11. Cannulate the heart through aorta using artery

cannula and mount the heart on Langendroff's apparatus by securing firmly in place with button thread. Perfuse the heart at a constant pressure, maintained at 37°C and pH 7.4 and saturated with a gas mixture of 95% 0_2 5% CO_2. Maintain a perfusion rate of 5 ml/minute. Saturate the perfusion solution with oxygen bubbles at a constant and slow pace. Attach a heart clip complete with a light thread to the tip of the ventricle. Locate the thread around a pulley about 4 mm vertically below the heart. Attach the thread to the transducer for recording the contractions of the heart beats. Record the heart rate and force of contractions on by using Biopac data aquisation. Inject (0.1 ml) the test drugs and record the heart rate and the heart contractions for a minute. The rat heart was washed with the Kreb's Hanselet solution after administration of every dose of extracts and drugs till it was brought back to the normal state.

Bio Chemical Studies:

Wistar albino rats were divided into 7 groups of 6 animals each[19]. Group I received 5% gum acacia suspension which served as control; Group II is treated with digoxin. Group III to Group VII, each group was treated with different extracts at a dose of 400 mg/kg p.o. for 7 days .On the 8th day the animals were sacrificed and the serum was separated from the blood. The heart washed with ice cold saline and 30% tissue homogenate was prepared in chilled 0.1 M Tris –HCl buffer in pattern Elejhem Teflon homogenizer. The serum was assayed for clinical marker enzymes like CPK[20], LDH[21] and transaminase AST[22] and ALT[23]. Heart homogenate samples are also assayed for Na^+/K^+ ATPase, Ca^{++} ATPase and Mg^{++} ATPase.

Tissue preparation

The heart was excised, rinsed in the ice cold isotonic saline, blotted with filter paper, weighed, Homogenized in 0.1 M Tris – HCL (PH 7.4) buffer solution. The homogenate was centrifuged at 300 rotations for 5 minutes. The supernatant was used for the estimation of various biochemical parameters.

Histopathology studies[24]:

A portion of heart from each group was stored in 10% formalin, for processing and section. By following routine histological techniques, these samples were put onto paraffin and serial cross sections of 5µm, which were taken from tissue blocks stained with hematoxylin and eosin. The preparations were evaluated under a photomicroscope and were photographed.

Statistical analysis

The results were expressed as Mean± SEM. The data were analyzed by using one-way ANOVA followed by Dunnet's Test. $P<0.05$ was considered as statistical significance.

3. Results and Discussion

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 and 3.

Table 1. Evaluation of Phytochemicals

S.No	Name of the Test	*EXTRACT*				
		Pet. Ether	Chloroform	Methanol	70% alcohol	Aqueous
1	**Test for Carbohydrates**					
	Molisch's test					
	A)Test for reducing sugars					
	i) Fehling's test	—	-	+	_	+
	ii) Benidict's test	_	_	_	-	_
	B)Test for monosaccharides					
	i)Barfoed's test	_	_	_	_	+
	C)Test for Hexose Sugar					
	i)Selwinoff's test	-	-	-	-	-
	ii)Cobalt chloride test	_	_	_	_	_
	D)Test for Non-reducing Sugar polysaccharides(starch)					
	i) Iodine test	_	_	_	_	_
	ii)Tannic acid test for starch	_	_	_	_	+
2	**Test for proteins**					
	I)Biuret test(General test)	_	_	-	+	-
	ii)Millons test for Proteins	_	_	_	_	_
	iii)Xanthoprotein test	_	_	_	_	+
	iv)Test for proteins containing	_	_	_	_	_

	sulphur					
	v)Precipitation test	_	_	_	_	_
3	**Test for Amino acids**					
	i)Ninhydrin test	_	_	+	+	+
	ii)Test for Tyrosine	_	_	+	_	+
	iii)Tryptophan	_	_	_	_	_
	iv)Test for Cysteine	_	_	+	+	+
4	**Test for Steroids**					
	i)Salkowski reaction	-	-	+	+	-
	ii)Liebermann reaction	+	_	_	+	+
	iii)Liebermann-Burchard reaction	+	+	-	+	-
5	**Test for Triterpenoids**					
	i)Salkowski reaction	-	-	+	+	-
	ii)Liebermann-Burchard reaction	+	+	_	+	_
6	**Test for Glycosides**					
	A)Test for cardiac glycosides					
	i) Baljet test	_	_	_	_	_
	ii)Legal's test (Test for Cardenolides)	_	_	+	+	+
	iii)Test for deoxy sugars (Keller Killiani test)	+	-	_	_	-
	iv) Liebermann's test (Test for butenoloids)	_	_	_	_	_
	B)Test for Anthraquinolide Glycosides					
	i)Borntrager's test	_	_	_	_	_
	ii) Modified Borntrager's test	+	-	_	_	_
	C)Test for Saponin glycosides					
	i)Foam test	_	_	_	_	_
	ii)Haemolytic test	_	_	_	_	_
	D)Test for Coumarin Glycosides					
	i)Alkaline reagent test	_	_	_	_	_
	ii)NaOH soaked paper test	_	_	_	_	_
7	**Test for flavonoids**					
	i)Ferric chloride test	_	_	+	+	+
	ii)Shinoda test	_	_	+	+	+
	iii)Alkaline reagent test	_	_	_	_	_
8	**Test for Alkaloids**					
	i) Dragendorff's test	_	_	_	_	_
	ii)Mayer's test	_	_	_	_	_
	iii)Hager's test	_	_	_	_	_
	iv)Wagner's test	_	_	-	_	-
	v)Murexide test for Purine alkaloids	_	_	_	_	_
9	**Test for Tannins and Phenolic compounds**					
	i)5% $FeCl_3$ solution	_	_	+	+	+
	ii)Lead acetate solution	_	_	+	+	+
	iii)Gelatin solution	_		+	+	+
	iv)Bromine water	_	_	+	+	+
	v)Acetic acid solution	_	_	_	_	_
	vi)Dilute iodine solution	+	_	+	+	+
	vii)Dilute HNO_3	_	_	+	+	+
	viii)Dilute potassium permanganate solution	_	_	+	+	_
10	**Test for Fats and Oils**					
	Solubility test	+	_	+	+	_

Ethanolic extract of flowers produced significant positive inotropic and negative chronotropic actions, when compare to the normal. Ethanolic extraction increased force of contraction, as compared to other extracts i.e. methanol, aqueous, pet ether and chloroform. Methanol, aqueous, pet ether, chloroform reduced the chronotropic effects compared to normal. Ethanolic extraction showed potent cardiotonoic activity. Adrenaline increased force of contraction and heart rate. Digoxin showed positive inotropic and negative chronotropic (Graph 1). Here two doses are used i.e. 20mcg/ml 0.1ml, 0.2ml and 1 mg/ml 0.1ml. In 20µg/ml 0.1ml and 0.2ml, 0.2ml dose increased force of contraction as compared to 0.1ml. It is dose dependent (Graph 2).

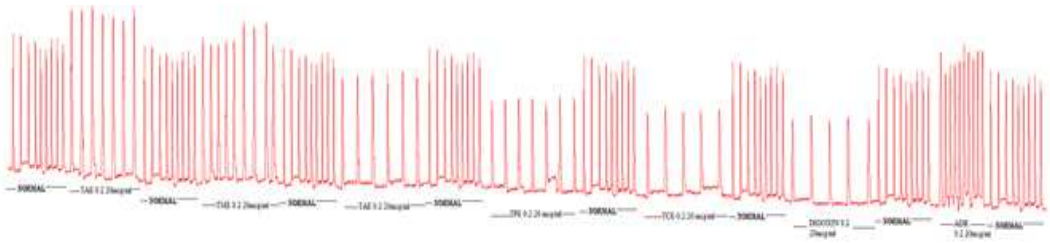


Fig.1 Effect of alcoholic extract (20mcg/ml) of *Rosa centifolia* Linn at 0.2ml

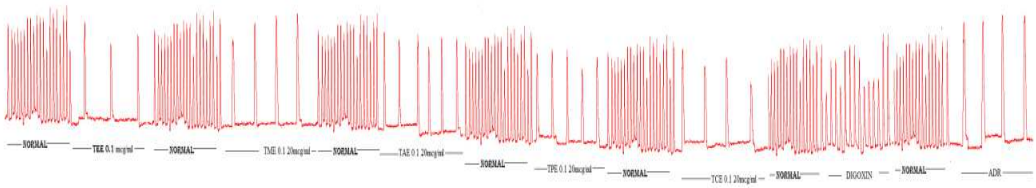


Fig.2 Effect of alcoholic extract (20mcg/ml) of *Rosa centifolia* Linn at 0.1ml

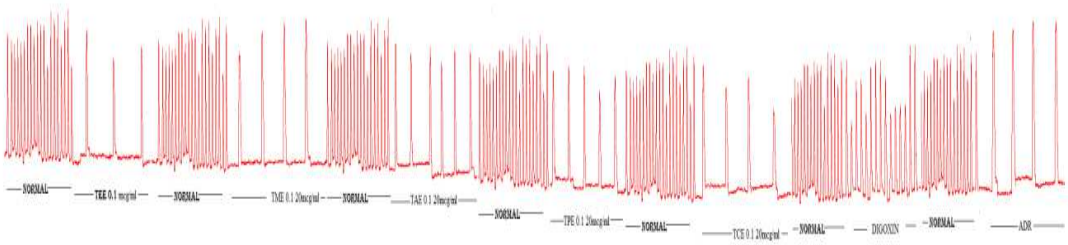


Fig.3 Effect of alcoholic extract 1mg/ml of *Rosa centifolia* Linn

All the extracts failed to produce significant changes in the levels of SGOT, SGPT, LDH, and CPK in heart and serum when compared to that of the control (Table.11). Therefore, it indicated that all the extracts did not alter the physiological conditions on the heart. CPK is found in high concentration in skeletal muscle, myocardium, and brain but not found in liver and kidney, small amounts are found in lungs. LDH is useful in the diagnosis of certain cardiovascular disease conditions. AST level increase markedly in conditions of extensive damage to muscle especially cardiac muscles. In pathological conditions, the enzymes such as CPK, LDH, SGOT, SGPT leak from the necrotic heart cells to the serum, which are important measures of cardiac injury. Levels of CPK, LDH, SGOT, SGPT of all the values did not change when compared to that of the control both in the serum. Therefore, it confirms that the extracts altering the physiological conditions of the heart when given at 400mg/kg body weight. The extracts are found to be safe.

A significant decrease in membrane Na^+/ K^+ ATPase, Mg^{2+} ATPase and increase in Ca^{2+}ATPase (Table 12) in digoxin, ethanol, methanol,aqueous. This inhibition of Na^+/ K^+ ATPase is similar to the action of cardiac glycosides. Na^+ /K^+ ATPase inhibion by cardiac glycosides leads ultimately to increase intracellular Ca^{2+} concentractions through Na^+/Ca^+ exchange and an associated increase was slow inward Ca^{2+} current as well as in transient Ca^{2+} current. The ethanol, methanol and aqueous extracts showed inotropic action whereas in pet.ether and chloroform groups no significant changes were observed. Hence extracts might have failed to show inotropic action. The ethanolic extract found to be more effective as compared to methanol and aqueous extracts. The cardiotonic effect of ethanolic extract of *Rosa centifolia* Linn. is comparable with digoxin (Fig 1-3).

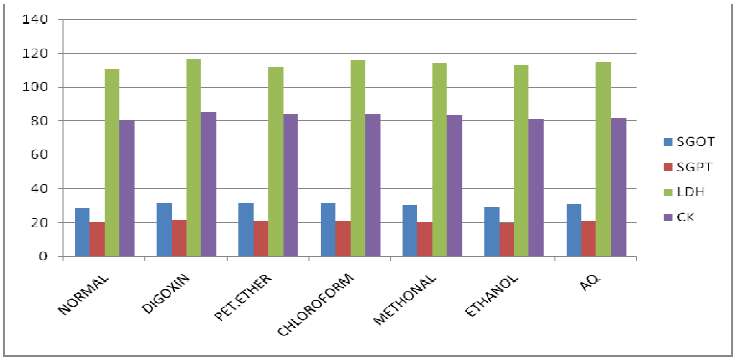
Table 2. Effects of the extracts from *Rosa centifolia* Linn. on the marker enzymes in rats 400mg/kg

GROUPS	SGOT (IU/L)	SGPT (IU/L)	LDH (IU/L)	CK (IU/L)
Normal	28.46±0.62	20.06±0.16	110.8±3.72	80.48±1.33
Digoxin	31.8±0.52 ^{ns}	21.28±0.06 ^{ns}	116.44±1.34 ^{ns}	85.35±088 ^{ns}
Pet. Ether	31.2±1.38 ^{ns}	21.11±0.38 ^{ns}	112±1.76 ^{ns}	84.22±1.84 ^{ns}
Chloroform	31.06±0.412 ^{ns}	21.02±0.02 ^{ns}	116.07±0.06 ^{ns}	84.31±0.34 ^{ns}
Methanol	30.62±0.68^{ns}	20.18±0.11 ^{ns}	114.2±1.84 ^{ns}	83.75±0.97 ^{ns}
Ethanol	29.20±0.06 ^{ns}	19.82±0.92 ^{ns}	112.0±1.83 ^{ns}	81.2±1.56 ^{ns}
Aqueous	30.8±1.63 ^{ns}	21.02±0.055 ^{ns}	114.8±0.13 ^{ns}	82.0±1.42 ^{ns}
ns: non-significant all the values were found to be suggest as compared to control				

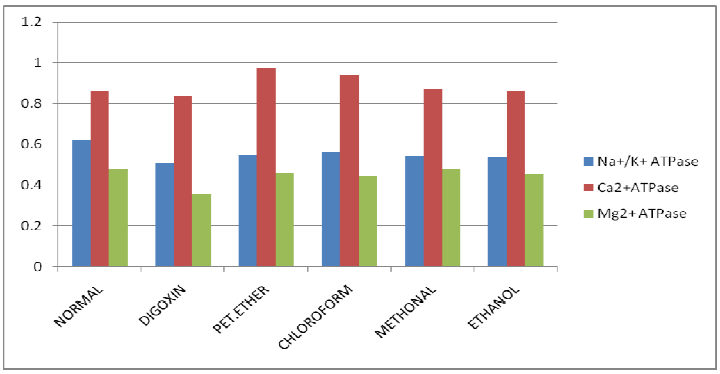
Table 3. Effects of the extracts of *Rosa centifolia* Linn. On heart tissue ATPase of wistar albino rats. 400mg/kg.

GROUPS	NA^+/K^+ ATPase (µmoles of phosphorus liberated/min/mg protein)	Ca^{2+} ATPase (µmoles of phosphorus liberated/min/mg protein)	Mg^{2+} ATPase (µmoles of phosphorus liberated/min/mg protein)
Normal (control)	0.622±0.01	0.866±0.013	0.478±0.015
Digoxin	0.0508±0.013**	0.835±0.012***	0.361±0.032***
Pet ether extract	0.549±0.014^{ns}	0.976±0.021^{ns}	0.456±0.027^{ns}
Chloroform extract	0.562±0.035^{ns}	0.939±0.045^{ns}	0.446±0.030^{ns}
Methanol extract	0.542±0.017*	0.872±0.013*	0.478±0.031*
Ethanol extract	0.538±0.035*	0.864±0.014**	0.453±0.030**
Aqueous extract	0.525±0.017**	0.870±0.025*	0.462±0.026*

D: $\uparrow\downarrow_{(Block)}$ NA^+/K^+ ATPase
Values are expressed as Mean±SEM, ns= non-significant
*P<0.05;**P<0.01;***P<0.001 as compared to control.



Graph 1. Effects of the extracts from *Rosa centifolia* Linn. on the serum marker enzymes in rats 400mg/kg



Graph 2. Effects of the extracts from *Rosa centifolia* Linn. on the heart tissue ATPase of wistar albino rats 400mg/kg

Histopathological studies of *Rosa centifolia* extracts

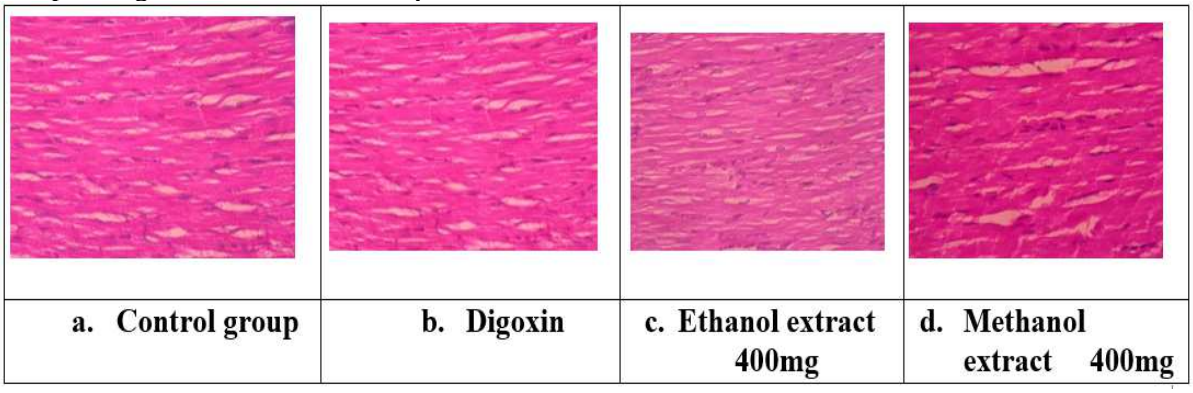


Fig.4. Histopathological results

Histopathological studies (Fig.4) of all extracts did not produce any significant changes in the heart when compared to that of the control, dogoxin . Mild changes in vascular congestion and there was no change in sporadic early necrosis of fibre, Eosinophilia of cytoplasm, Vascular congestion, Interstitial odema, Mononuclear inflammatory cells, Intravascular hemolysis, Myofibrils fragmentation, Focal haemorrhage, Cytoplasmic vacuolation. Therefore it is indicated that all the extracts did found to be safe, as they did not show any pathological changes in the histopathology.

4. Summary and Conclusion

The results of pharmacological activity led to the conclusion that the ethanol extract exhibited more significant activity than the petroleum-ether, chloroform and methanol, ethanol extract. For assessing cardiotonic activity isolated rat heart by using Langendroff method was used and also marker enzymes CPK, LDH, SGOT and SGPT were estimated in serum and also performing membrane bound enzyme studies such as NA^+/K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase were determined on heart tissue of albino rats. Finally histopathological observation was done on the basis of histopathological heart sections of photomicrographs of rat heart, stained with haemotoxylin-eosin. The results obtained reveal that the therapeutic efficacy of extract of *Rosa centifolia* Linn is dose dependent and similar to that of Digoxin. Limitation of using Digoxin may be overcome by using an alcoholic extract of *Rosa centifolia* Linn which has been reveals a cardiotonic activity. It may be a safe alternative to Digoxin in the treatment of congestive cardiac failure. The present result indicated that alcoholic extract of *Rosa centifolia* Linn showed similar therapeutic index like cardiac glycosides.

5. Acknowledgement

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