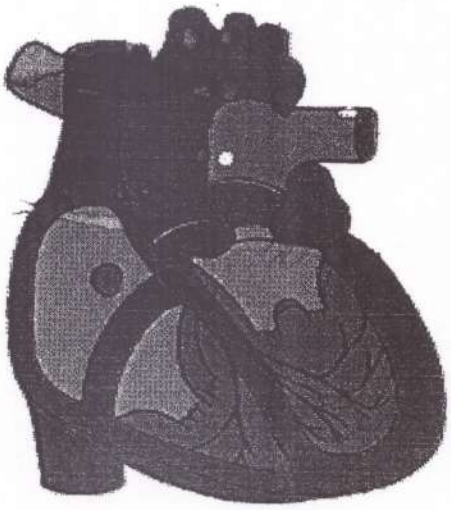
REPORT FOR

ANTI-ARRHYTHMIC ACTIVITY OF TEST FORMULATION BRIHATVATACHINTAMANI



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TATEMENT OF COMPLIANCE

Test Article: Brihatvatachintamani

Study Title: Anti-arrhythmic activity of test formulation Brihatvatachintamani

This is to certify the report entitled as the **"Anti-arrhythmic activity of test formulation Brihatvatachintamani"** contains the information, which is correct, authentic, and accurate to the best of our knowledge. The investigations were conducted according to the direction of the sponsor and compliance with the protocol submitted to the sponsor. The investigations on animals performed at registered animal house facility by 'Committee for the Purpose of Control and Supervision of the Experimental Animals' (87 / 1999/ CPCSEA.) at UICT, Matunga, Mumbai.

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The heart of animals as the foundation of their life, the sovereign of every thing within them; the sun of their microcosm that upon which all growth depends, from which all power proceeas.

-- William Harvey

The twentieth century witnessed the dramatic rise in cardiovascular diseases (CVD) as a leading cause of mortality. Initially appearing in industrialised countries , CVD s are now emerging in the rest of the world. CVDs are major contributors to mortalityand morbidity in india. Prevalence rates , according to community and school surveys, range from 1 to 22 per 1000 children. Conservative estimates suggest that in 2000 CVD caused 3.0 million deaths & the nation incurred aloss of 30.6 million disability adjusted life year and it has been projected that 3.8 million & 4.8 million death during 2010 & 2020 respectively due to CVD. CVD is likely to account for 33.5% of total deaths at all ages by 2020. Epidemological transitions with increasing life expectancy and demografic shift of population age profile with life style related increase in the level of cardiovascular risk factors, is accelerating the CVD's in India. Studies from various contries show that there is 1.5 to 8 folds excess risk of CVD deaths in persons of indian origin. Therefore the search for better and safer drugs for the treatment of cardiovascular disease has been continued to be an area of major research interest.

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Chapter

1

INTRODUCTION:

THE HEART

In adult human being, the heart is the size of man's closed fist. The pump beats at an average of about 70 times per minute without rest, day & night, in excess of 2.5 billion times during 70 average years of life. It is essential that the heart continuously function in this manner, because even after only several minutes of blood deprivation, irreversible changes occur that permanently impairs brain function.

CARDIAC RATE AND RHYTHM

The mammalian heart circulates blood through the lungs and systemic circulatory system . the heart is provided with special system for generating impulses at regular intervals to cause rhythmic contraction of the heart and for conducting these impulses rapidly throughout the heart. The normal cardiac rhythm is cotrolled by the rate of discharge of the sino-atrial node (S-A node) which is known as the pace maker of the heart . An impulse generated at the S-A node passes throughout the cardiac muscle to generate a contraction using a special conducting pathway which is as follows :

The impulse generated at the S-A node spreads throughout the atrial muscle mass and simultaneously travels rapidly through the internodal tracts to the A-V node. It takes about 50-60 msec. for the impulse to travel from the SA node to AV node. The impulse then travels through the AV node, causing a delay in its transmission which enables the atria to empty their content into the ventricles before the ventricular contraction starts. From the AV node, the impulse travels through the right and the left branches of the bundle of His and then it spreads

via Purkinje network throughout the ventricular mass & contraction of ventricals occur.

ELECTRO PHYSIOLOGY OF HEART

- ***Phase 0, The rapid depolarisation,*** occurs when the membrane potential reaches the critical firing threshold (about –60mV) at which point the inward current of sodium ions flowing through the voltage dependent sodium channels becomes large enough to produce regenerative (all-or-nothing) depolarisation. The activation of these sodium channels by membrane depolarization is transient , and if the membrane remains depolarised for more than a few milliseconds, they close again. They are therefore closed during the plateau of the action potential, and remain unavailable for the initiation of another potential until the membrane repolarises.
- ***Phase 1, the partial repolarization,*** varies markedly in prominence in different parts of the heart, and occurs as the Na^+ current is inactivated. There may also be transient voltage sensitive outward current.
- ***Phase 2, the plateau,*** results from an inward calcium current, the slow inward current. The calcium channels show a pattern of voltage-sensitive activation and inactivation. Activation of contractile machinaries due partly to the increase in the intracellular concentration of Ca^{+2} concentration that results directly from this influx and partly from the release Ca^{+2} from sarcoplasmic reticulum. The plateau is assisted by a special property of the cardiac muscle membrane, known as the inward going rectification, which means that the potassium conductance falls to a low level when membrane is depolarized. Because of this, there is little tendency of outward potassium current to restore the resting membrane potential during the plateau, so a relative small inward Ca^{+2} current suffices to maintain the plateau.

- ***Phase 3, repolarisation,*** occurs as the Ca^{+2} current inactvates. It happens abruptly because the inward going rectification causes the potassium permiability to increase as soon as the membrane begins to repolarise, and the membrane potential flips abruptly back to the resting level, close to the potassium equilibrium potential.
- ***Phase 4, the pace maker potential,*** is a gradual depolarisation during diastole. This has been ascribed to a gradual increase of sodium permiability. When the membrane potential reaches threshold, the fast Na^{+} current is activated again (phase 0).

CARDIAC ARRHYTHMIASIS :

Disturbances in normal cardiac rhythm termed as arrythmias. The normal rhythm of the heart may be altered by disturbances in pacemaker activity of the pacemaker S.A.node or by another part of the heart usurping the pacemaker function. For example, the A.V. node or purkinje fibpes may begin to generate impulses more rapidly than the S.A.node and there by set a new pace for the heart. As a result, the atria and ventricals contract independently and at different rates, disrupting normal cardiac rhythm and causing the heart to become less efficient. Some of these arrhythmias may prove fatal to patients.

TECHNIQUES USED IN PRODUCTION OF EXPERIMENTAL ARRHYTHMIAS :

The techniques so far reported for the production of experimental arrhythmias can be classified as follows:

- Drugs and other chemical agents
- Electrical stimulation of the heart
- Production of the ectopic foci in the heart.

Production of Experimental arrhythmias by drugs or other Chemical agents:

Various substances with different chemical structures have been employed for the production of experimental arrhythmias. Arrhythmogenic agents can either be applied locally, introduced directly in to the heart or into the blood stream. Some of the arrhythmogenic agents commonly employeed are

1. Barium Chloride
2. Aconitine
3. Cardiac Glycoside (Ouabain)

OUABAIN :

Ouabain is a cardiac glycoside which is frequently used to produce experimental arrhythmias. Ouabain is potent and highly selective inhibitor of the active transport of Na^+ and K^+ across cell membranes,by binding to specific site of Na^+,K^+-ATPase, the enzymatic equivalent of the cellular Na^+ pump. Inhibition of Na^+,K^+-ATPase results in increase in intracellular Ca^{+2} levels which in turn causes increase in force of contraction. Ouabain is found to produce ventricular fibrillation and ventricular tachycardia. The ventricular fibrillation and tachycardia is prevented by quinidine.

Almotrefi and Baker[1981] reported that ouabain when infused into the isolated heart of guinea pig produced cardiac arrhythmia.the antiarrhythmic potency of the test drug was determined by comparing the duration of the infusion required to produce cardiac arrhythmias in the drug treated and non treated hearts.

QUINIDINE:

Quinidine is used for maintenance of sinus rhythm in patients with atrial flutter or atrial fibrillation and in the prevention of recurrence of ventricular tachycardia and ventricular fibrillation.

Quinidine blocks Na^+ current and multiple cardiac K^+ currents. Quinidine's Na channel blocking properties result in an increased threshold for excitibility and

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decreased automaticity. As a consequance of K^+ blocking actions ,quinidine prolongs refractoriness in most tissues , probably as a result of both prolongation of action potential duration and its Na^+ channel blockage .

Chapter

2

MATERIALS AND METHODS

MATERIALS :

Ouabain was purchased from Sigma chemical company ,USA. All chemicals used to make CK solution were of analytical grade and were obtained from Loba-chemie ans S.D. fine chemicals ,Bombay. Rats were obtained from Haffkine Biopharmacutical Incorporation Ltd. Indusrial oxygen company Ltd, patalganga, supplied carbogen gas. Feed for rats was purchased from amrut suppliers, Mumbai. Quinidine was obtained from B. Wellcome, Mulund.

METHOD :

Rats (Wistar albino) of either sex weighing between 200-250g were used throughout the study. The rats were housed in polypropylene cages (3 to 4 rats per cage) and were given food and water ad libitum.

CALIBRATION OF THE STUDENT PHYSIOGRAPH.

Calibration of the student physiograph was achieved by suspending 1 gram and 2 gram weights and the corresponding deflection of the writing pen was adjusted to give a 0.5cm and 1cm deflection for 1 gram and 2 gram weight respectively on the polyrite chart paper using the calibration adjustment knob at a sensitivity setting of 500 μv/sec. After the transducer and the polyrite channel were calibrated, the amplitude of the heartbeats was directly interpreted in grams of force generated.

PREPARATION OF SOLUTIONS:

- **Solution of quinidine sulfate:** A stock solution of 10^{-3} M quinidine sulphate was prepared in distilled water. The required concentration was prepared by serial dilution technique and an appropriate volume of diluted stock solution was added to the reservoir to obtain the final concentration of 10^{-7} M in the perfusate.
- **Solution of Ouabain:** A stock solution of 4mg/ml was prepared by dissolving Ouabain in distilled water. The required concentration of 4µg/ml was prepared by diluting appropriate volume of stock solution in physiological solution.

EXPERIMENTAL:

Animals satisfying the conditions for body weight, age, and non-infected/ non-wounded, showing no abnormal behavior was included in the study. The animals were divided in four groups.

- Group I:- Animals received 1 ml/rat, saline, served as negative control.
- Group II :- Animals treated with Ayurvedic formulation, dose was 4.5 mg/rat for one day.
- Group III:-Animals treated with Ayurvedic formulation, dose was 0.9 mg/rat for seven days.
- Group IV:- Animals treated with Ayurvedic formulation, dose was 0,9 mg /rat for forty five days.

ISOLATED RAT HEART PERFUSION:

Rats were anesthetized by ether inhalation and killed by cervical dislocation. The hearts were exposed and quickly excised and kept in physiological salt solution bubbled with carbogen. The preparation was squeezed several times when first placed in Chenoweth-Koelle (CK) solution, so as to remove as much blood as

possible. The aorta was located and dissected free and all other vessels connected to the heart were trimmed away. The aorta was cut just below the point where it divides and the heart was transferred to the perfusion apparatus, Lagendroff's apparatus, where the aorta was tied to the cannula. The heart was perfused at a constant pressure, 40cm of water filling pressure.

The constant pressure perfusion apparatus consisted of a movable jacketed reservoir connected to the perfusion cannula through a jacketed water condenser. The perfusion pressure was controlled by adjusting the height of the movable reservoir. The perfusate level in the reservoir was maintained by periodic addition of the perfusate to make up to the required level. The perfusate was constantly bubbled with a mixture of 95% oxygen and 5% carbon dioxide (Carbogen). The temperature of the perfusate was maintained constant by circulating water at 37 ^{0}C through the jacketed reservoir and condenser by means of a thermostatic water-circulating pump. The perfusate used was Chenoweth-Koelle (CK) solution which contained the following mill moles per litre: NaCl - 119.8, KCl - 5.63, $CaCl_2$ – 2.18, $MgCl_2$. $6H_2O$ – 2.0, Glucose – 9.9 and $NaHCO_3$ – 19.

The hearts were secured to the inflow cannula through the aortic stump by means of a silk thread. The cannula was tied in such a way that it did not occlude the opening of coronary arteries. A silk thread was attached to the apex of the heart by means of palmer clip. The other end of the thread was attached to a force displacement transducer (Inco Model T 305) through a pulley. The resting tension was adjusted to 1 gram and the heart rate and tension developed were recorded on polyrite recorder (Inco Model 201). The hearts were allowed to equilibrate for 15 mins. before any recording. The recording of the normal heart were taken for few seconds, ouabain was infused at the rate of 4µg/ ml /min, the ouabain infusion was continued till the heart failed. The ouabain infusion was stopped after heart failure, and time was recorded till the heart was fully recovered.

Chapter 3

RESULTS

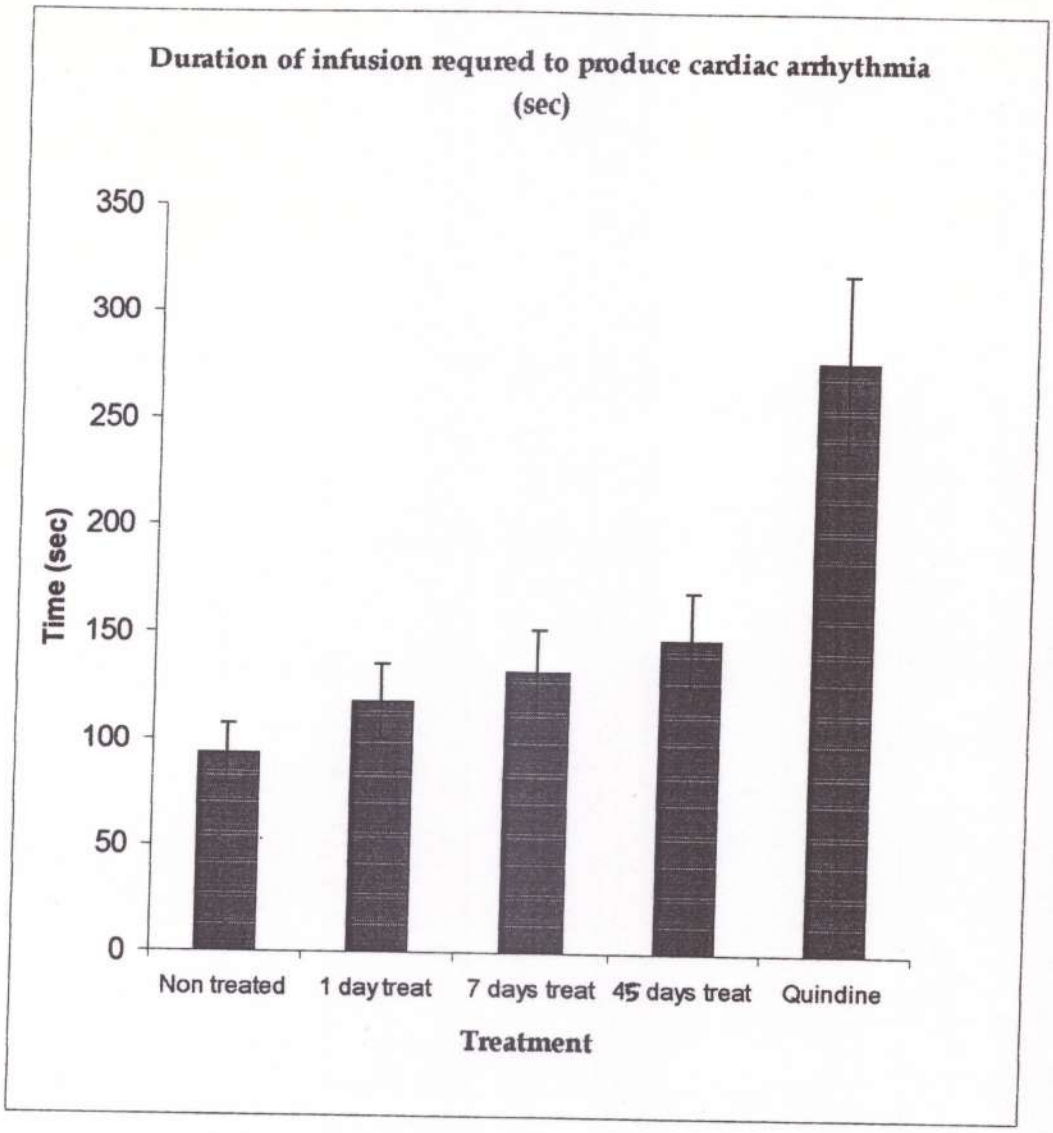
Effect of pre-treatment on Duration of infusion required to produce cardiac arrhythmia and heart failure

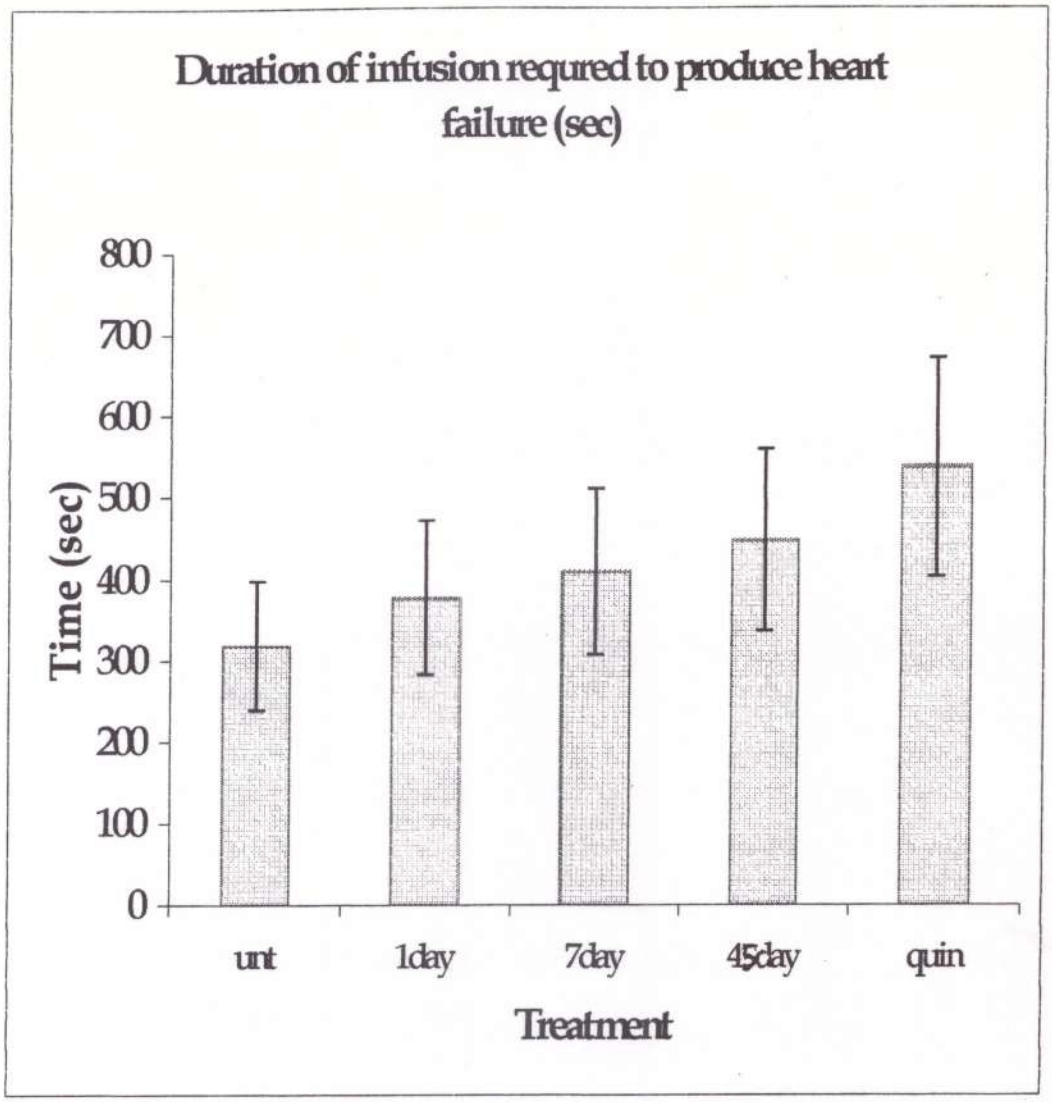
Group	Duration of infusion required to produce cardiac arrhythmia (sec)	Duration of infusion required to produce heart failure (sec)	Time required to recover the heart (sec)
Non treated	93.14 (± 14.392)	317.71 (± 73.01)	590.00 (± 57.60)
Treated for 1 day	118* (± 44.57)	376.66* (± 46.45)	600.00 (± 60.00)
Treated for 7 days	131.33* (± 7.571)	409.01* (± 97.35)	340.00* (± 91.20)
Treated for 45 days	147* (± 21.037)	447.28* (± 102.95)	228.00* (±72.00)
Infused with Quinidine	277.5* (± 41.445)	537.33* (± 64.027)	204.60* (± 55.02)

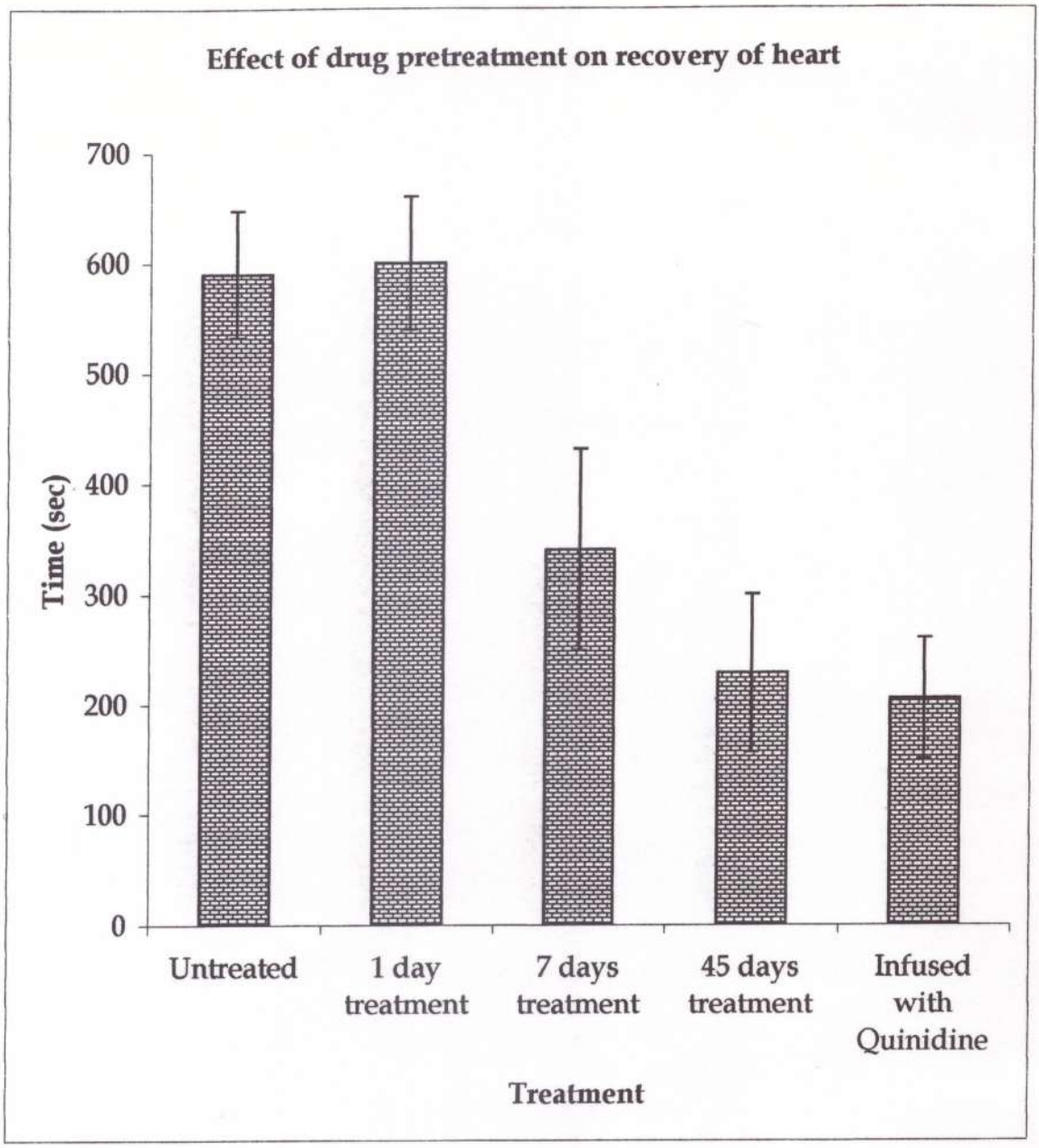
No. of animals in each group (n) = 6

A value in bracket represents standard deviation

* Significant difference as compared control at $P < 0.05$.







Effect of 1 day treatment on force of contraction in isolated rat heart preparation

Time (min)	1 day treatment	Untreated
0	0.933 (± 0.11)	1.13 (± 0.11)
2	1.00 (± 0.01)	1.33 (± 0.11)
4	1.73 (± 0.41)	1.53 (± 0.50)
6	1.13 (± 0.61)	

Effect of 7 days treatment on force of contraction in isolated rat heart preparation

Time (min)	7 days treatment	untreated
0	1.00 (±0.20)	1.13 (±0.11)
2	1.26 (±0.30)	1.46 (±0.11)
4	1.73 (±0.46)	2.20 (±0.25)
6	2.00 (±0.28)	

Effect of 45 days treatment on force of contraction in isolated rat heart preparation

Time (min)	45 days treatment	Untreated
0	1.05 (± 0.09)	1.14 (± 0.39)
2	1.54 (± 0.56)	1.42 (±0.43)
4	1.51 (± 0.44)	1.60 (± 0.91)
6	1.52 (± 0.57)	

Effect of Quinidine (10^{-7} M) on force of contraction in isolated rat heart preparation

Time (min)	Quinidine
0	1.06 (± 0.16)
2	1.16 (± 0.15)
4	1.76 (± 0.42)
6	1.90 (± 0.21)
8	1.20 (± 0.23)

No. of animals in each group (n) = 6

Effect of 1 day treatment on heart rate in isolated perfused rat heart

Time (min)	1 day treatment	untreated
0	78 (±15.87)	88 (±3.46)
2	78.66 (±26.02)	94 (±10.06)
4	64 (± 6.92)	70 (±22.71)
5	44.66 (± 8.08)	

Effect of 7 days treatment on heart rate in isolated perfused rat heart

Time (min)	7 days treatment	untreated
0	74.66 (± 12.85)	60.66 (± 11.05)
2	77.33 (± 11.54)	80.66 (± 16.16)
4	78.67 (± 16.65)	44.00 (± 14.14)
6	53.00 (± 1.414)	

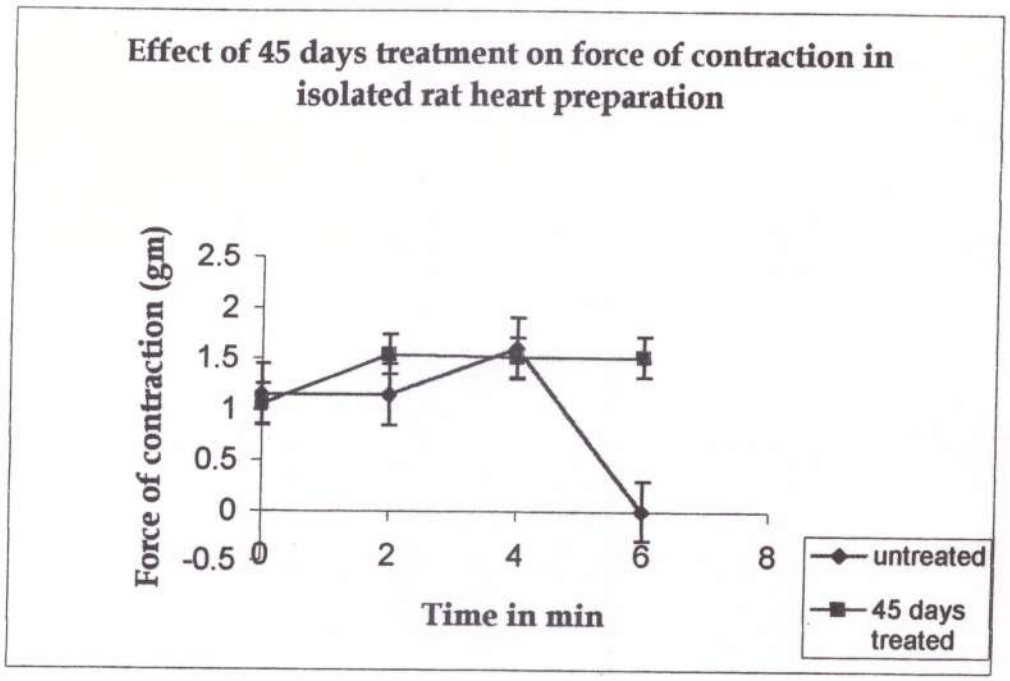
Effect of 45 days treatment on heart rate in isolated perfused rat heart

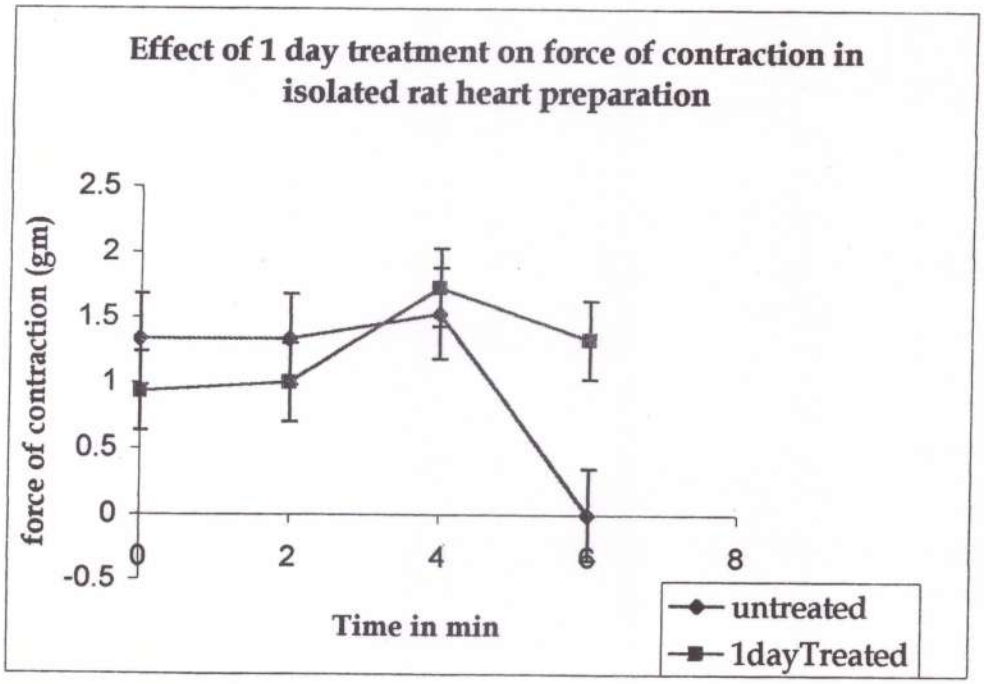
Time (min)	45 days treatment	untreated
0	86.57 (± 3.20)	81.42 (±12.89)
2	99.71 (± 8.97)	93.71 (± 22.78)
4	86.85 (± 20.52)	72.00 (± 18.19)
6	75.20 (± 18.25)	

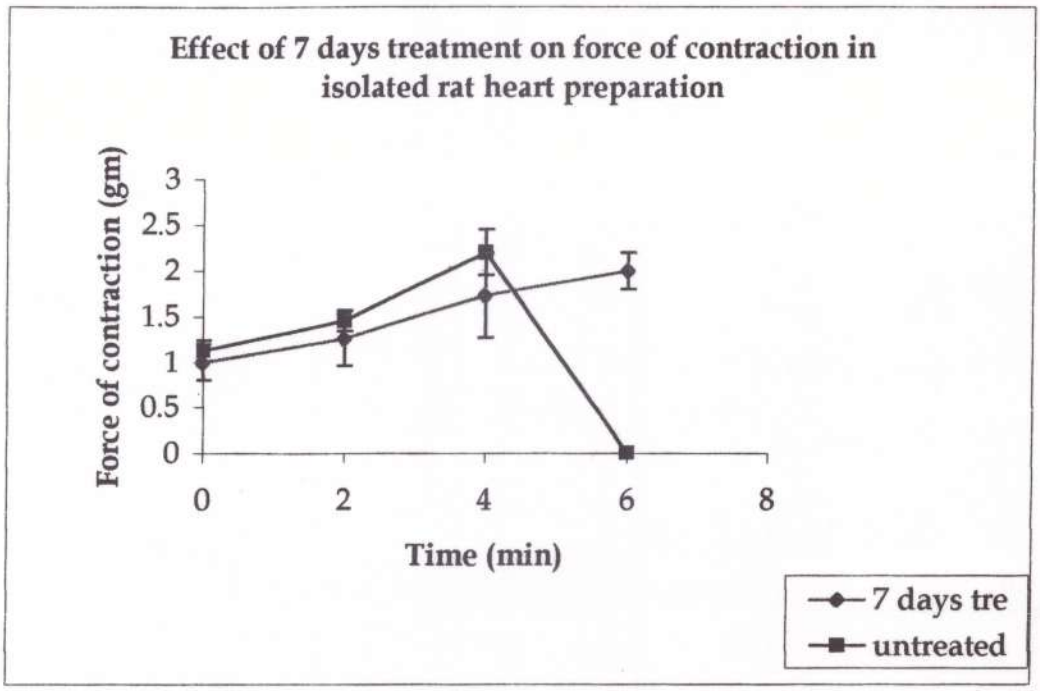
Effect of Quinidine (10^{-7} M solution) on heart rate in isolated perfused rat heart

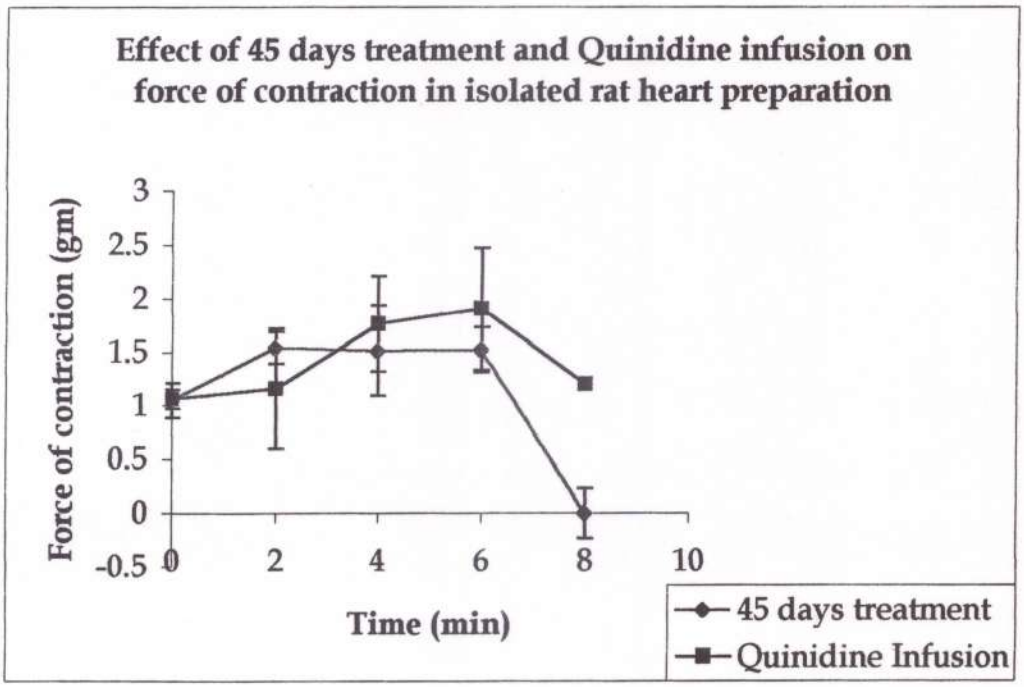
Time (min)	Quinidine
0	81.00 (± 4.69)
2	82.67 (± 6.02)
4	81.67 (± 11.69)
6	57.33 (± 23.45)
8	46.40 (± 12.28)

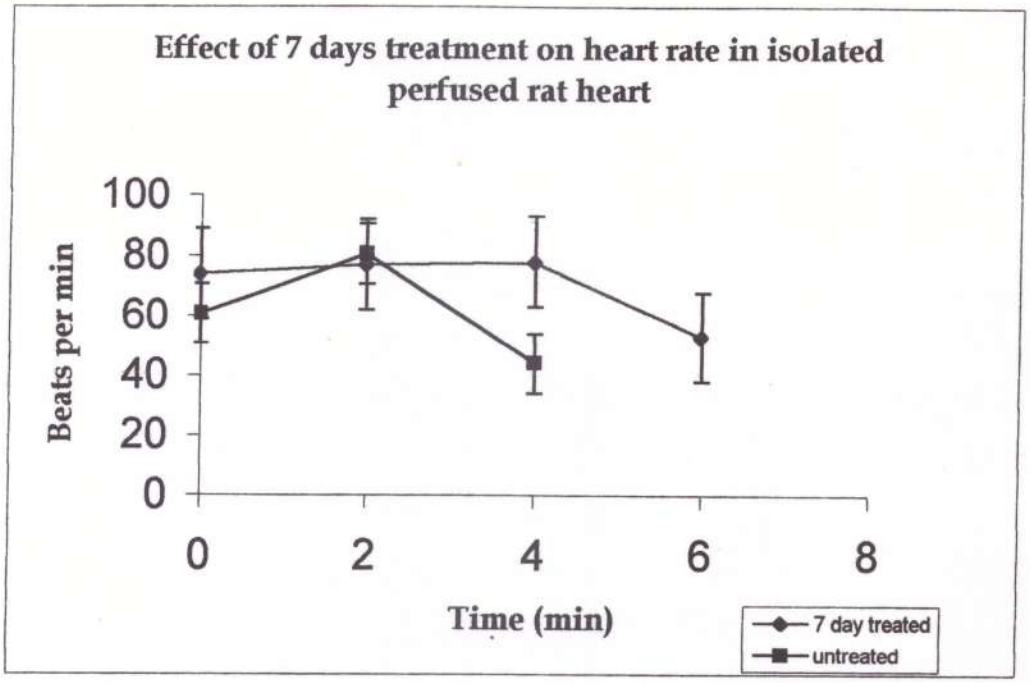
No. of animals in each group (n) = 6

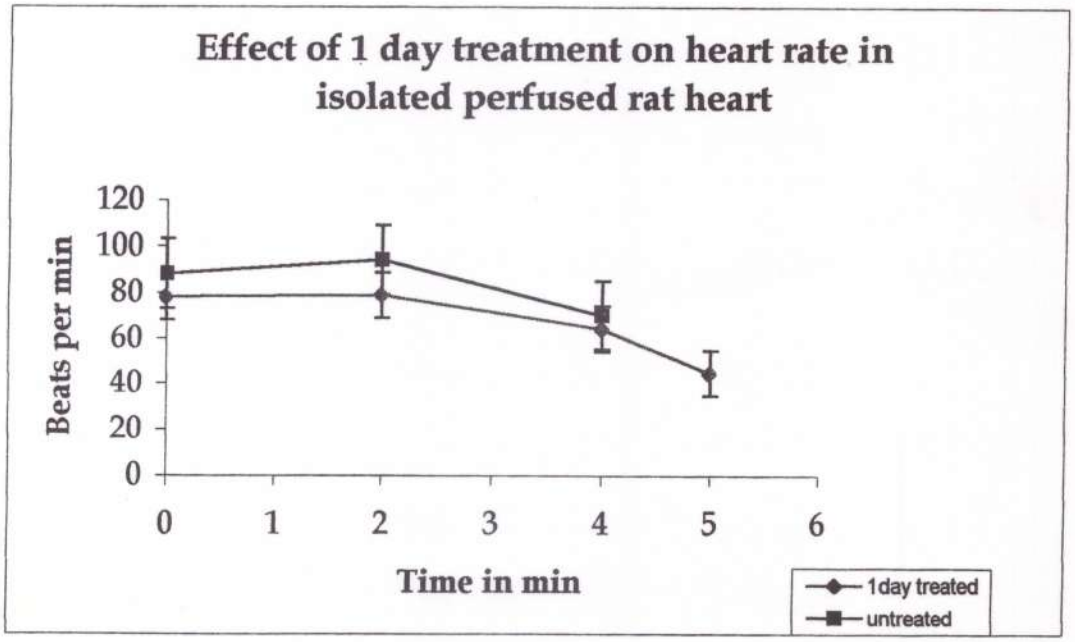


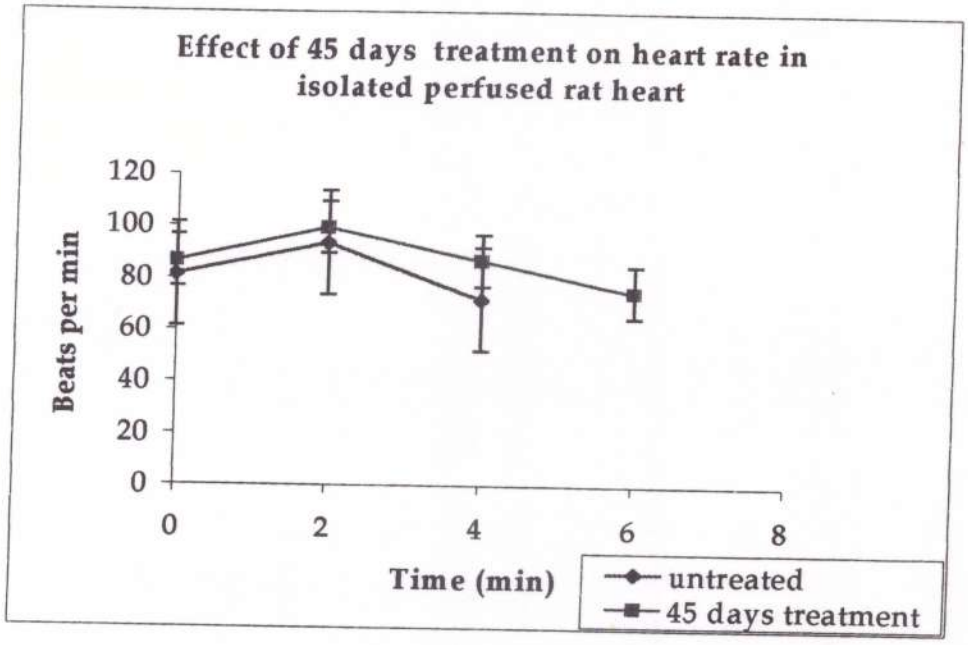


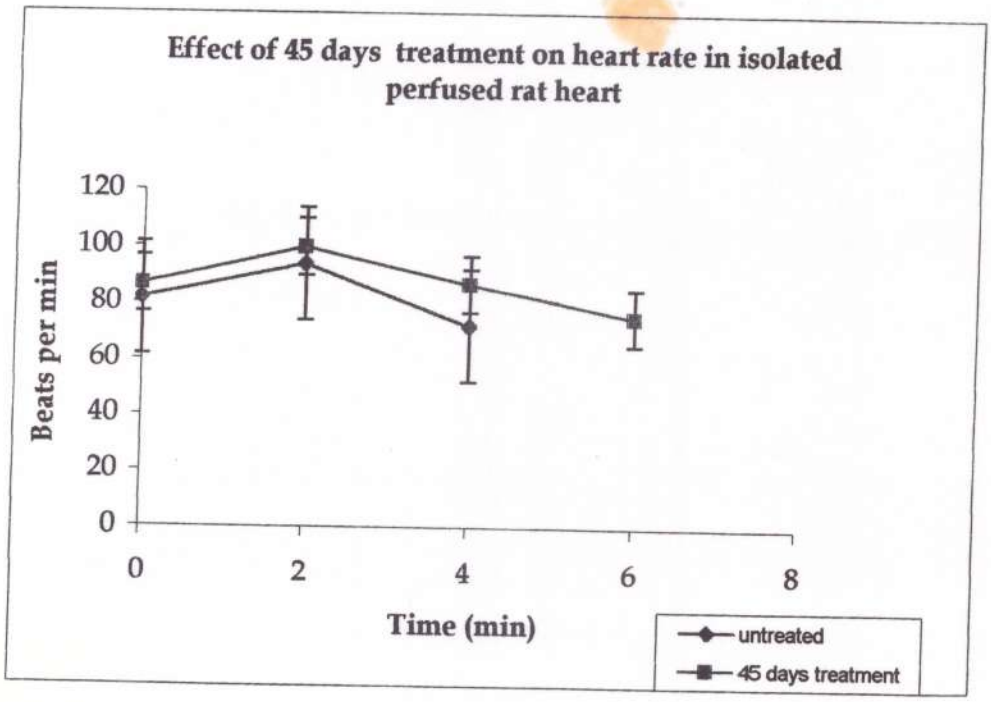












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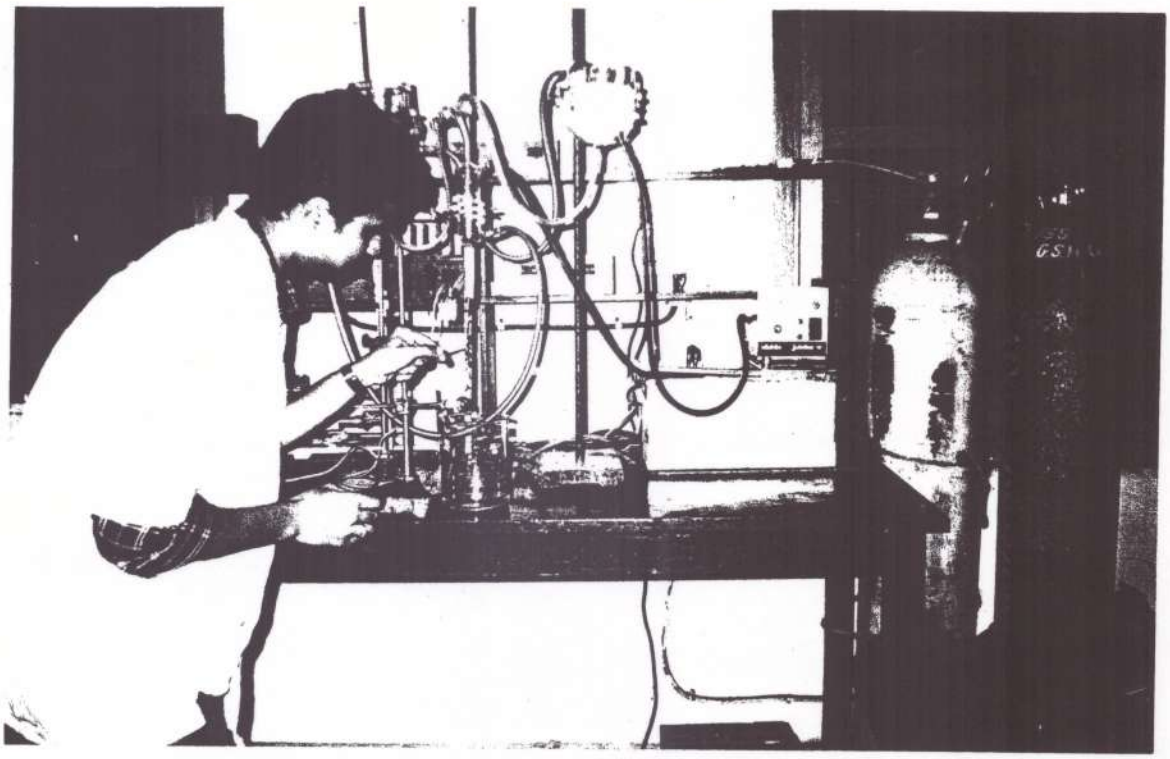
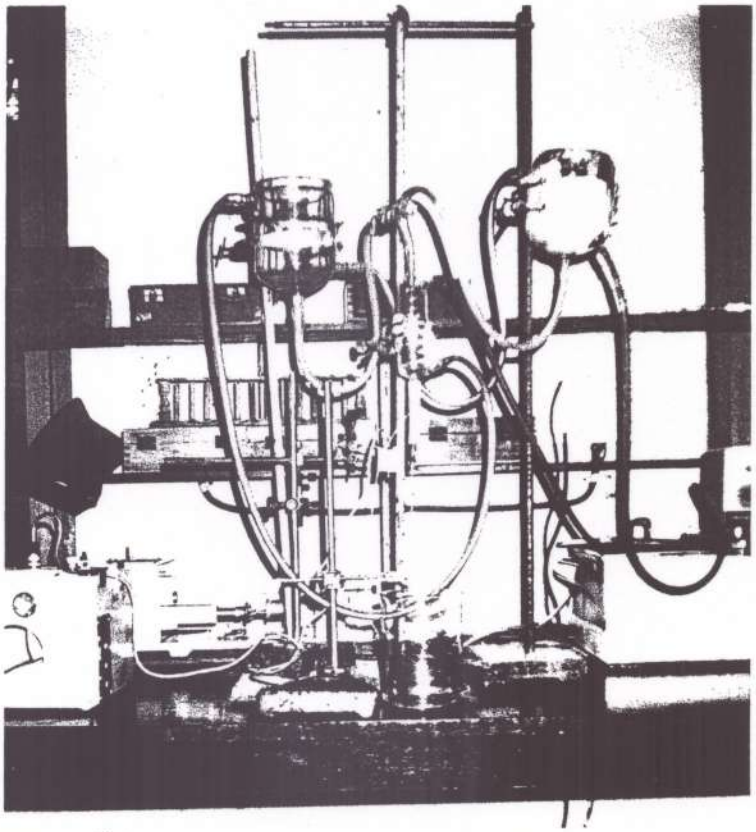
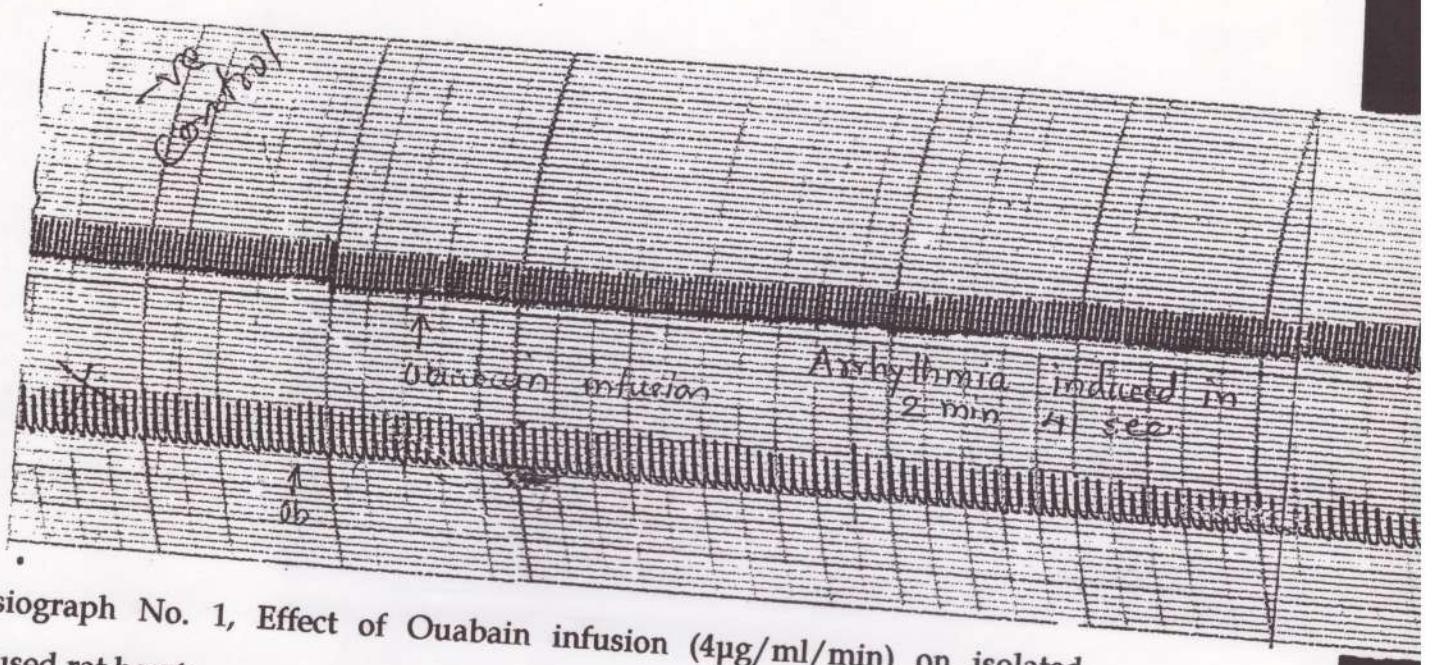
Photographs of Lagendroff's Preparation

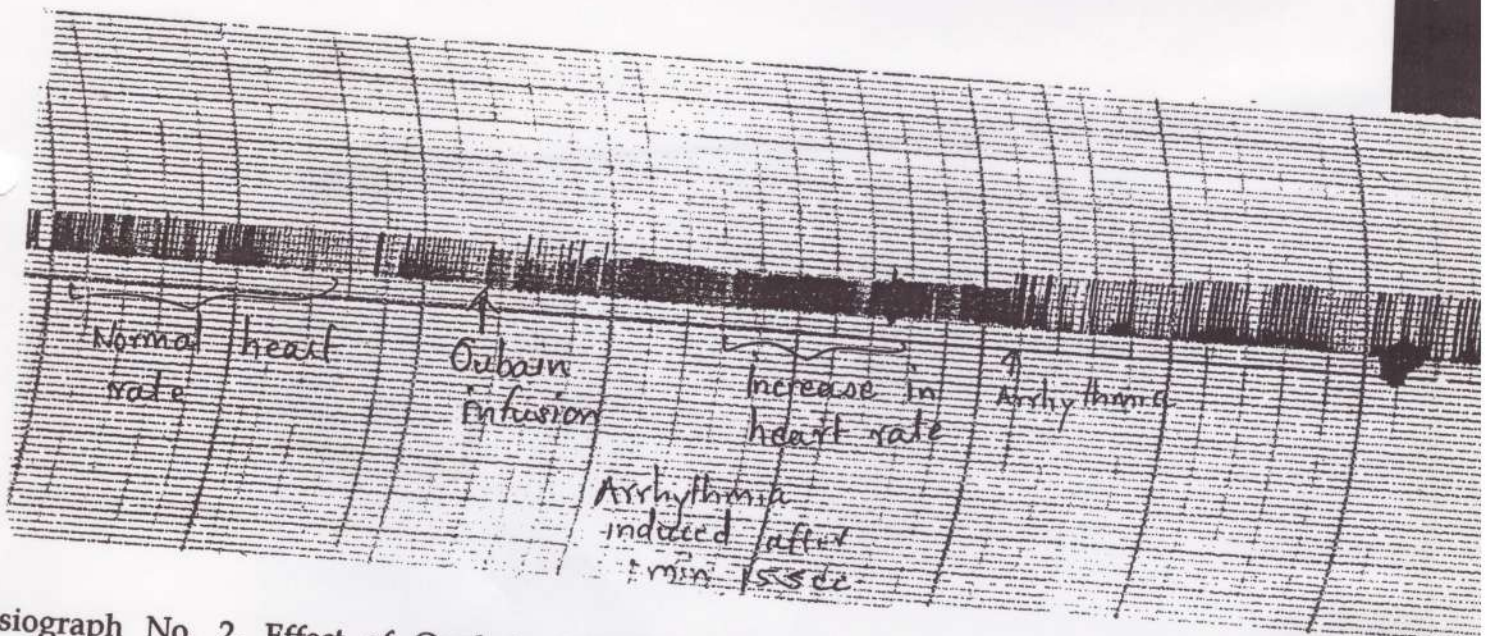




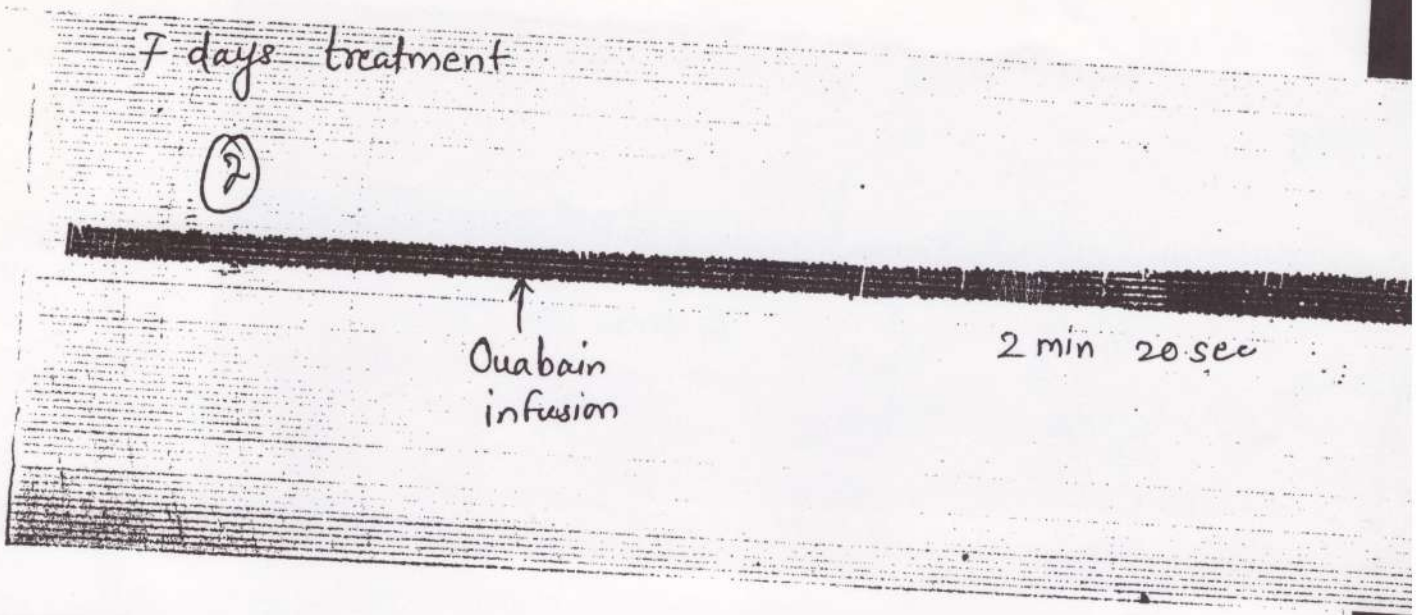
The following graphs represent the physiograph recordings of isolated rat heart preparation.



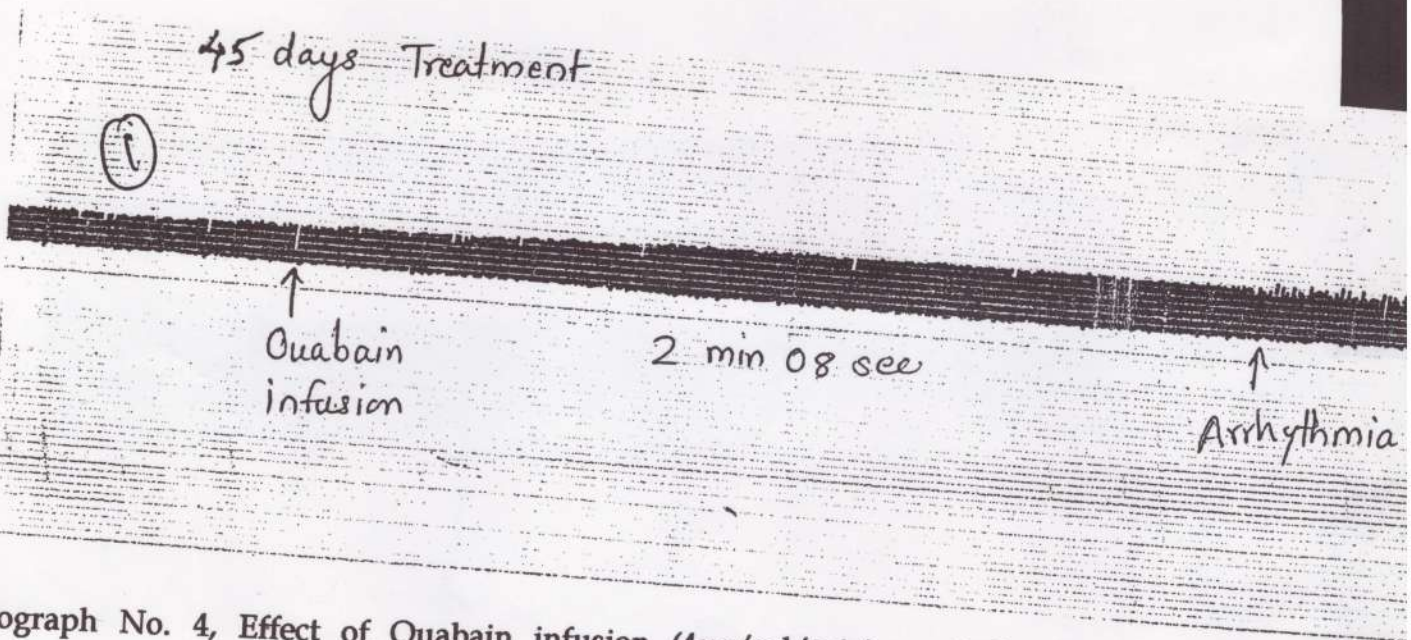
Physiograph No. 1, Effect of Ouabain infusion (4µg/ml/min) on isolated perfused rat heart.



'hysiograph No. 2, Effect of Ouabain infusion (4µg/ml/min) on isolated erfused rat heart pretreated (one day) with Brihatvatachintamani.



Physiograph No. 3, Effect of Ouabain infusion (4µg/ml/min) on isolated perfused rat heart pretreated (seven days) with Brihatvatachintamani



Physiograph No. 4, Effect of Ouabain infusion (4µg/ml/min) on isolated perfused rat heart pretreated (forty-five days) with Brihatvatachintamani.

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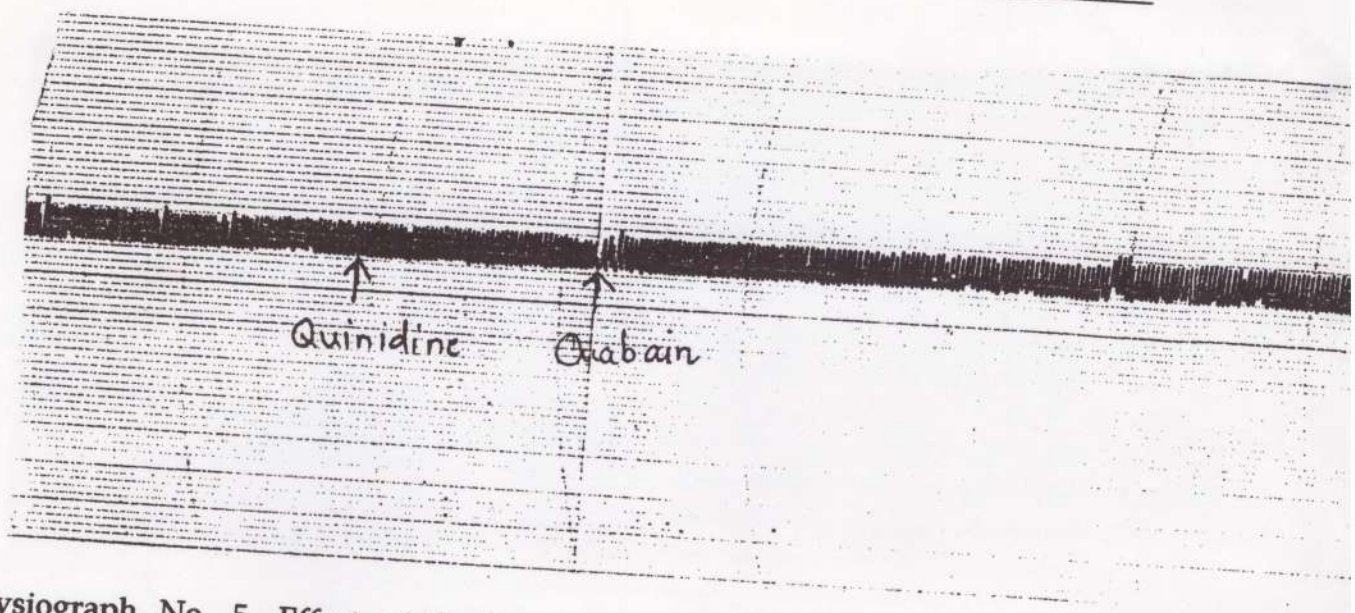

Physiograph No. 5, Effect of Ouabain infusion (4µg/ml/min) on isolated perfused rat heart in the presence of quinidine (1x 10 $^{-7}$ M).

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Chapter

4

CONCLUSIONS

SupraVentricular Tachycardia- a marked predictor of Cardiac arrhythmias was induced onto isolated perfused rat heart by using Oubain Infusion (4 µg/ml) for a period of 90 seconds. The arrhythmic changes were characterised by an initial elevation of both the heart rate (10-20 beats per minute) as well as the force of contraction (Ionotropic and Chronotropic effects) followed by a rapid decline in the heart rate with a milder decrease in the force of contraction. A simulataneous evaluation of standard anti-arrhythmic agent, Quinidine sulphate, focussed on correcting the arrhythmic alterations was also carried out with a view point to characterise the comparative profile with the test formulation. It was observed that the onset of arrhythmia was delayed by Quinidine at a concentration of 10^{-7}M.

An encouraging data was obtained with respect to effect of the test formulation (Brihatvatachintamani) on Cardiac arrhythmias. It was observed that pre-treatement of animals with test formulations significantly augmented the cardiac response which was indicated by its ability to inhibit the Oubain induced Cardiac arrhythmias. The Cardiac reponse observed in the tested animals was exponentially correlated with the duration of pre-therapy of the test formulation. A testimony to prove this fact can be well understood by considering the data obtained on animals pretreated for a single day to animals pretreated for a maximum of 45 days. The duration of infusion of Oubain required to produce cardiac arrythmias with one day, seven days and forty days pretreatment increased by 13%, 20.61% and 29% respectively. Moreover, the duration of infusion of Oubain required to produce heart failure with one day, seven days

and forty days pre-treatment increased by 26%, 41% and 58.9% respectively. The time taken to revive the heart was shortened as the pre-treatment period was increased.

The present investigation thus brings to light the significant role of test formulation (Brihatvatachintamani) in fortifying the overall cardiac responses of the treated animal lot. Delay of Oubain induced arrythmias and heart failure are evidences cropped out to prove this aspect. The effect was more pronounced with a duration-dependent characteristic profile for the test formulation.

It can be thus ardently concluded that the test formulation (Brihatvatachintamani) possesses significant anti-arrythmic activity at tested concentrations.