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THE STUDY OF PRECLINICAL EVALUATION OF HYDROALCOHOLIC EXTRACT OF *CATHARANTHUS ROSEUS* LEAVES FOR IT'S TROPIC EFFECT ON ISOLATED HEART OF ALBINO WISTAR RAT

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ABSTRACT

Catharanthus Roseus is one of the prime plants in ayurveda. It is also known as Madagascar periwinkle which is belong apocynaceae family. It is used to treat of fatal disease, also have a good antioxidant. This plant is very much essential for its therapeutic effects like anticancer property, antidiabetic property, antimicrobial property, hypolipidemic property etc. The aim of the experiment to established preclinical evaluation of hydroalcoholic extract of Catharanthus roseus leaves for its tropic effect on isolated heart of Albino Wister rat. The powder sample collected from hydroalcoholic extract of leaves by percolation process. *Catharanthus Roseus* itself showing its cardiotropic action. The effect of *Catharanthus Roseus*'s extract on the heart was very satisfactory in this experiment.

Keywords: *Catharanthus Roseus*, hydroalcoholic extract, albino Wistar rat, isolated heart preparation, cardiotropic activity

INTRODUCTION:

According to WHO, medicinal plants are the best sources of various new herbal drugs. Traditional medicine is used by 80% of individuals from developing countries. Earlier due to lack of knowledge about the reason of illness or proper information about

the medical property of a plant, cure attempts were mainly based on experience **[1-4]**.

In ancient times, people looked to nature for drugs for their diseases. In view of the fact that at the time there was not sufficient information about the reason for the illness or concerning which plant and how it could be utilised as a cure, everything was based on experience. Then the gradual usage of medicinal plants was abandoned after the reason for the usage of a specific medicinal plant for the treatment of certain diseases was discovered **[5, 6]**.

Catharunthus Roseus also known as Madagascar periwinkle. *Catharunthus Roseus* is a important medicinal plant. *Catharunthus Roseus* belonging to the family apocynaceae is used in the treatment of fatal diseases. It also have a good antioxidant potential as well as different therapeutic Properties like- Anticancer property, Ant diabetic property, Antimicrobial property, Anti ulcer property, Hypertensive property, Hypolipidimic property **[7]**.

METHODOLOGY:

Collection of samples:

The fresh leaves of *Catharanthus roseus* brought from the tree from the local areas of Netaji Subhas Chandra Bose Institute of Pharmacy. Washed them by clean and fresh water for removing the unwanted particles.

Subject Collection:

Albino Wister rats of around 200 gm of either sex was collected from Saha Enterprise (follows CPCSEA guidelines).

Preparation of extract: [6, 8]

After cleaning the leaves, it was kept in 400 ml of 70% hydroalcoholic solution in a percolator for 36 hours. After that the liquid extract was separated from the leaves by filtration in a fresh beaker and they were kept in vacuum extractor for evaporation for 36 hours. After the final extraction, the product was solidified and formed powder like appearance. Finally, the powder of the leaves extract of was kept in cool and dry place in laboratory.

Animal:

Albino Wistar rats, weighing between 200 and 220g were purchased from an authorised breeder and utilised in this experiment. Animals were kept in animal housing under conventional conditions with a 12-hour alternating cycle of light and dark.

Chemical Test: [9]

The leaves extract was subjected to different phytochemical assessment.

Krebs-henseleit's solution preparation:

The experimental subject was albino Wister rat heart. The proper solution to make heart alive is Kerbs-henseleit's solution. The composition of the solution is shown in **Table 1**.

Table 1: Components of krebs-henseleit's solution

S. No.	Name of the compound	Amount (in 1000 ml)
1.	NaCl	6.9 gm.
2.	KCl	1.28 gm.
3.	$NAHCO_3$	2.1 gm.
4.	$CaCl_2$	0.28 gm.
5.	$MGSO_4, 7H_2O$	0.35 gm.
6.	DEXTROSE	2.0 gm.
7.	$K_2H_3PO_4$	0.16 gm.

To make krebs-henseleit's solution, 1000 ml of distilled water is subjected, and temperature is maintained 20°c-25°c. All the materials were mixed one by one except $CaCl_2$. $CaCl_2$ is measured as per the previous table and made solution is separate beaker. After that the solution was slowly poured into the remaining solution and stirred well to avoid formation of chelates. By mixing well the krebs-henseleit's solution was prepared. When the solution is ready for experimental work, then the temperature was elevated to 37°C.

Dose preparation:

As all organ of a living system receive both parasympathetic and sympathetic innervations simultaneously, the extract of *Catharanthus roseus* needs to be compared with both standard compound of autonomic nervous system like acetylcholine and adrenaline. For that reason, acetylcholine and adrenaline stock solution need, be prepared for in vitro study.

To make the dose of acetylcholine at first, we have taken a conical flux and measured 100 ml of distilled water. Then we have added 2 equal drops of acetylcholine by insulin syringe into 100 ml of water to make the concentration of 100 μl/100 ml

As adrenaline was available in powder form, we have taken 1mg (1000 μg) and dissolved it in 1 lit (1000ml) of distilled water. To make the stock solution concentration of 100 μg/100ml we have taken 100 ml solution from 1000 ml of total solution.

Heart model: [8]

Albino Wister rat weight of 200 gm was sacrificed by cervical dislocation method. The thoracic cavity was opened so that the heart can be exposed and isolated. Then the vena cava cannulated followed by the heart isolated and mounted along with adequate supply of the *kerbs-henseleit's* solution and aeration. The reservoir of the solution was maintained at 37◦±2 C by using thermostat. The flow of physiological solution controlled by a regulator and to maintain the uniform flow through the heart. The apex of the ventricle attached with sterling heart lever and the inotropic and chronotropic activity recorded on Sherrington rotating drum.

RESULT AND DISCUSSION:

Phytochemicals: (Table 2-5)

Dose Response Curve of Adrenaline:

Adrenaline known as sympathetic neurotransmitter produce action in most of the organ. Adrenaline produces action by α, β receptors, the α receptors are divided into α1 and α2 receptor. α1 is mostly present in postjunctional on effector organ and α2 present in prejunctional on nerve ending. β receptor is divided into β1, β2, β3. β1 is dominant in heart, β2 is present in smooth muscle and β3 is located in adipose tissues **[10]**.

Adrenaline shows high affinity towards α1, α2, β1 and β2 but very poor affinity to β3 receptor. All cardiac actions are predominantly β1 receptor mediated.

Adrenaline increases heart rate by increasing the slope of slow diastolic depolarization of cells in the SA node. It also activates the latent pacemaker in AV node and purkinje fibres, arrhythmia can occur with high dose. Raised BP, SA node is depressed and unmasks the latent pacemaker **[10]**, idioventricular rate is increased in patient with complete heart block.

Cardiac contraction force is increased. Development of tension as well as relaxation are accelerated, thus systole is shorten more than diastole. Cardiac output and oxygen consumption of heart increases **(Figure 1)**.

Here in the experimental model, we have used Adrenaline 100µg/ml as a stock solution and Adrenaline shows positive inotropic and positive chronotropic effect in isolated rat heart as usual. The response was gradually increases in dose dependent manner.

As the dose increases the response length (force of contraction) and number of peak /minute (rate of contraction) both increases. In the dose of 0.2 ml the adrenaline shows its highest effect (maximum response) on isolated rat heart., But as the number of receptors is limited in isolated tissue, in higher dose(0.4ml) it has shown its saturation peak (supramaximal response) **(Figure 2)**.

Dose Response Curve of Acetylcholine:

Here in the experimental model, we have used Acetylcholine 100µg/ml as a stock solution and Acetylcholine shows negative inotropic and negative chronotropic effect in isolated rat heart as usual. The response was gradually decreases in dose dependent manner.

Dose Response Curve of *Catharanthus Roseus*:

Here in the experimental model, we have used *Catharanthus roseus* 100µg/ml as a stock solution and *Catharanthus roseus* shows negative inotropic and positive chronotropic effect in isolated rat heart. The response was gradually increases in dose dependent manner.

On the very first day of the experiment, we have applied 0.2 ml of *Catharanthus roseus* extract on isolated rat heart. The nature of the graph indicated that the activity of the

heart was increased and obviously the tropic action was increased **(Figure 3)**.

On the 2nd day of experiment we have applied 0.4ml (100ug/ml) *Catharanthus roseus* extract and the result in the graph showed very satisfactory.

The peak on the responses recorded by kymograph was shortened. The extract decreased the inotropic activity of the heart in very controlled manner. But the chronotropic action was elevated, and the heart showed total cardiac arrest after some time **(Figure 4)**.

Dose Response Curve of Catharanthus Roseus Over Acetylcholine:

In this experiment we have used acetylcholine over the *Catharanthus roseus* extract to determine the alteration of the peaks obtained from both acetylcholine and *Catharanthus roseus* extract with same dose and same concentration. After getting the peaks we can say that at first after applying *Catharanthus roseus* extract the peaks were demoted as we obtained previously but as soon as we applied acetylcholine over it, the distance between the peaks were elongated. It is obvious that acetylcholine can have an action to reduce both tropic action in heart but in this case acetylcholine has no effect on inotropic action. Only the chronotropic action was altered. And irregular heartbeat was reported and arrythmia was produced **(Figure 5)**.

Dose Response Curve of Catharanthus Roseus Over Adrenaline:

In this experiment we have used adrenaline over the *Catharanthus roseus* extract to determine the alteration of the peaks obtained from both acetylcholine and *Catharanthus roseus* extract with same dose and same concentration. After getting the peaks we can say that at first after applying *Catharanthus roseus* extract the peaks were demoted as we obtained previously but as soon as we applied adrenaline over it, the distance between the peaks were shortened and the length of the peaks was elevated. In this case the peak lengths were increased and the time interval between two peaks was shortened. The overall cardiac activity was increased more than individual peaks of both adrenaline and *Catharanthus roseus* extract. So here in this experiment the *Catharanthus roseus* is showing synergistic action when adrenaline was introduced. But after some time the synergism causes cardiac arrhythmia **(Figure 6)**.

Table 2: Test For Alkaloids

S. No.	TEST NAMES	RESULTS
1	Dragendorff test	Positive
2	Tannic acid test	Positive

Table 3: Test For Carbohydrate

S. No.	TEST NAMES	RESULTS
1	Molish Test	Positive
2	Pentoses Test	Negative

Table 4: Test For Cardiac Glycosides

S. No.	TEST NAMES	RESULTS
1	Zinc hydrochloride test	Negative
2	NaoH+Dilute HCL	Positive

Table 5: Test For Glycoside

S. No.	TEST NAMES	RESULTS
1	Methanolic KOH	Negative
2	Baljet's	Negative

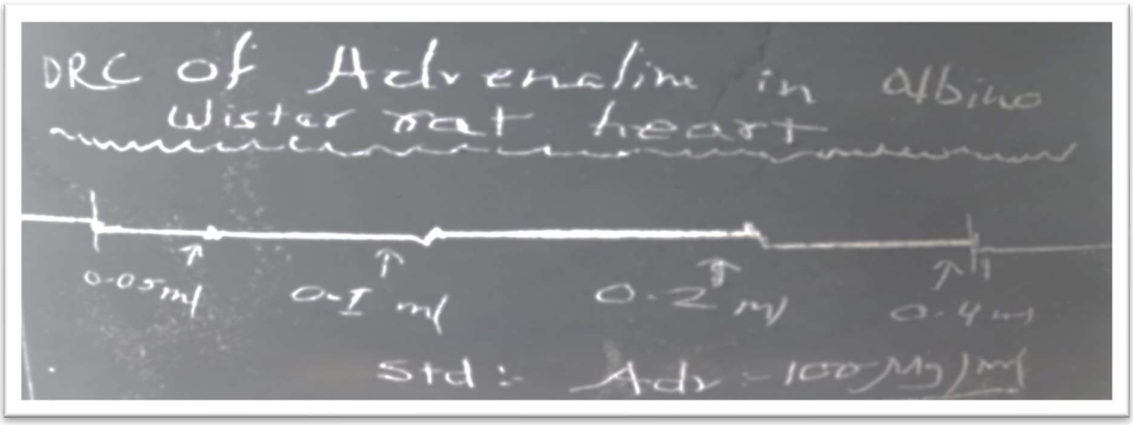

Figure 1: Dose Response Curve of Adrenaline

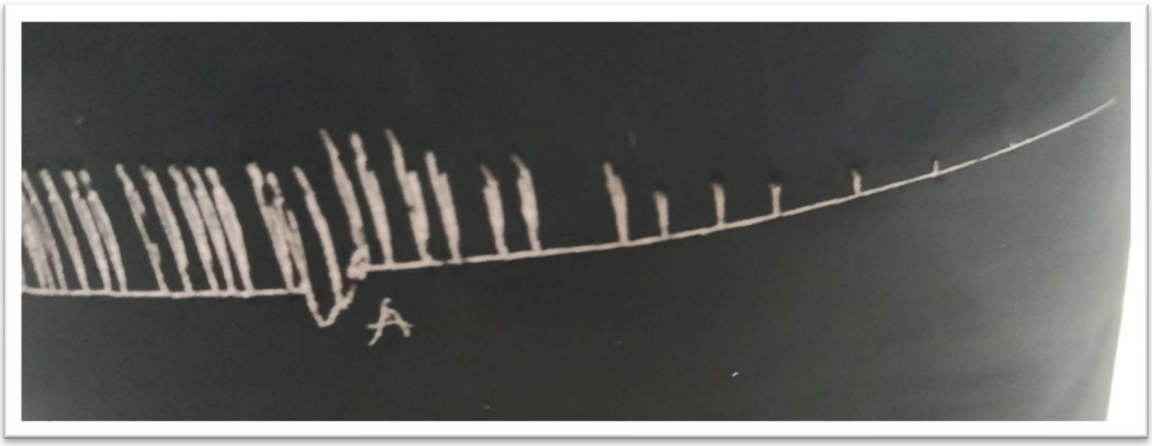

Figure 2: DRC of Acetylcholine

Figure 3: DRC of *Catharanthus Roseus*

Figure 4: DRC of *Catharanthus Roseus*

Figure 5: DRC of *Catharanthus Roseus* over Acetylcholine

Figure 6: DRC of *Catharanthus Roseus* over Adrenaline

CONCLUSION:

The effect of the extract of *Catharanthus roseus* on the heart was very satisfactory in this experiment. *Catharanthus roseus* itself showing its cardiotropic action. Comparing with the effect with acetylcholine. The heart reduced its chronotropic action along with cardiac arrest and arrythmia was reported.

Comparing with the effect with adrenaline, *Catharanthus roseus* extract showing its positive synergistic action. It may overcome from the failing heart also.

Catharanthus roseus is very common plant in India. In this preclinical studies, we can conclude that the extract of *Catharanthus roseus* is very effective in heart. The activity of the heart was increased but the proper activity of the *Catharanthus roseus* extract is still unknown in molecular level and also the effective dose and the therapeutic window should be determined for its prominent effect. In this stipulated time we have found some valuable data on this matter but still more research work to find out more detail are always will be appreciable in cardiac pharmacology.

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