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PRECLINICAL EVALUATION OF HYDRO-ALCOHOLIC EXTRACT OF *HYGROPHILA AURICULATA* LEAVES FOR CARDIO-TROPIC ACTIVITY

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ABSTRACT

Aim of the present study was to evaluate the cardio tropic activity of hydro-alcoholic extract of *Hygrophila auriculata* leaves on Albino wistar rat. In this study the leaves of *Hygrophila auriculta* were subjected to cold percolation extraction by using 70% ethanol for 48 hours and 6.57% yield was obtained. The presence of various phytochemical constituents like alkaloid, carbohydrate, cardiac glycoside, tannin, saponin, flavonoid and protein were found by phytochemical investigation of the extract. Albino wistar rats weighing around 100-120 g were used in this study. Different doses of acetylcholine, adrenaline and hydro-alcoholic extract of *Hygrophila auriculata* leaves were given on heart of the experimental animals. The cardio tonic response supports the traditional use of *Hygrophila auriculata* in the treatment of heart failure. Due to the presence of cardiac glycoside, *Hygrophila auriculata* has produced cardio tonic effect and may be effective in the treatment of heart failure. This finding establishes the traditional use of *Hygrophila auriculata* in heart failure.

Keyword: *Hygrophila auriculata*, Cardiac glycoside, Acetylcholine, Adrenaline, Cardiotropic activity

INTRODUCTION:

Cardiovascular diseases (CVDs) have long been seen as a serious public health issue worldwide. Men and women both have the risk of cardiovascular disease and the chance is more than 50%. Hypertension, hypercholesterolemia, obesity, diabetes, stress, air pollution, cigarette smoking and family history etc. are all key risk factors of

cardiovascular diseases. Despite the fact that current medications are useful in treating and preventing cardiac diseases, their usage is often limited due to side effects and adverse reactions. So, there are continuous searches for new cardiotonic drugs from natural origin.

Research on herbal plants is a result of many years of struggles against many types of diseases and illnesses **[1]**. From ancient times people depended on plants for food and medicinal purposes. The relation between human and their search for drugs in nature is very old. According to WHO, medicinal plants are the best source for various new herbal drugs. Nowadays, about 85% of individuals from developing countries use traditional medicines derived from medicinal plants **[2, 3, 4]**.

Hygrophila auriculata belongs to Acanthaceae family is a spiky bush that can be found all over India. Though the entire plant has been used medicinally, the root and leaves are the most commonly used parts. Leaves of *Hygrophila auriculata* contain alkaloids, carbohydrates, proteins, steroids, glycosides, flavonoids, tannins, phenols, fats, oils, vitamin C, sodium, copper, potassium, calcium, ß-carotene and riboflavin **[5]**. It is traditionally used in the treatment of jaundice, arthritis, itching, edema, pain, inflammation, urinary infection and sleeplessness **[6]**. Various studies show that it has anti-inflammatory, analgesic and antipyretic action, hematopoietic, anti-neoplastic, hepatoprotective, diuretic, antidiabetic, antibacterial **[11]**, anthelmintic and antioxidant activity **[5, 6]**.

After a thorough study of the literature, no scientific evidence was found regarding cardiotropic action of *Hygrophila auriculata* leaves. Hence, it was considered to evaluate cardiotropic activity due to the presence of cardiac glycoside in it. The cardiotropic activity of hydro-alcoholic extract of *Hygrophila auriculata* leaves was performed on the isolated heart of Albino Wistar rat.

MATERIALS AND METHODS:

Preparation of extract formulation:

Fresh leaves of *Hygrophila auriculata* were collected from local area. Then the leaves were washed gently with distilled water and dried under the shed. Dried leaves were crushed by hand and subjected for extraction process.

The extraction of dried *Hygrophila auriculata* leaves were done with 70% ethanol by using the cold percolation technique for consecutive 48 hours. The extract was collected and evaporated to dryness inside a vacuum desiccator. The dried extract was stored in a refrigerator at 4°C **[7, 8]**.

Preliminary phytochemical investigations of the extract: Hydroalcoholic extract of *Hygrophila auriculata* leaves was evaluated for phytochemical investigation **[8]**.

Experimental animals:

Albino Wistar rats weighing 100-120 g were used in this study. Animals were purchased from an authorized animal breeder. The animals were well maintained under standard hygienic conditions, at a temperature of 22±2°C, 65% relative humidity, and 12-hours light and dark cycle at animal house of Netaji Subhas Chandra Bose Institute of Pharmacy. The animals were fed with rodent standard diet of pellets along with water *ad libitum*. The IAEC (Institutional Animal Ethics Committee) of Netaji Subhas Chandra Bose Institute of Pharmacy approved all the experimental methods and protocols used in this study as per CPCSEA guidelines. The experiment was performed between 10 am to 6 pm.

Dose preparation:

10 mg of leaves extract, acetylcholine, adrenaline and atropine were weighted and dissolved in 100 ml of distilled water. The concentration of stock solution was 100 µg/ml for each drug.

Krebs-Henseleit's solution preparation: [9]

The Krebs-Henseleit's solution was used to keep the isolated heart alive. At 20°C-25°C temperature all the materials were mixed one by one except $CaCl_2$ in distilled water and separate solution of $CaCl_2$ was prepared in a separate beaker. Then the $CaCl_2$ solution was poured slowly into the remaining solution and stirred well to avoid formation of chelates. Total volume of the Krebs-Henseleit's solution was 1000 ml. When the solution was ready for the experimental work, the temperature was elevated to 37°C. The composition of the solution is given in the table below.

Table 1: Composition of Krebs-Henseleit's solution

Sl.no	Name of the compound	Amount (in 1000ml)
1	NaCl	6.9 g
2	KCl	1.28 g
3	$NaHCO_3$	2.1 g
4	$CaCl_2$	0.28 g
5	$MgSO_4,7H_2O$	0.35 g
6	Glucose	2.0 g
7	$K_2H_3PO_4$	0.16 g

Isolated heart model: [9]

Albino Wistar rat was sacrificed by cervical dislocation method. Then the rat was dissected from the lower abdominal towards the throat. The thoracic contents were excised and the heart was cleared of as much tissue as possible. Finally, the vena cava was cannulated, the heart was isolated and mounted. Adequate supply of Kerbs-Henseleit's solution and aeration were maintained. The reservoir of the solution was maintained at 37°C by using thermostat. The flow of the solution passing through the heart was controlled by a tap and observed

in a drop-chamber. The apex of the ventricles was attached by a thread to a sterling lever writing on a smoked drum. Both maximal and submaximal stimulation of the myocardium were obtained by this method. The drugs like adrenaline, *Hygrophila auriculata* leaves extract, acetylcholine and atropine were administered in the injection port as per the protocol to obtain their activity on heart. Kymograph was maintained at 0.25 rpm. Graphs were obtained by the lever attaching with smoke drum. Different doses of extract of *Hygrophila auriculata* leaves were applied in the same heart.

RESULTS AND DISCUSSION:

Preliminary phytochemical investigations:

The phytochemical examination of ethanolic (70%) extract of *Hygrophila auriculata* leaf material reveals the presence of alkaloids, carbohydrates, tannins, saponins, flavonoids, and glycosides.

Effects of various agents along with extract on heart model:

In this investigation, the cardiotropic potential of an extract from *Hygrophila auriculata* leaves was assessed. It was contrasted with the parasympathetic and sympathetic pathways to more clearly illustrate its mode of action on the heart. In an isolated rat heart, a prototype of parasympatholytic, sympathomimetic, and parasympathomimetic drugs were tested together.

Response of acetylcholine:

Both somatic and autonomic neurotransmitters include acetylcholine. Acetylcholine interacts with the muscarinic (M1, M2, M3) and nicotinic (NN, NM) receptors to exert its effect. The M2 receptor found in the heart regulates the effects of acetylcholine on the heart. Acetylcholine, a parasympathomimetic stimulant, causes the SA node to become hyperpolarized, which slows the pace of phase IV depolarization and reduces the generation of impulses. A bradycardia-like condition could be the situation. Increased refractoriness on the AV node and bundle of his results in a poor chronotropic impact **[10, 11]**. Evidence from the ECG graph of decreased A-V nodal conduction or block. A certain extent of an increase in the PR interval is followed by a detrimental inotropic impact. When conduction is homogenous, vagal innervation reduces the cardiac fiber's resting potential, which causes arterial fibrillation. Vascular dilatation is a result of acetylcholine. Endothelium-dependent relaxing factor (EDRF)-mediated vasodilation results from the presence of the muscarinic receptor (M3) on vascular endothelial cells.

Acetylcholine was administered to the isolated rat heart in this experiment in several dosages, including 0.0125 ml,

0.0250 ml, 0.0500 ml, and 0.1 ml. In 0.0125 ml of acetylcholine, the heart generated 20 beats per minute, as well as 30 drops. 8 beats and 13 drops/minute were seen in the 0.0250 ml dose. 3 beats and 6 droplets per minute were produced by the 0.05 ml dosage. After receiving 0.1 ml of acetylcholine, the heart displayed complete cardiac failure. Acetylcholine caused a negative inotropic (droplet count) as well as negative chronotropic (beats/min) response that was dose-dependent. At higher doses, like 0.1 ml, cardiac failure due to SA nodal hyperpolarization led to the heart failing.

Table 2: Observation of Heartbeat at different dosage of acetylcholine

Acetylcholine (ml)	Drops/min	Beats/min
0.0125	30	22
0.0250	13	8
0.0500	6	3
0.1	-	Cardiac failure

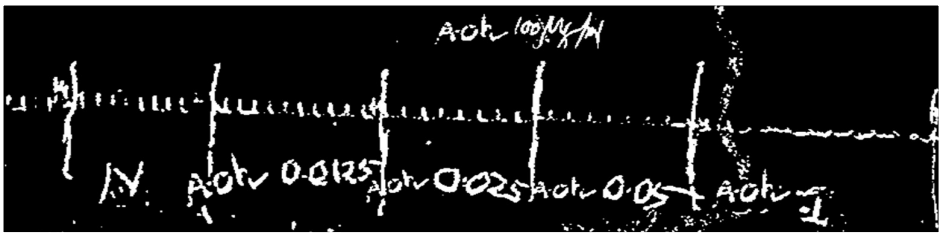

Figure 1: Dose Response Curve of Acetylcholine

Response of atropine over acetylcholine:

Atropine, an anti-cholinergic medication, inhibits the SA node's M2 receptor (competitive inhibitor), which causes a decrease in vagal tone and tachycardia. In the absence of acetylcholine, atropine has no effect since it is an antagonist of the parasympathetic nervous system **[10, 11]**. To compare the effects of atropine and acetylcholine in this experiment, this model was developed.

42 beats and 56 drops per minute were detected after the addition of 0.025 ml of atropine and 0.025 ml of acetylcholine. This finding made it obvious that atropine caused tachycardia within the acetylcholine-induced heart.

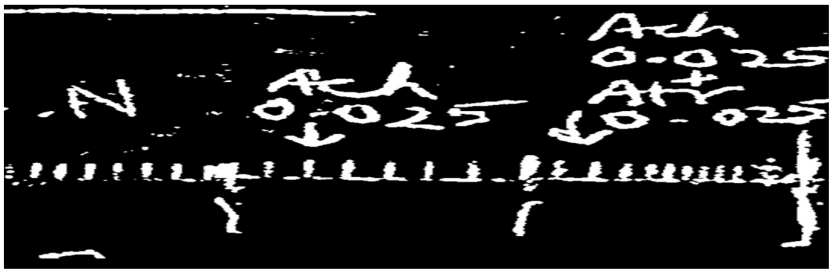

Figure 2: Response Curve of Atropine Over Acetylcholine

Response of adrenaline:

The sympathetic portion of the autonomic nervous system receives a lot of adrenaline transmission. Noradrenaline and dopamine, two catecholamines that are connected to one another, influence the sympathetic

nervous system. In the adrenergic nerve terminal, noradrenaline is kept in synaptic vesicles. As a result of the stimulation, catecholamines are generated by exocytosis, subsequently followed by noradrenaline or adrenaline, which are then released and bind to an abundance of adrenergic receptors in our body. Adrenergic receptors are divided into types (α1, α2) and β (β1, β2, β3). The intracellular generation of second messengers, such as cyclic AMP or IP3/DAG, as well as the regulation of ion channels, can all be changed by the G-protein coupled receptors. K+ or Ca++ are both present in the heart, and blood vessels also contain β2. The heart and blood vessels are post junctional or effector organs that contain α.

Because nodal cells in the SA node's phase 4 depolarization slope rise with adrenaline, as a result heart rate increases. Blood pressure rises as a result of arrhythmia, which is caused by increased latent pacemaker activity in the AV node and purkinje fibre. The pacemaker activity is revealed by a sudden surge in blood pressure response. Systole shortens more than diastole as a result of the positive inotrope. The heartbeat rate goes up. Conduction through the AV node, purkinje fibre, and SA node all provide higher impulse, which is followed by a favorable chronotropic effect. Positive inotropic and chronotropic effects on the heart are mostly caused by molecule β1 in the body.

Depending on the medication, its dose, and the effector organ, catecholamines have a vasoconstriction (α1) or vasodilation (ß2) impact on blood vessels. While the diastolic pressure falls as a result of adrenaline, the systolic pressure rises. Despite having no ß1 effect, noradrenaline raises systolic, diastolic, and mean blood pressure. Isoprenaline causes the mean blood pressure to decrease while causing the systolic pressure to rise (ß1 cardiac stimulation, ß2 vasodilation) **[10,11]**.

When adrenaline was administered to the heart, a normal dose-dependent sympathomimetic response was observed. Here, we employed a stock solution of 100 g/ml of adrenaline in the experimental model. An isolated rat heart was given 0.05 ml of adrenaline, and the least increase in contraction force and rate was found to be 96 drops and 68 beats per minute. While 0.1 ml of adrenaline caused 111 drops and 88 beats per minute, 0.2 ml of adrenaline caused 130 drops and 98 beats per minute as its maximum reaction. However, due to the restricted number of receptors in isolated tissue, the subsequent larger dose (0.4 ml) demonstrated 119 drops and 92 beats per minute (supramaximal response).

Table 3: Observation of Heartbeat at different dosage of Adrenaline

Adrenaline (ml)	Drops/min	Beats/min
0.05	96	68
0.1	111	88
0.2	130	98
0.4	119	92

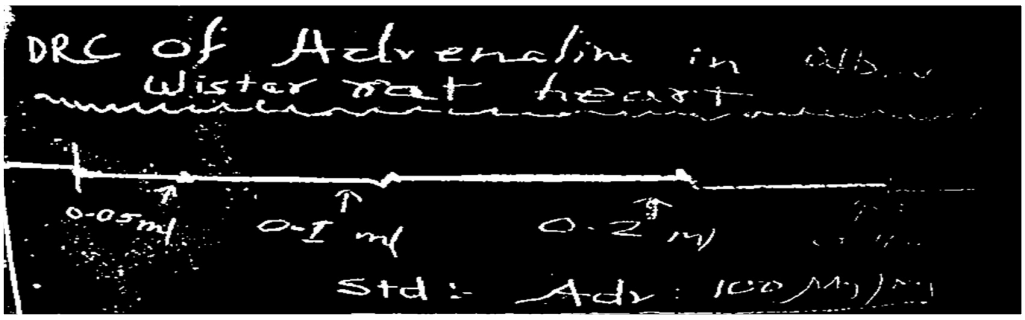

Figure 3: Dose Response Curve of Adrenaline

Dose Response curve of *Hygrophila auriculata:*

The leaf extract of *Hygrophila auriculata* was dissolved in distilled water to produce µg/ml of stock solution, from the stock solution 0.1 ml was applied on isolated rat heart and 80 drops/minute (inotropic action) and 52 beats/min (chronotropic effect) was observed. In 0.2 ml 90 drops and 60 beats and in 0.4 ml 144 drops and 76 beats were produced.

From the above finding it is clear that hydroalcoholic extract of *Hygrophila auriculata* leaves has shown positive inotropic and positive chronotropic effect in dose dependent manner. It has also been observed *Hygrophila auriculata* leaves extract has produced the cardiac activity resemble with adrenaline. From here it can be stated that *Hygrophila auriculata* is having significant cardiotonic activity. Cardiotonic activity mostly exhorted through cardiac glycoside. The increase in cardiac impendence in isolated heart in dose dependent manner due to the presence of cardiac glycoside in *Hygrophila auriculata* .[7]

In the present research *Hygrophila auriculata* was evaluated for cardiotropic activity on heart. The response curve of *Hygrophila auriculata* was obtained and compared with sympathetic and parasympathetic agents. The cardiotonic response supports the traditional use of *Hygrophila auriculata* on retarded heart.

Table 4: Observation of Heartbeat at different dosage of *Hygrophila ariculata*

Hygrophila auriculata extract (ml)	Drops/min	Beats/min
0.1	80	52
0.2	90	60
0.4	144	76

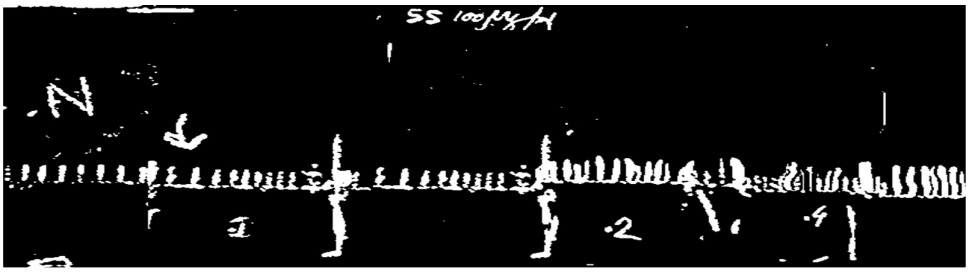

Figure4. Dose Response Curve of *Hygrophila ariculata*

CONCLUSION:

The leaves of *Hygrophila auriculata* were gathered, cleaned, dried in the shade, and extracted with 70% ethanol. To make the stock solution (100 g/ml), distilled water was used. On an isolated rat heart, acetylcholine, adrenaline, and a leaf extract of *Hygrophila auriculata* were applied, and the dose response curves of the various compounds were noted. Due to the presence of cardiac glycoside, *Hygrophila auriculata* has a cardio tonic action that may help alleviate heart failure. The traditional usage of *Hygrophila auriculata* in heart failure is confirmed by this research.

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