

## Preclinical Evaluation of Tropic Activity on Isolated Frog Heart by Using Volatile Oil of *Nigella sativa* Seeds

Sanchita Subhendunath Samadder<sup>1</sup>, Shristi Singh<sup>2</sup>, Anumita Saha<sup>3</sup>, Aishi Chatterjee<sup>4</sup>, Dr. Mrityunjoy Majumdar<sup>5</sup>, Sourav Roy<sup>6</sup> and Prodip Roy<sup>7</sup>

<sup>1</sup>Student, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdah, Nadia, West Bengal - 741222, INDIA.

<sup>2</sup>Student, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdah, Nadia, West Bengal - 741222, INDIA.

<sup>3</sup>Student, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdah, Nadia, West Bengal - 741222, INDIA.

<sup>4</sup>Student, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdah, Nadia, West Bengal - 741222, INDIA.

<sup>5</sup>Professor (H.O.D), Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdah, Nadia, West Bengal - 741222, INDIA.

<sup>6</sup>Assistant Professor, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdah, Nadia, West Bengal - 741222, INDIA.

<sup>7</sup>Assistant Professor, Department of Pharmacology, Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdah, Nadia, West Bengal - 741222, INDIA.

<sup>2</sup>Corresponding Author: shristisingh16503@gmail.com



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### ABSTRACT

The lifestyle of people has changed over the time. Before the revolution days, people suffered from various diseases and the availability of medicines were not available. Today's life is busier. Healthcare system has improved a lot but at the same time the number of people getting affected by different sources is still on a rise. The number one cause of death worldwide is due to heart diseases. The death rates due to cardiovascular disease increased. In order to improve the health conditions, there is a continuous development of synthetic and herbal medicines. Recently, rise in the use of herbal medicines have due to its natural origins and less side effects. One of the emerging herbs is *Nigella sativa* described as the remedy of all diseases. The tropic activity of the extracted volatile oil from *Nigella sativa* is observed on the isolated frog's heart. The heart was isolated from the frog and was treated with adrenaline and acetylcholine to study its activity respectively. The isolated heart was also treated with the volatile oil of *Nigella sativa* seeds which produced dose dependent bradycardia like situation followed by cardiac arrest.

**Keywords-** cardiotropic, herbal remedies, bradycardia, cardiac arrest, hypotensive, volatile oil.

### I. INTRODUCTION

Since the dawn of civilization, herbs have been utilized to cure illnesses and are now a staple of modern pharmacology. Prior to now, the focus on using medicinal herbs had been on disease treatment rather than prevention. However, there have been a lot of recent studies on the use of medicinal plants and the

substances found in them in the prevention of disease reported in the literature.

There were numerous herbs that had been used for centuries to treat cardiovascular disorders. Cardiovascular illnesses (CVDs), which include coronary heart disease (heart attacks), cerebrovascular disease (stroke), hypertension, peripheral artery disease, rheumatic heart disease, congenital heart disease, and

heart failure, are brought on by problems with the heart and blood arteries.<sup>[1]</sup>

*N. sativa* L. is a spice plant belonging to the family Ranunculaceae<sup>[2]</sup>. It is a medicinal plant with dark seeds that has been used as a home treatment for a number of conditions. It has been demonstrated that the seeds of *N. sativa* L. contain more than 30% of a fixed oil and between 0.40 and 0.45% of a volatile oil (VO). The VO has been demonstrated to have bronchodilator<sup>[3]</sup>, bactericidal<sup>[4]</sup>, diuretic<sup>[5]</sup>, and hypotensive<sup>[6]</sup> as well as immune-potentiating<sup>[7]</sup> activities. It also contains 18.4–24% thymoquinone and a total of 46% of several monoterpenes, including p-cymene and -pinene<sup>[8]</sup>.

## II. MATERIALS AND METHODOLOGY

### *Nigella sativa* seeds Collection:

Seeds of *N. sativa* were collected from near market of Netaji Subhas Chandra Bose Institute of Pharmacy, cleaned, and stored.

### Extraction:

With the use of a mixer grinder, the seeds of *N. sativa* were reduced to a fine powder. By using the steam distillation procedure, the oil was separated from the powder and kept in a collection.<sup>[9]</sup>

### Animals:

Frogs (*Bufo melanostictus*) were collected and housed in NSCBIP animal houses. They were fed regular food pellets and water, and they had a 12-hour light/dark cycle. Prior to doing the experiment, the animals spent seven days acclimating in the animal home.

### Procedure:

Firstly pith the frog and pin it to the frog board. Then give a midline incision on the abdomen. By removing the pectoral girdle and expose the heart carefully has to remove the pericardium and put a few drops of frog Ringer over the heart. Then tracing of the inferior vena cava is done and, a thread is put around it, and gives a small cut in order to insert the venous cannula which is in turn connected to a perfusion bottle containing frog Ringer. After this inserted the cannula in the vein and tied the thread to assure the cannula is in place. A small cut was given in one of the aortae for the perfusate to come out. Adjustment of a proper venous pressure of 2-4 cm was done by altering the height of the perfusion bottle. The effective venous pressure is the height in cm from the level of the venous cannula and the Ringer level in the perfusion bottle. Start the perfusion by opening the screw clamp attached to the tube. A thin pin hook is passed through the tip of the ventricle and with the help of a fine thread attached to a hook, ties it to the free limb of the universal lever, which is fixed to a stand. Adjustment of proper tension and magnification by altering the height of the lever is done. Recording the normal contraction of the heart was taken on the smoked drum. Injection of 0.1, 0.2, 0.4, and 0.8 ml of the stock solution of each drug in sequential order

was observed to note the change in the rate and amplitude of contraction. Keeping at least five to six minute gap between the administrations of each dose of the drug is required here. The drug is administered by injecting the drug into the perfusion tube very close to the venous cannula. Precautions must be taken to avoid any leakage of the drug from the tube and the injection of air bubbles. Labelling took place and fixes the solution with the fixing solution.

## III. RESULT AND DISCUSSION

### *Dose-response curve of adrenaline*

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 frog heart. The response was gradually increasing in dose dependent manner. As the dose increases the response length (force of contraction and number of peak/minute (rate of contraction) both increases.<sup>[10][11][12]</sup> In the dose of 0.2ml the adrenaline shows its highest effect (maximum response) on isolated frog heart, but as the number of receptors is limited in isolated tissues, in higher dose (0.4ml), it has shown its saturation peak (supramaximal response).

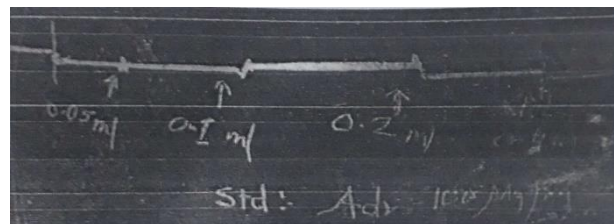


Figure 1: Dose response curve of Adrenaline on frog heart model.

### *Dose-response curve of Acetylcholine*

Ach produces arrhythmia at 0.0125ml (concentration 100µm/ml) on the frog heart. But total cardiac arrest could not occur. But when applied 0.025 ml of acetylcholine, total cardiac arrest occurred. There was no peak in the graph. Cardiac contractility totally fall down and beats were disappeared.<sup>[10][13][14]</sup>

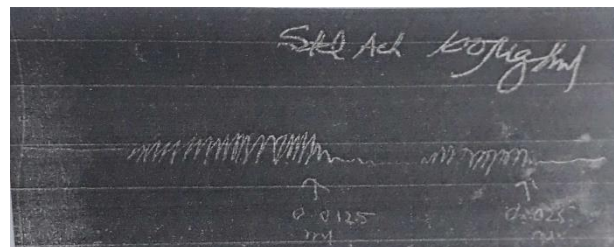
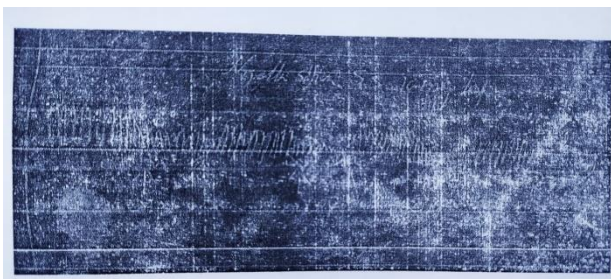


Figure 2: Dose response curve of Acetylcholine on frog heart model

### *Dose-response curve of Nigella sativa*

*Nigella sativa* has shown typical activity when applied in isolated frog heart preparation. This drug has

shown negative inotropic and negative chronotropic activity when applied in the heart, but in dose dependant manner. The volatile oil of *N.sativa* was applied in 0.1, 0.2, 0.4, 0.8 ml of doses and in all the doses it has shown decrease in tropic activity<sup>[15][16][17]</sup>



**Figure 3: Dose response curve of *Nigella Sativa* seed volatile oil extraction on frog rat model**

#### IV. CONCLUSION

The seeds of *Nigella sativa* were taken and grinded in the coarse powder. The volatile oil from that powder was extracted by using steam distillation method & was collected & stored. The heart was isolated from the frog & was treated with adrenaline & acetylcholine which showed the characteristics effects respectively. The isolated heart was also treated with the extracted volatile oil of *Nigella sativa* and the effect was also seen. It produced dose dependent bradycardia like situation followed by cardiac arrest.

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