SHORT COMMUNICATION

Effect of *shilajit* on the heart of *Daphnia*: A preliminary study

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ABSTRACT

Shilajit is a mineral-rich complex organic compound used in the traditional system of Ayurvedic medicine for treating hypertension and improving the cardiac function with many herbomineral preparations. However, very little experimental evidence is available about its effect on the cardiac function. We used *Daphnia* as a model organism for observing the effect of *shilajit* on its heart due to its myogenic properties and its response to number of cardioactive drugs that are known to affect human heart function. Genome of *Daphnia* shows the strongest homology with the human genome. These characteristics of *Daphnia* make it an ideal organism for biomedical research. Our results suggest that this complex organic compound lowers the heart beats as its concentration increases from 1.0 to 100 ppm. The beats come to near normal condition at 1000 ppm. Above 1000 ppm, the beats are very fast and impossible to count. These results indicate a negative chronotropic effect on the *Daphnia* heart at low concentrations and a positive chronotropic effect to arrhythmia and finally failure at increasing higher concentrations of *shilajit*.

Key words: Chronotropic effect, *Daphnia*, *shilajit*

INTRODUCTION

Shilajit (L. *Asphaltum*) also known as "mineral pitch" is a pale brown to blackish brown rock exudates found in many mountain ranges of the world, especially the Himalayas and Hindukush ranges of the Indian subcontinent.[1] *Shilajit* is a complex mixture of organic humic substances as well as plant and microbial metabolites which occur in the rock rhizospheres.[2] Substances identified in *Shilajit* include moisture, gums, albuminoids, resin, vegetable matter, benzoic acid, silica, minerals, vitamins, and many other substances.[3,4] It is used for the last thousands of years as a rejuvenator and as an adaptogen in traditional medicinal systems of many countries[5,6] and has been attributed with miraculous healing properties.[7] Recent reports have revealed that it has antioxidant,[8] anti-inflammatory,[9] and anxiolytic activity.[10] It has also demonstrated spermiogenic and ovogenic effects in mature rats.[11] Although many herbomineral preparations aimed to treat hypertension and cardiac hypertrophy use *shilajit* studies related to its effect on blood pressure and cardiac hypertrophy have not been reported so far which prompted the present investigations.

In this study, we used *Daphnia* [Figure 1] as a model due to several reasons such as their high responsiveness to

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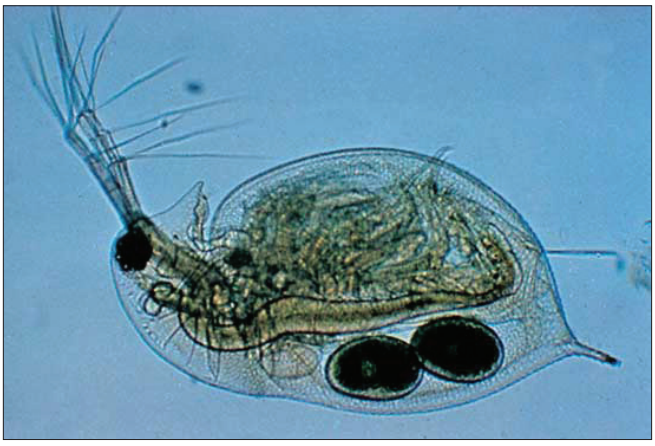

Figure 1: *Daphnia* (Encyclopedia Brittanica Image)

pharmacological agents added to culture water, [12] their transparent body enabling easy observations of heart beats, heart being myogenic with a myogenic pacemaker inhibited by extracardiac cholinergic nerves,[13] similarity of the ultrastructural features to striated and cardiac muscles of other species.[14] In addition, the genome of *Daphnia* shows strong homology with the human genome[15,16] which provides a provisional clinical relevance. *Daphnia magna* responds to many cardioactive drugs known to affect human heart function.[17,18] Thus, the organism is suitable to be used as a model for studying disease process, toxicological studies, and for biomedical research; However, there are many species in the genus which are poorly described taxonomically at the species level.

MATERIALS AND METHODS

Preparation of different concentrations of *Shilajit*

Shilajit Sat was purchased from a local distributor and stored at ambient room temperature. Different concentrations were prepared in distilled water. Due to lack of previously reported information on its lethal dose in *Daphnia* and in humans, a starting concentration of 1 ppm was chosen so that the highest concentration did not exceed 100–200 mg/L. The concentration of *shilajit* was logarithmically increased (1, 10, 100, and 1000 ppm). The stock and working solutions of *shilajit* were maintained under refrigeration at 4–8°C during the course of this study [Figure 2].

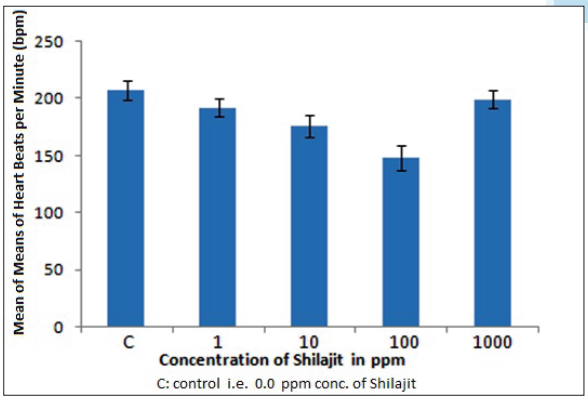

Figure 2: Observed mean of means of heart rate of daphnia following exposure to Shilajit at different concentrations

Collection of *Daphnia*

Daphnia were collected from the pond water from Ahmednagar College Campus without ascertaining the exact species. After the settling of debris, mud, and particulate matter, *Daphnia* were transferred to cavity block containing filtered pond water. Organism was maintained using pond culture method at ambient temperature in the laboratory till the completion of this study.

Counting of heart beats

After acclimatization for 10 minutes, an individual *Daphnia* was carefully transferred, to a cavity slide containing filtered pond water. Its heart beats were counted for 10 seconds, under the low power objective of Olympus compound optical microscope using a countdown timer with audible signal. In all, 25 such observations were taken and their mean was taken as a control reading. The organism was then carefully transferred to another cavity block containing 1 ppm *Shilajit* solution, acclimatized for 10 minutes, and 25 observations of heart beats for 10 second intervals were noted. The procedure was repeated for *shilajit* concentrations of 10, 100, and 1000 ppm using the same organism. Data were obtained for 20 individuals of *Daphnia* as described above.

Data analysis

The mean value and standard error per organism per *shilajit* concentration were calculated. Similarly, the mean of means of heart beats per 10 seconds were calculated in each case and were converted to heart beats per minute with standard error. Both the data were tested using Students' distribution (*t*-test) at 95% CI. The data of mean of means are shown in Table 1.

RESULTS AND DISCUSSION

The individual variations in the rhythm observed may be a result of body size, sex, and physiological factors. However, the individual rhythm appears to be fairly constant during treatment with *shilajit* concentrations of 1, 10, and 100 ppm. A rapid increase in heart beat frequency was observed when the organism was treated with *shilajit* concentration above 1000 ppm. The frequency increase was so rapid that heart beats could not be measured manually.

Table 1: Mean of means of heart beats per minute of *Daphnia* at different concentrations of shilajit with standard error at 95 % confidence interval*

Concentration of Shilajit in ppm	Mean of means of heart beats per minute (bpm)	Standard error at 95 % CI
0.00	207.46	8.84 Control (pure pond water)
1.00	191.59	7.85
10.00	176.11	9.86
100.00	148.43	10.64
1000.00	199.26	8.20

*Note: $N = 20$ and df = 19. For df = 19, $t = 2.09$ at 0.05 level

The data represented in Table 1 are the mean of means of heart beats of *Daphnia* per minute under control and test conditions with 95% CI. It was revealed that the frequency decreases by 7.65% at 1 ppm, 15% at 10 ppm, and 28.45% at 100 ppm treatments, respectively, indicating a negative chronotropic effect at low *shilajit* concentrations, whereas treatment with 1000 ppm showed a positive chronotropic effect. Although the exact mechanism of action is not yet analyzed, it is possible that the negative chronotropic effect may result from a direct effect on muscle or stimulation of the cholinergic nerves to the pacemaker.

The probable reason for the positive chronotropic effect may be due to mimicking of adrenaline- and noradrenaline-like effect or a change in Ca^{2+} levels which needs to be assessed during further studies. At increasing higher concentrations, *shilajit* may be leading to hypercalcemia in daphnia. This condition is associated with abnormal heart rhythm (arrhythmia) leading to cardiac arrest. We feel that the accelerating action of this drug on daphnian heart is of less physiological significance because it occurs only with high concentrations (>1000 ppm). This study being a preliminary investigation does not satisfactorily explain the related phenomena, but the results are still encouraging and of provisional clinical relevance prompting further in-depth studies for which authors intend to include echocardiographic analysis to strengthen their observations along with biochemical studies.

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