Effect of *Withania somnifera* and *Nardostachys jatamansi* on heart rate of *Moina mongolica*

Ms. Veenatai Dongare, Ms.Vishakha Chodanakar and *Shashibhal Pandey

*Smt. CHM College, Ulhasnagar, Dist: Thane, Maharashtra 421003, India

*Correspondence: pandey.shashibhal@gmail.com

Abstract.

Many Cladocerans are established as model organisms to study effects of various toxicants, phytochemicals. The effect of different chemicals like caffeine, ethanol, aspartame (artificial sugar), and lactose on the heart rate of different species of *Daphnia* have been studied earlier. Likewise the procedures to carry out those experiments have been optimized. But its cousin *Moina* belonging to family *Moinidae*, has rarely been used as model organism for pharmacological studies. In the present study we have examined the effects of phytochemicals like *Withania somnifera* and *Nardostachys jatamansi*, on the heartbeat of *Moina mongolica*. Both of these phytochemicals have been used as medicine in Ayurveda since ancient times and are consumed by humans, but their effect on the heart rate of this small Arthropod was not explored. We prepared extracts of these phytochemicals and observed their effect on heart rate of *Moina.* Our study showed that 1% extract of *Withania* had no effect on heart rate of *Moina,* but showed positive effect on the growth of *Moina.* In contrast to this, *Moina* couldn't survive in 1% extract of *N. Jatamansi.* Whereas lower concentrations of *jatamansi* showed decrease in heart rate of *Moina*. The hypotensive effect of *N.jatamansi* in lower organism like *Moina,* is novel observation which indicates a promising scope for extending such studies on higher organisms, so that safer dose for the consumption can be determined. Our study showed that *Moina* can also serve as model for pharmacological studies.

Key words: *Moina mongolica, Withania somnifera, Nardostachys jatamansi,* Ayurveda, hypotensive effect, ecotoxicology.

1. INTRODUCTION

Withania somnifera (family: Solanaceae) is popularly known as Ashwagandha. It has been used in Ayurveda-the Indian system of traditional medicine since times immemorial. The human doses of *Withania somnifera* (Ashwagandha) are generally in the range of 4-6 g/day and are safe and non-toxic. Steroidal alkaloids and lactones known as 'withanolides' are the active ingredients present in withania and they are responsible for most of the biological actions of *Withania*[1]. It possesses adaptogenic, anti-inflammatory, antioxidant, anti-hypersensitive and hypoglycemic effects which contribute to its cardio protective properties[2]. Ashwagandha also shows antibacterial and antifungal properties. *W.somnifera* extract shows significant inhibition of some important bacteria's like *Staphlococcus aureus, Bascillus subtilis, E.coli, Raoultella planticola, Pseudomonas aeruginosa, Enterobactor aerogens.*It also inhibits yeast *Candida albicans* & a fungi *Aspergillus flavus*[3]. Studies indicate that the Ashwagandha (*Withania somnifera* Dunal) possess antistress property. It also appears to exert a positive influence on the endocrine, cardiopulmonary & Central Nervous System [4].

Nardostachys jatamansi is a commonly used ayurvedic herb. The roots are the main medicinal part which contains aromatic compounds responsible for its therapeutic effect in mood and stress disorders. Jatamansi has cardio protective properties and enhances the functions of heart and regularizes heart rate. The decoction of roots is used in mental disorders, insomnia and disorders of blood and circulatory system[5]. It consists of

sequiterpenes such as jatamansic acid and jatamansone, lignans and neolignans in the roots of the plant. In addition to this, it also shows the presence of volatile oils like jatamansic oil in the roots and rhizomes of the herb[6]. *Nardostachys jatamansi* protect heart from ISO (isoproterenol hydrochloride) induced toxicity. The cardio protective activity is due to antioxidant and hypolipidemic activity. Jatamansi is hypolipidemic as well as cardio protective[7]. In animal (rats, cats, dogs) studies, essential oil of rhizome of *N.jatamansi* . As well as Jatamansone displayed significant and prolonged hypotensive effect. Jatamansone also possess antiarrhythmic activity in certain animal models, besides its sedative and tranquilizing activities[8].

Although these phytochemicals are used from a long time for treatment of various disorders, its direct effect on the cardiac activity has not been studied. Hence we carried out the present study to test its effect on simple organisms like *Moina.* It is convenient to use and easy to maintain the culture in laboratory. As it is an invertebrate and hence no ethical permission is required for carrying out the research experiment. Also it is easier because the dose of the drug can be directly introduced in the culture medium and do not require any special equipment.

Daphnia, a water flea is used as a modal organism, as it has a myogenic, four chambered heart like mammals and a transparent body which will enable easy monitoring of the cardiac activity/ heart beats. In addition, *Daphnia magna* responds to many cardio active drugs that are known to affect human heart[9].

Moina belonging to class Crustecea lies within the family *Moinidae*. They are also water fleas but are smaller in size than *Daphnia*. They are easy to work with than higher organisms like mammals (rats, mice). The heart is readily visible under a compound microscope because of their transparent body. *Moina* tolerates low dissolved oxygen levels of about 0.14-0.93 mg l^{-1}[10]. *Moina mongolica* is a eurythermal species, which can survive in 2–35 ◦C[10]. The maximum intrinsic rate (rm=0.32) and longest life span of 10 days occur at 25 ◦C while the shortest spawning interval is 1 day, and maximum lifetime fecundity (20.4 eggs per female) occur at 28 ◦C. Between 25 and 28 ◦C, M. *mongolica* reaches maturity in 4–4.8 days and spawns every 1–2.4 days. In the lifetime, *M. mongolica* reproduces 1.5–2.8 times and produces 16–20.4 offspring[11].

To observe the antistress and cardio protective effect of *W.somnifera* and hypotensive effect of *N.jatamansi* this study was carried out on *Moina.*

2. Materials & Methods

2.1Materials

Initial Moina culture, 1 liter water bottles (dechlorinated water), Powder of roots of Ashwagandha and Jatamansi, simple microscope, algae, Milk, stop watch, cotton, cavity slide.

2.2Methods

2.2.1 Preparation of W.somnifera and *N.jatamansi* extract

The powder of roots of *W.somnifera* and *N.jatamansi* was used for the preparation of water extract. 1g of powder was added in 100ml distilled water to prepare solution of 1% concentration of both the phytochemicals and kept overnight. The solution was centrifuged and the supernatant (extract) was used.

2.2.2 Culture of *Moina*

At room temperature (i.e. 28^0C), in a one liter bottle of dechlorinated water, 5 Moina were added along with few drops of milk as a source of food (As bacteria present in the milk supports growth of *Moina)*. Initially they were red in color due to the presence of hemoglobin pigment in the blood which tends to increase during less amount of dissolved oxygen in the water. As only few were added in a liter of water and availability of enough dissolved oxygen, *Moina* turned white/transparent in color.

As the number of Moina increased there was an increased demand for oxygen and hence *Moina* started turning red. Algae were added at this point to provide oxygen.

2.2.3 Counting of heartbeats

The heart beats were counted for 20 seconds and then by correlating, the data was converted to beats/min. Initially the heart beats of *Moina* in the untreated sample were observed by selecting a random individual. 10 such observations were made. The average heart rate of the untreated *Moina* was recorded.

Moina were introduced in 1% extract of *Withania somnifera* and *Nardostachys jatamansi* and the heart was observed again after 30 minutes. No change was observed. The heart beats of *Moina* in *W. somnifera* extract were measured again after 2 days by selecting 10 random *Moina*. The average heart rate was recorded.

Moina introduced in 1% extract of *Nardostachys jatamansi* were observed after 2 days. The 1% extract of *N. jatamansi* was toxic for *Moina.* Hence solution of low concentrations viz. 0.1%, 0.2% and 0.8% were prepared. Moina were added to these solutions and the heart beats were recorded after 30 minutes.

3. OBSERVATIONS

Sr.no	Beats /20 sec	Beats/ min
1	88	264
2	103	309
3	110	330
4	99	297
5	106	318
6	88	264
7	94	282
8	90	270
9	87	261
10	94	282
mean		287.7

Table1. Counting of heart beat of *Moina* before the treatment with *W.somnifera* and *N.jatamnasi*

Sr. no	Beats/20 sec	Beats/min
1	100	300
2	96	288
3	100	300
4	100	300
5	96	288
6	104	312
7	107	321
8	122	366
9	104	312
10	102	306
mean		309.3

Table2. heartbeat of Moina after treatment with 1% *W.somnifera*

Sr. no	Beats/ 20sec	Beats/ min
1	190	327
2	111	333
3	114	342
4	100	300
5	114	342
6	97	291
7	83	249
8	101	303
9	96	288
10	90	270
mean		303.5

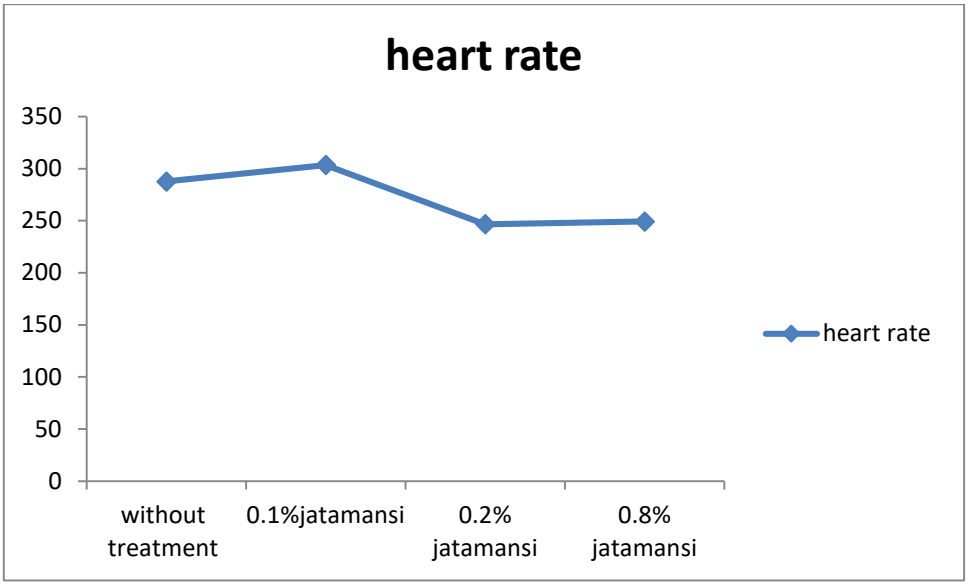
Table 3:Counting heartbeat of Moina after treatment of 0.1% of N. jatamansi extract

Sr. no	Beats/20sec	Beats/min
1	94	282
2	84	252
3	82	246
4	84	252
5	84	252
6	80	240
7	82	246
8	82	246
9	71	213
10	78	234
mean		246.3

Table 4 : :Counting heartbeat of Moina after treatment of 0.2% of N. jatamansi extract

Sr. no	Beats/ 20sec	Beats/min
1	81	243
2	80	240
3	71	213
4	70	210
5	74	222
6	90	270
7	93	279
8	94	282
9	88	264
10	90	270
mean		249.3

Table5. Counting of heartbeat of Moina after treatment of 0.8% of *N.jatamansi*



Graph showing difference in heart rate of Moina after treating with N.jatamansi

4. Results and discussion

The average heart rate of untreated *Moina* was observed to be 287.7 beats/min (table 1). The heart beats of *Moina* in *W. somnifera* extract were measured again after 2 days by selecting 10 random *Moina*. The average heart rate was 309.3 (table 2).

Moina introduced in *N. jatamansi* did not survive as long as it did in the *W.somnifera* extract. The 1% extract of *N. jatamansi* was toxic for *Moina.* Hence solution of low concentrations viz. 0.1%, 0.2% and 0.8% were prepared. Moina were added to these solutions and the heart beats were observed after 30 minutes. For 0.1% the average heart rate was 304.5 beats/min (table 3), and for 0.2% and 0.8% it was 246.3 beats/min (table 4) and 249.3 beats/min (table 5) respectively. Paired T-test was carried out to check whether the increase in heart rate in case of *W.somnifera* extract and decrease in heart rate in case of *N.jatamansi* extract is up to a significant level or not.

Withania somnifera extract of 1% concentration when tested on *Moina* it showed an increase in the heart rate from 287.7 beats/min to 309.3 beats/min , which is approximately 20% increase if examined non-statistically. However on statistical analysis by applying paired T-test by taking null hypothesis [H_O] = *W.somnifera* has no significant effect on the heart rate and alternative hypothesis [H_A] = *W.somnifera* has significant effect on the heart rate, the calculations showed that H_O is accepted i.e. *W.somnifera* has no significant effect on the heart rate of *Moina (*as P value >0.05). It was observed that there was a rapid increase in the number of individuals as they proliferated at a higher rate. It did not affect *Moina* and was nontoxic at 1% concentration level. *Moina* survived for about a week in the solution without any source of food provided to it. This shows that *W.somnifera* has some components on which *Moina* was surviving and deriving its nutrition as well as oxygen. This result can be of great significance as it gives us a component which can be used in future in the culture medium of *Moina.*

Nardostachys jatamansi extract of 1% concentration proved to be toxic to *Moina*. This suggested us that unlike *W.somnifera, N.jatamansi* is showing some effect on *Moina*. To test that effect,

1% solution was diluted to make three solutions of lower concentrations viz. 0.1%, 0.2% and 0.8%. The 0.1% treatment of *N.jatamansi* extract showed an increase in the heart rate of *Moina* from 287.7 beats/min to 303.5 beats/min. If examined non-statistically there was a significant decline in the heart rate in 0.2% and 0.8% solutions which was 246.3 beats/min and 249.3 beats/min respectively. On statistical analysis of the data by applying paired T-test and taking null hypothesis [H_O] = *N.jatamansi* has no significant effect on the heart rate and alternative hypothesis [H_A] = *N.jatamansi* has significant effect on the heart rate the calculations shows that H_O is rejected i.e. *N.jatamansi* has no significant effect on the heart rate of *Moina* ***as*** there is a 40% decline observed which proves the hypotensive effect of *Nardostachys jatamansi.* Thus the present study concludes that *N.jatamansi* also shows its hypotensive effect on simple crustaceans like *Moina.* To obtain concrete evidences of these effects more detailed studies of this drug should be carried out in the future.

5. Conclusion

From the conducted study we can conclude that as Moina shows ability to respond to concentrations above 0.1% of jatamansi by showing hypotensive effect. Hence it can serve as the model organism to study effect of various drugs, toxicants etc. on their cardiac activity & further to optimize their dose. These data can serve as fundamental material for further studies for dose optimization for animals having myogenic heart. Ability to tolerate low oxygen levels, less spawning interval of 5-6 days, transparent body, myogenic heart which is able to respond to phytochemicals, ease to culture makes Moina a suitable model organism in research.

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