Antioxidant Activity of Water Soaked in Saligrama Stone – A Preliminary Report

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Abstract
Saligrama otherwise called Shaligram or Salagrama is a fossilized shell stone usually recovered from the banks of the Gandaki river in Nepal. It is supposed to be a sacred symbol of Hindus. Soaking the stone in water for twenty to thirty minutes and partaking the water was considered to give good health. Hence, we mobilized two stones which were authorized as original by experts. The first one was worshipped for more than 5 years and the other one for two years. The water soaked was around 25 ml which was analysed for antioxidant activity by DPPH assay with plain water without soakage as control. The antioxidant property was significantly found in both the stones with more activity in the stone worshipped for more than 5 years. We did not standardize the volume and the duration of soakage to exact values while it was approximated to around 20 – 30 minutes. We suggest more refined studies with estimation of rare elements needs to be performed to establish the beneficial effects of this sacred saligrama stone.

Keywords: Antioxidant, Saligrama, Soak, Stone

1. Introduction
Saligrama or Shaligram refers to a fossilized shell used in South Asia as an iconic symbol and as an incarnation of the God Mahavishnu by some Hindus. Saligrams are usually black coloured stones with different sacred marks. They are the fossilized remains of currently extinct sea dwelling ammonites. Hence, they are found in river beds and other regions that were underwater earlier, the most common being in Himalayas and Nepal. It is found in the river banks of the Gandaki river. Historically, the use of Saligrama in worship can be traced back to the time of Adi Sankara through his work. It is important to remember that even the water that has touched the Saligrama becomes holy water. This belief currently has no scientific evidence. Hence, we tried to find out the antioxidant efficacy of water soaked in saligrama. The aim was to find out the presence of antioxidant substances in water soaked in saligrama stone and whether the years of puja has any effect on such activity.

2. Methods
Two stones with Sudarshan carvings which have been worshipped for different years were considered. The first one was worshipped for more than 5 years with the second one being around 2–3 years. They were brought from the banks of the Gandaki river long time back. Both were certified by a specialist in analysing Saligrama stone and found to be genuine stones from the river (Figure 1). The stone is approximately two inches / one inch with a height of 3/4th of an inch. The two stones which were considered for testing antioxidant activity were being soaked in water to perform puja for twenty to thirty minutes daily.
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for more than two and five years respectively. The water used for soakage was got by a standard Reverse Osmosis (RO) system. On the day of testing, the same puja was performed by soaking the stones in 25 ml of RO water for thirty minutes separately. The samples were labelled stone 2 and stone 5 respectively. At the same time, the plain water (control) collected from the same RO system. All the three samples were subjected to (1, 1-diphenyl-2-picryl-hydrazyl) DPPH assay for antioxidant activity. The procedure was as follows. Briefly, 0.1mM solution of DPPH in methanol was prepared. One mL of this solution was added to 3 ml of the solution of all methanol extracts at variable concentration (50, 100, 200, 400 and 800μg/mL). The mixtures were shaken and then allowed to stand at room temperature for thirty minutes. later the absorbance was measured at 517 nm using a UV-VIS spectrophotometer according to standard protocol. Ascorbic acid was used as the reference. The percentage of inhibition was calculated by (1 – A1÷A0) × 100, where A1 is the experimental water while A0 is ascorbic acid. This was repeated twice to find the average. The DPPH assay was performed on plain RO water. It has to be clearly differentiated that the control group i.e. plain RO water is different from the control ascorbic acid.

3. Results

The results are shown in Tables, 1, 2 and 3. There were three groups. The control and the Saligrama stone worshipped for two years and five years. From the data, we can deduce that there is significant antioxidant activity in water soaked in the stone. Stone 2 showed significantly more antioxidant activity than the control in higher concentrations (400 and 500 µL) while the stone 5 had better antioxidant activity in all the concentrations. The antioxidant activity of stone 5 is significantly more than stone 2.

4. Discussion

The Saligrama stone is usually worshipped by a sect of Hindus. It is supposed to be an avatar of the Lord. The classical puja of the stone is usually done daily by sprinkling water and then soaking the stone in water for around twenty minutes. The same puja was performed for more than five years in stone 5 while in stone 2 it was 2 years. We can detect a significant difference between plain water and the stone 2 water. But the water from stone 5 had more antioxidant activity. Usually stones are tested for minerals and rare metals. Such study of antioxidant activity of rare stones is being carried out for the first time. A detailed search on the internet did not reveal any references of any abnormal significant health benefits of

Table 1. DPPH assay results of RO water plain

| Concentration (µL) | I    | II   | Average | Inhibition (%) |
|-------------------|------|------|---------|---------------|
| Control           | 0.926| 0.923| 0.9245  | 0             |
| 100               | 0.917| 0.924| 0.9205  | 0.432666306   |
| 200               | 0.916| 0.911| 0.9135  | 1.189832342   |
| 300               | 0.895| 0.902| 0.8985  | 2.81233099    |
| 400               | 0.889| 0.883| 0.886   | 4.164413196   |
| 500               | 0.876| 0.87  | 0.873   | 5.570578691   |
| Concentration (µL) | I   | II  | Average | Inhibition (%) |
|-------------------|-----|-----|---------|---------------|
| Control           | 0.926 | 0.923 | 0.9245 | 0             |
| 100               | 0.912 | 0.919 | 0.9155 | 0.973499189   |
| 200               | 0.899 | 0.905 | 0.902  | 2.433747972   |
| 300               | 0.878 | 0.872 | 0.875  | 5.354245538   |
| 400               | 0.855 | 0.86  | 0.8575 | 7.247160627   |
| 500               | 0.827 | 0.823 | 0.825  | 10.76257436   |

Table 3. DPPH assay of stone 2 years

| Concentration (µL) | I   | II  | Average | Inhibition (%) |
|-------------------|-----|-----|---------|---------------|
| Control           | 0.926 | 0.923 | 0.9245 | 0             |
| 100               | 0.922 | 0.918 | 0.92   | 0.486749594   |
| 200               | 0.909 | 0.916 | 0.9125 | 1.297998918   |
| 300               | 0.899 | 0.893 | 0.896  | 3.082747431   |
| 400               | 0.856 | 0.865 | 0.8605 | 6.922660898   |
| 500               | 0.835 | 0.843 | 0.839  | 9.248242293   |

There was neither decaying nor any problem during the assay. The 5 years stone showed more activity than the 2 years’ stone which was significant.

Saligrama stone being proved in a scientific manner. It is of immense importance that the sample is only water unlike many DDPH assays only being done on plant and leaf extracts. But in our study, the sample is water only. Hence, as such, with this, we were able to find a significant antioxidant effect. Our limitation lies in the fact that we did not standardize the volume of water to a minimum. We had placed each of the stones in 25 ml of water.

5. Conclusion

The water soaked in the Saligrama stone obtained from the river Gandaki showed significant antioxidant activity. The activity increased with the years of puja on the stone.

6. References

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