Poll Res. 39 (2) : 304-308 (2020)
Copyright © EM International
ISSN 0257–8050

AN AYURVEDIC PURIFICATORY METHOD TO REDUCE THE LEAD ACCUMULATION IN ROOT TUBERS OF MUSTA-*CYPERUS ROTUNDUS* LINN (A POTENT MEDICINAL PLANT)

SAILAKSHMI V.J., RAIBY P. PAUL AND PRIYALATHA B.

Department of Dravyaguna Vijnana (Ayurvedic Pharmacology)
Amrita School of Ayurveda, Amritapuri, Amrita Vishwa Vidyapeetham, India

(Received 6 December, 2019; accepted 2 January, 2020)

ABSTRACT

***Musta* (*Cyperus rotundus* Linn.) is a potential Ayurvedic medicinal plant which has got a hyper accumulatory action on lead (Pb). In *Cakradatta* (An Ayurvedic textbook), *Sodhana* (Ayurvedic purification) of Musta has been mentioned. The objective of the present study was to analyze and compare the change in lead content in root tuber of *Musta* before and after *sodhana* (Ayurvedic purificatory method). The study comprises of seven samples of *Musta*. Out of this, 3 were collected from heavy traffic area and 3 from shooting ranges and the non polluted sample was collected from natural source atleast 10 km away from the source of contamination. The samples were subjected to analysis of lead content using Inductively Coupled Plsama - Mass spectrometry before and after *sodhana* (Ayurvedic purificatory method). Results were statistically analyzed using Wilcoxon signed rank test and Mann whitney U test in IBM SPSS Ver 20. Statistical analysis revealed that the lead content is significantly higher in heavy traffic area (17.3 ± 11.9) compared to shooting range (4.32 ± 0.92) and lead content is significantly reduced after purification (1.79 ± 0.80) compared to the lead content before purification (9.55 ± 10.1). Tetra methyl lead is one of the most significant heavy metal contaminant in recent years which is eliminated from motor vehicle exhaust. This might have contributed to the high level of lead in heavy traffic area in the study. The specific *sodhana* (Ayurvedic purification) procedure mentioned in *Cakradatta* is effective in reducing the lead content accumulated in root tuber of *Musta*.**

KEY WORDS : *Musta, Cyperus rotundus,* Lead, Lead contamination, Sodhana, Purification.

INTRODUCTION

Medicinal plants have been a major source of cure for human diseases since time immemorial. These plants have a commanding role in the prevention and mitigation of the diseases through their unique strength.

But in the present scenario, these plants are seriously affected owing to the hazardous effect of environmental pollution. Among the different types of environmental pollution, plants are commonly affected by soil pollution. Contamination of the soil by heavy metals is of prime concern nowadays. Among heavy metals, lead (Pb) is a potential pollutant that readily accumulates in soil and sediments. Soils near highways, smelting areas and shooting ranges have higher levels of lead than soils in other areas because of their exposure to lead dust, which accumulates over time. Although, lead is not an essential element for the growth of plants, it gets easily absorbed and accumulated in different plant parts.[1] According to World Health Organization, the permissible limit of lead in medicinal plants is 10 parts per million. But many plants posses an affinity towards heavy metals. Due to this affinity, these plants will absorb the heavy metals from soil and results in various adverse effects. Accumulation of lead in plants can cause a number of toxic symptoms in plant itself, i.e. growth retardation, chlorosis and blackening of the roots, disturbed mineral nutrition, water imbalance and alteration in membrane permeability. In humans, the exposure to high levels

of lead may cause vomiting, constipation, anemia, high blood pressure, foot drop, hearing loss, memory loss, mood disorders, reduced sperm count, nephropathy and encephalopathy.

Musta (*Cyperus rotundus* Linn.) is one such plant which has got a hyper accumulator effect on lead.[2] Since the habitat of Musta is roadsides, waste lands, paddy fields, water lodged areas etc, the chances of pollution by heavy metals are more. So the Musta collected from these areas will be prone to heavy metal contamination. Musta is a highly potent medicinal plant which is included in more than 260 formulations mentioned in Sahasrayoga (An Ayurvedic text book of formulations). In Chakradutta (A text book on Ayurveda), the purification of Musta has been mentioned. But unfortunately the pharmaceutical companies are not performing purification for Musta. Hence in this study, an attempt has been made to find out the variations in lead (Pb) content in the root tuber of Musta before and after purification.

MATERIALS AND METHODS

The study comprises of one group contains seven Musta (*Cyperus rotundus* Linn.) samples. Out of the 7 samples, 3 samples were collected from heavy traffic roads, (Table 1) 3 from shooting ranges Table 2) and 1 sample from natural source at least 10 kms away from contaminated areas and this was considered as the non-polluted sample in the present study (Table 3). All the samples were taxonomically identified.

Preparation of Root Tubers

Seven samples collected from different places were cleaned off mud and washed thoroughly under tap water. The root tuber was taken and remaining portion was discarded. The root tubers were allowed to dry under shade. After attaining proper dryness, required amount of the samples were made into fine powder and kept in a clean transparent polythene cover and labeled properly for pharmacognostical, physico-chemical, and for the analysis of lead content. Required quantity of root tubers were kept as such in a clean transparent polythene cover for the purification procedure.

Purification of Musta

Root tubers of Musta from each samples were crushed separately and kept aside.

- **Nimajjana in Kanjika (Dipping in fermented cereal)**

Root tuber crushed in khalwayantra (pestle and mortar) were taken in separate vessels and kanjika (fermented cereal) is poured into the vessels above the level of musta. This is kept undisturbed for 3 days. On the 4th day, the samples were taken out and washed in hot water.

Table 1. List of districts and area specification of polluted samples collected from shooting range.

Sl.No.	Polluted sample	District	Area specification (Shooting range)
1	Sample 1	Trivandrum	A R Police camp
2	Sample 2	Kollam	NCC shooting range, Fahimamatha College
3	Sample 3	Kollam	NCC Shooting range, Thevally

Sample 1 was collected on 02/10/2018 and the Sample 2 and 3 were collected on 30/09/2018.

Table 2. List of districts and area specification of polluted samples collected from heavy traffic area.

Sl. No.	Polluted sample	District	Area specification (Heavy traffic area)
1	Sample 4	Pathanamthitta	Pandalam
2	Sample 5	Alappuzha	Mavelikkara
3	Sample 6	Kottayam	Kodimatha

Sample 4 and 5 were collected on 18/11/2018 and Sample 6 was collected on 24/11/2018.

Table 3. Name of district and area specification of non-polluted sample.

Sl. No.	Non-polluted sample	District	Area specification
1	Sample 7	Kollam	Pattazhy

Non Polluted sample was collected on 25/11/2018

- **Dola kashaya Swedana in Panchapallava Kashaya (Boiling in Panchapallava decoction)** Samples were made into a potali (a bundle tied with cloth) and placed in dola yantra (special boiling vessel) Panchapallava kashaya (decoction prepared with leaves of 5 medicinal plants) is poured into the dolayantra above the level of potali. This is kept under low fire for 1 hour. After cooling, samples are taken out and washed with hot water.
- **Atapa soshana (Drying under Sunlight)**

The root tubers were spread on a clean cotton cloth and kept under sunlight for drying.

- **Gudambu Sechana (Washing with jaggery water)**

The dried root tubers were washed with gudambu (Jaggery water)

- **Bharjana (Frying)**

  Root tubers were fried in an iron vessel and then powdered.

- **Bhavana (Trituration)**

  The powdered sample of root tuber of musta was taken in a khalwayantra (pestle and mortar) added with ajamutra (Goat's urine) and sigru twak kashaya (Decoction of stembark of *Moringa oliefera*) . Trituration was carried out for 1 yama (3 hours).
  Thus the samples were dried under shade and packed in a clean transparent polythene cover and labeled.

Analysis of Lead (Pb)

Sample Preparation

Samples of musta before and after the purification procedure were taken and about 0.2 gms from each sample was taken weighed by electronic weighing balance. These samples were taken in MDS tubes. 1 ml Nitric acid (HNO_3), 0.5 mL Hydrochloric acid (HCl) and 1 mL Hydrogen peroxide (H_2O_2) were added to these samples. And one more solution of HNO_3, HCl and H_2O_2 was prepared omitting sample in order to rule out the presence any elements in the acid. All the samples were placed on a water bath for 10 minutes. After 10 minutes the samples were taken out of the water bath. MDS tubes were closed with their lids and the tubes wiped thoroughly. Then they were placed into CEM Microwave digester Mars Xpress model under 175 °C for 1 hour. Digested samples were taken out. Required number of polypropylene tubes were taken and washed thoroughly with 20% HNO_3 followed by ultra pure water. The digested samples were transferred to these clean polypropylene tubes. To these samples again 1 mL HNO_3 was added. These solutions were made up to 50 mL by adding ultra pure water. These 50 mL of samples were then subjected to Rotex Laboratory Centrifuge in 2000 RPM for 4 minutes.

Analysis of Lead (Pb)

Standard solution was prepared in different concentrations such as 0.5 ppb, 5 ppb, 50 ppb, 100 ppb, 200 ppb and 250 ppb. Using the standard solution ICP-MS was calibrated. Then each sample was analyzed for lead content.

Statistical Analysis

The data obtained were presented as Mean ± SD of 7 samples of Musta. Comparison of lead content before and after purification was done by Wilcoxon Signed Rank Test and comparison of lead content between different areas were done by Mann Whitney U Test using IBM SPSS Ver. 20 with a minimum level of significance at $P<0.05$.

RESULTS

Results of Analysis of Lead (Pb)

The result of lead analysis is shown in Table 4.

Results of Statistical Analysis

Comparison of Lead Content (Before Purification) Between Groups

Here the p-value is less than the significant level 0.05; the difference in lead content between shooting range and heavy traffic area is significant. That is,

Table 4. Results of analysis of Lead content (ppm) in root tuber of Musta before and after purification.

Sl. No.	Samples	Lead content (ppm)	
		Before purification	After purification
2	Sample 1	5.22	1.38
3	Sample 2	3.38	1.74
4	Sample 3	4.36	0.98
5	Sample 4	30.2	2.27
6	Sample 5	15.06	2.12
7	Sample 6	6.71	3.15
8	Sample 7	1.93	0.89

Table 5. Comparison of Lead content (before purification) between shooting range and Heavy traffic area.

Group	Mean± SD	Median (Range)	p – value
Shooting Range	4.32 ± 0.92	4.36 (3.38 - 5.22)	0.050
Heavy Traffic Area	17.3 ± 11.9	15.1 (6.71 - 30.2)	

Table 6. Comparison of Lead content before and after purification.

Lead Content (mg/kg)	Mean± SD	Median (Range)	p – value
Before	9.55 ± 10.1	5.22 (1.93 - 30.2)	0.018
After	1.79 ± 0.80	1.74 (0.89 - 3.15)	

there is significant difference in lead content between shooting range and heavy traffic area. The table reveals that the lead content is significantly higher in heavy traffic area (17.3 ± 11.9) compared to shooting range (4.32 ± 0.92). Median lead content confirms that the lead content is significantly higher in heavy traffic area (15.1) compared to shooting range (4.36) (Table 5).

Difference in Lead Content after Purification

Here the p-value is less than the significant level 0.05; the difference in lead content after purification is significant. That is, there is significant difference in lead content after purification. The table reveals that the lead content is significantly reduced after purification (1.79 ± 0.80) compared to the lead content before purification (9.55 ± 10.1).Median lead content confirms that the lead content is significantly reduced after purification (1.74) compared to the lead content before purification (5.22) (Table 5).

Graphical representation of difference in Lead Content before and after Purification

In this graph, X-axis represents the sample and Y-axis represents amount of Lead. Lead content before purification was plotted by blue colour and after purification with red colour (Fig. 1).

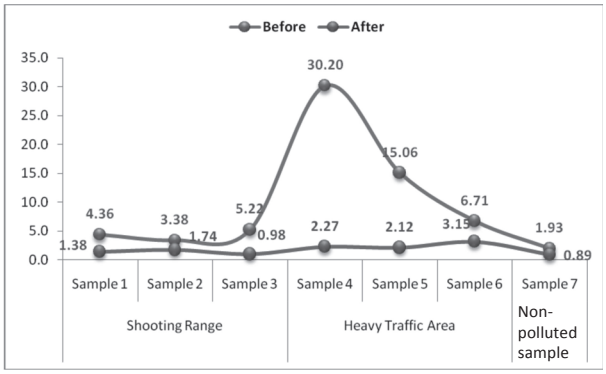

Fig. 1. Graphical representation of difference in Lead content before and after purification.

DISCUSSION

Discussion on analysis of lead

In the present study, among the three samples collected from heavy traffic areas, the greater amount of lead was noted in Sample 4 (Pathanamthitta), i.e 30.2 ppm followed by sample 5 (Alappuzha) i.e 15.06 ppm. Both the values were greater than the permissible limit of lead (10 ppm). Lead content in sample 6 (Kottayam) was 6.71 ppm. The high levels of lead could be due to higher emission of gasoline lead into atmosphere via motor vehicles.

While considering the drug collected from shooting ranges, all the three samples contain lead with in the permissible limit. On comparing them, Sample 1 (Shooting range, A R Police camp) contain relatively more amount of lead (5.22 ppm) followed by Sample 3(4.36 ppm) and then Sample 2 (3.38 ppm). When the 3 sites were compared, it was observed that A R police camp, Trivandrum was more often used as a training ground for shooting than other two sites. Therefore the amount of lead content in that shooting range soil may be higher which might have contributed for the relatively high level of lead in the root tuber.

Discussion on purification

- In Cakradutta, purification of root tuber of Musta has been described in the context of Gandha Dravya sodhana. In that chapter, it was mentioned that the gandha dravyas like Haridra, Vaca, Pippali, Kushta, musta etc should be subjected to purification before using it in a formulation.
- Therefore the particular purification procedure mentioned in Cakradutta was taken for the present study to know whether the purification

has any effect on reducing the lead content accumulated in the root tuber of Musta.

- Lead is a heavy metal which posses the chemical propery of insolubility in water and solubility in mild acids. Here Kanjika which was used for the nimajjana is acidic in nature. The physico chemical analysis of kanjika proved the pH as 5.42 (Acidic). So the kanjika might act as a solvent for the pollutant. Further in dola yantra swedana with pancapallava kashaya, both physical and chemical impurities which are soluble in boiling liquid media could be removed. Here pancapallava kashaya also had acidic pH i.e 5.90. Heat also helps in accelerating the solubility of impurities. This could also be a reason for the reduction in lead content. Moreover the root tuber of Musta exhibited more fragrance after the boiling which indicate that the drug may acquire the property of the media used. Atapa soshana and bharjana of the root tuber might have helped in reducing the moisture content thereby increase the shelf life. Gudambu was used for the washing. Guda (Jaggery) which has Kshara guna (property of alkali) might have absorbed the liquid content from the drug. In Rasâr Guava (a text book on Ayurveda), it is explained that a kshara dravya (drugs with alkaline property) when used as a media can remove all types of impurities. Bhavana was carried out using two medias ajamutra and sigru twak kashaya. Ajamutra and sigru twak kashaya showed alkaline pH as 7.88 and 9.27 respectively. Here the bhavana dravya (medias used for trituration) may help in eliminating the pollutant and also help in reducing the particle size of the drug thereby increase the bioavailability when internally administered. Kshara property of both the media might have contributed for the removal of toxins from the root tuber.
- Altogether, the lead content in all the samples were significantly reduced after purification indicating the overall effect of the purification procedure.

CONCLUSION

Based on the observations and analysis of results in the study, following conclusions were obtained;

1. The specific purificatory procedure mentioned in the Ayurvedic classics is effective in reducing the lead content present in the root tubers of Musta.
2. The heavy traffic area contains comparatively higher level of lead content than the shooting range.
3. The values obtained after the study were alarming category which shows the impending toxicity of lead in near future.

REFERENCES

Sharma, P. and Dubey, R.S. 2005. Lead Toxicity in Plants. *Brazilian Journal of Plant Physiology*. 17(1) : 35-52.

Subhashini, V.V. and Swamy, A.V.V.S 2004. Screening Potential of Three Native Grass Species for Phytoremediation of Heavy Metals. *European Academic Research.* 2(4)