



Original Research Article (Clinical)

Ayurveda intervention for brass-induced heavy metal toxicity: A report from the single-arm pilot study

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ABSTRACT

Background: Brass is a copper-zinc alloy that also contains additional elements, including lead. Industrial operations on brass produce dust and fumes that can be harmful to an individual's health. Ayurveda recommends *Dadima Svarasa* and *Triphala Churna* for the management of symptoms caused due to brass toxicity.

Objective: To obtain preliminary evidence on the efficacy of *Triphala Churna* (powder mixture of three myrobalans) and *Dadima Svarasa* (pomegranate juice) in the management of brass-associated high serum level of copper, zinc, lead, and their harms through an open-label single-arm pilot study.

Materials and methods: A total of 20 workers with brass toxicity and increased level of one or more of the serum ions including copper, zinc and lead were prescribed. 5gm *Triphala* powder once daily and *Dadima Svarasa* 40 ml in two divided doses were administered daily for 28 days. Changes in serum concentration of heavy metals and common health problems like anorexia, headache, dizziness, weakness, nausea, nose irritation, eye irritation, epigastric pain, abdominal distention, chest pain, dyspnea, cough, lumbar pain, body ache, numbness, and occupational dermatitis were reported at baseline and end of the trial.

Results: Trial drugs were found to produce significant decrease in mean values of serum copper ($p < 0.001$) and serum lead ($p < 0.001$) whereas significant increase in mean values of serum zinc ($p > 0.001$) was observed. Significant relief was observed in nausea ($p = 0.005$), anorexia ($p = 0.000$), epigastric pain ($p = 0.001$), abdominal distention ($p = 0.014$), weakness ($p = 0.005$) and body ache ($p = 0.005$).

Conclusion: *Triphala Churna* along with *Dadima Svarasa* are safe and effective in the management of brass-associated high serum levels of copper and lead and their health hazards.

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1. Introduction

Among industrial workers, heavy metal toxicity has proven to be a major threat as it is associated with significant health risks. A variety of human activities such as urbanization and industrialization have resulted in serious environmental contaminations by heavy metals globally.

The brass industry of Jamnagar is one of the largest industries in India. The brass parts industries are mostly concentrated in and around the Jamnagar district which caters to the requirement of around 70% of the machine brass components of India and also exports to various countries [1].

Brass mainly consists of 60–70% copper (Cu) and 20–30% zinc (Zn). The amounts of zinc and copper are the responsible for the strength and versatility of the brass metal [2]. Lead, which is categorized as trace element and toxic metal, is used in the third highest quantity (up to 4%) to improve high machinability, strength and to improve casting characteristics of brass [3]. Chronic brass poisoning has been reported and is characterized by the appearance of anemia and green line at the base of the teeth (due to a large proportion of copper) along with palpitation, dyspnea on exertion, dyspeptic symptoms, anorexia and epigastric pains, nausea, vomiting, and colic [4].

Chelation therapy is the standard treatment to remove toxic metals by using chelating agents; [5] however, the chelating agents have a considerable risk of side-effects including renal failures, arrhythmias, tetany, hypocalcemia, hypotension, bone marrow depression, prolonged bleeding time, convulsions, respiratory arrest, etc. [6] The risk of such serious side-effects has led researchers

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to develop safer alternatives. Ayurveda-based antioxidants and herbal medicines may serve as a relatively safer and better alternative.

Textbooks of Ayurveda, the traditional system of medicine of India, mentioned the traditional way of removing toxins from the body using herbs as antidotes. Poisons/toxins are termed as *Visha* in Ayurveda [7]. Information about toxic and poisonous substances originated from metals, minerals, herbs/foods, or animal products along with their pathophysiology and management are described in detail in classical Ayurvedic texts [8]. Metal toxicity falls under the *Sthavara Visha* (inanimate poison) category in Ayurveda and *Charaka* categorized management for *Visha* into twenty-four categories [9]. *Anupana manjari*, an exclusive Ayurvedic text on managing adverse effects of drugs, recommends the use of *Pakva Dadima Phala Svarasa* (juice of ripe pomegranate) for the poisonous effect of *Pinga* (brass) in particular and *Triphala Churna* for the symptoms caused by *Tridhatu* (a combination of three metal) i.e., *Naga* (lead), *Vanga* (tin), and *Yashada* (zinc) [10]. Pomegranate is one of the most commonly available and frequently consumed fruit across the globe. *Triphala* is an Ayurvedic herbal formulation composed of varied proportion of three myrobalans. Ayurvedic Formulary of India recommends the use of *Triphala* which contains dried fruits from three herbal plants in equal proportions i.e., *Terminalia chebula* Retz (*Haritaki*), *Terminalia bellerica* Roxb (*Bibhitaki*), and *Emblica officinalis* Gaertn (*Amalaki*) [11].

The aim of the present study was to obtain preliminary evidence on the efficacy of *Triphala Churna* and *Dadima Svarasa* in the brass toxicity.

2. Materials and methods

2.1. Study design

An open-label, interventional, single-arm pilot study was designed to achieve the study objectives. The study protocol was approved by the Institutional Ethical Committee (vide letter. no. PGT/7/-A/ethics/2018–19/3399 dated: 14-02-2019). The study was conducted according to good clinical practices (GCP) guidelines and registered in the Clinical Trial Registry, India (vide CTRI Reg. No. CTRI/2019/03/017973). Informed consent was obtained from each of the patients prior to enrollment.

2.2. Study site, sampling, and selection of participants

This study was conducted from August – October 2020 in brass industries of Jamnagar, Gujarat, India located in Dared village, M.P. Shah Industrial Area, Hapa Industrial Area, and Shankar Tekri. Based on the operation of brass units in Jamnagar, brass clusters are engaged in three different operations, viz., casting, machining, and electroplating. Casting units include foundry and extrusion [12]. The potential community-dwelling brass industrial workers were targeted through an observational study that is reported elsewhere. The selection criteria for study participants is briefly described here. One thousand brass industry workers were selected randomly and interviewed. The level of exposure of brass was accessed through a standard questionnaire designed to collect information about the working hours in exposed areas such as brass dust, fumes, minute brass particles, etc. at their workplaces. Three hundred and twenty two participants with a high level of brass exposure, irrespective of their working units in the factory, with symptoms/health complaints related to heavy metal poisoning were listed and further screened for eligibility. Among these sampling frames of 322 workers, 20 randomly selected workers were referred to the institute hospital, for further screening of toxicity of copper, zinc, and lead. Among these participants, the ones with

high serum levels for at least one of the heavy metals – copper, zinc and/or lead, were provided with clinical counselling and were enrolled in the study.

2.3. Inclusion criteria

Workers having age more than 18 years who were working for more than 3 years and had high level of brass exposure with increased serum levels of any one of the three i.e., copper (>140 µg/dL) [13], zinc (>120 µg/dL) [14], and lead (>30 µg/dL) [15,16] were included for the study.

2.4. Exclusion criteria

Workers having diagnosis of uncontrolled systemic and life-threatening diseases with serum levels, two times of upper normal ranges of copper (>280 µg/dL), zinc (>240 µg/dL), and lead (>70 µg/dL), severe anemic condition (Hb = < 7 g/dL) and impaired psychological health were excluded from the study.

All the screened individuals having high serum level of lead (>70 µg/dL) were referred to chelation therapy. However, among these individuals, those who refused to seek chelation therapy and were willing to provide consent for Ayurvedic treatment at their own risk were included in the present study.

2.5. Study procedure

The participants who fulfill the inclusion criteria were registered and examined for the baseline characteristic wherein demographic profile, medical history, data from general and systemic examinations were recorded and outcomes were analyzed. Intervention was provided to each participants for 28 days. At the end of the trial, participants were subjected to primary and secondary outcome analysis.

2.6. Investigational drug/intervention

All study participants received *Triphala Churna* powder 5 gm/day orally before 8 am prior to consumption of any food items and *Dadima Svarasa* (juice) 40 ml twice daily (after breakfast at 8 am and evening snacks at 8 pm) for 28 days. *Dadima Svarasa* was prepared from its fruit *Dadima* (*Punica granatum* Linn.) as per standard procedure in the departmental laboratory and 0.1% sodium benzoate, a United States Food and Drug Administration approved preservative [17] was added to the juice [18]. *Triphala Churna* was prepared by adding an equal quantity of *T. chebula* Retz. (*Haritaki*), *T. bellerica* Roxb. (*Bibhitaki*), and *E. officinalis* Gaertn. (*Amalaki*) procured from the institutional pharmacy. Along with the intervention, participants were also advised to take precautionary measures to minimize further exposures.

2.7. Outcomes

2.7.1. Primary outcome

Changes in the serum levels of copper, zinc, and lead were the primary outcomes of interest. The copper, zinc, and lead levels were assessed by using inductively coupled plasma-optical emission spectrometry (ICP-OES) under optimized conditions in all acid digested blood samples. The calculated samples were nebulized manually downstream to the plasma and therefore, the concentrations were automatically determined using the quality calibration graph prepared within the same plasma conditions [19].

2.7.2. Secondary outcome

Common health symptoms as per WHO guideline were the secondary outcomes. These symptoms were anorexia, headache, dizziness, weakness, nausea, irritation in eyes and nose, epigastric pain, abdominal distention, chest pain, dyspnea, cough, lumbar pain, body ache, numbness, and occupational dermatitis [20]. Grading of common symptoms was adopted from common terminology criteria for adverse events [21].

2.7.3. Safety outcome

Study participants were instructed to report any spontaneous adverse events during the trial period. Levels of Hb, FBS, SGPT, SGOT, and serum creatinine were assessed at the end of the trial.

Primary, secondary, and safety outcomes were measured at baseline and at the end of the trial.

2.8. Statistical methods

The collected data was entered and coded in MS Excel (version 2013; 15.0.4420.1017) and statistically analyzed using SPSS (version 26.0). Continuous data were presented in mean and standard deviation and categorical data as frequencies and percentages. Paired t-test was performed to analyze parametric data like laboratory investigations and heavy metal analysis. Wilcoxon signed-rank test was applied to assess non-parametric data like change in grade of symptoms. The test results were considered significant when p -value < 0.05 and confidence interval at 95%.

3. Results

A total 1000 workers had been surveyed. Among them, 652 workers were excluded because they did not belong to high exposure group, 5 workers declined to participate and 21 workers were having diseased conditions. 322 screened individuals eligible for study, 20 were randomly selected for the study. Four participants did not complete the study. Patients who dropped out after the baseline visit were excluded from the analysis (Fig. 1).

The mean age of the study group was 42.5 ± 7.4 years with 10 participants (50%) belonging to 44–56 years age group and 9 (45%) belonging to the 31–43 years age group. Majority of the workers were literate ($n = 16$; 80%). Thirteen workers (65%) were vegetarian and adopted regular dietary habit i.e. timely intake of breakfast,

lunch and dinner. Eighteen workers (90%) complained about poor appetite. The baseline mean value of serum copper (153.31 ± 19.98) and serum lead (49.23 ± 21.77) was higher than normal ranges whereas the mean value of serum zinc was slightly below (72.3 ± 9.01) to normal range. Among 20 selected workers, 11 (55%) workers were from casting units whereas rest of the workers ($n = 9$; 45%) were from machining units (Table 1).

3.1. Primary outcome

The study data indicate that a significant decrease was observed at the end of the trial in the mean values of serum copper ($p < 0.001$) and serum lead ($p < 0.001$) whereas a significant increase in the mean value of serum zinc ($p < 0.001$) from the baseline. (Table 2).

3.2. Secondary outcome

It was observed that anorexia and generalized weakness were the most frequently occurring health problems ($n = 16$) followed by body ache ($n = 15$), and nausea ($n = 12$). The other health problems observed among the participants were epigastric pain, dizziness, chest pain, and skin-related complaints found in 11 workers. Numbness was found in 7 workers followed by abdominal distention ($n = 6$) and headache ($n = 6$). Shortness of breath was reported by 5 workers, cough by 2 workers (Table 3).

Significant reduction in grading was observed in nausea ($p = 0.005$), anorexia ($p < 0.001$), epigastric pain ($p = 0.001$), abdominal distention ($p = 0.014$), generalized weakness ($p = 0.005$), and body ache ($p = 0.005$). No statistically significant changes were observed in lumbar pain ($p = 1.000$), headache ($p = 0.083$), dizziness ($p = 0.157$), numbness ($p = 0.083$), chest pain ($p = 0.083$), shortness of breath ($p = 1.000$), cough ($p = 0.317$), occupational dermatitis ($p = 0.083$), nose irritation ($p = 1.000$), and eye irritation ($p = 1.000$) (Table 3).

3.3. Safety outcome

No statistically significant changes were observed in Hb ($p = 0.09$), FBS ($p = 0.795$), SGPT ($p = 0.375$), SGOT ($p = 0.06$), and

Table 1
Workers' socio-demographic profile ($n = 20$).

S. No.	Socio-demographic variables	N (%)	
1.	Age (yrs.)	Mean \pm S.D	42.5 \pm 7.4
		Range	18–67
2.	Sex	44–56 years	10 (50%)
		31–43 years	9 (45%)
		Male	20 (100%)
3.	Religion	Female	0 (0%)
		Hindu	19 (95%)
4.	Education	Muslim	1 (5%)
		Illiterate	4 (20%)
5.	Working units	Literate	16 (80%)
		Casting	11 (55%)
6.	Types of diet	Machining	9 (45%)
		Vegetarian	13 (65%)
7.	Dietary Habits	Mixed	7 (35%)
		Regular	12 (60%)
8.	Appetite	Irregular	8 (40%)
		Poor	18 (90%)
9.	Copper ($\mu\text{g/dL}$) ($n = 16$)	Moderate	2 (10%)
		Mean \pm S.D	153.31 \pm 19.98
10.	Zinc ($\mu\text{g/dL}$) ($n = 16$)	Range	70–140 $\mu\text{g/dL}$
		Mean \pm S.D	72.31 \pm 9.01
11.	Lead ($\mu\text{g/dL}$) ($n = 16$)	Range	80–120 $\mu\text{g/dL}$
		Mean \pm S.D	49.23 \pm 21.77
		Range	>30 $\mu\text{g/dL}$

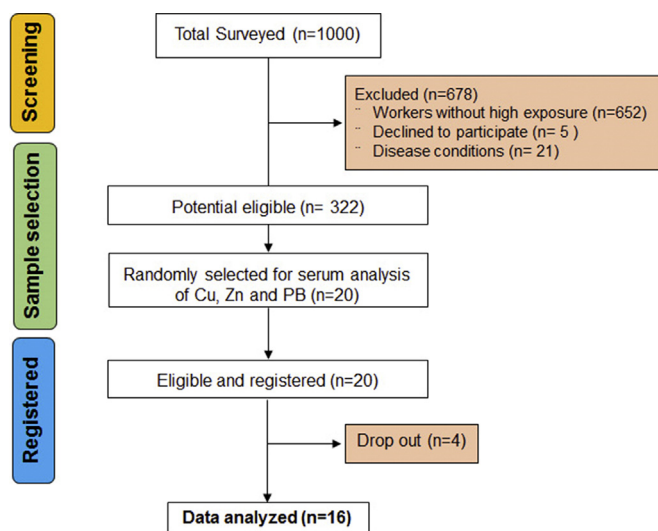


Fig. 1. Participants flow diagram.

Table 2

Effect of the intervention on blood serum heavy metal analysis of copper, zinc, and lead (n = 16).

Heavy metal	BT	AT	p
	Mean ± S.D	Mean ± S.D	
Copper (70–140 µg/dL)	153.31 ± 19.98	139.06 ± 22.37	p < 0.001 ^a
Zinc (80–120 µg/dL)	72.31 ± 9.01	87.31 ± 7.73	p < 0.001 ^a
Lead (>30 µg/dL-Exposure >60 µg/dL- Chelation Therapy)	49.23 ± 21.77	38.64 ± 23.15	p < 0.001 ^a

Paired t-test. BT- Before treatment, AT- After treatment, S.D- Standard Deviation.

^a p < 0.05 (Level of significance).

serum creatinine analysis (p = 0.240). Mean of baseline values and end of treatment values of blood and serum parameters were reported within their normal range (Table 4).

3.4. Adverse event

No adverse drug reactions were reported during the clinical trial.

4. Discussion

Evidence from the present study indicates that *Dadima Svarasa* and *Triphala Churna* decreased serum copper and lead levels and increased serum zinc level among registered brass industry workers. Some health complaints associated with heavy metal toxicity were also relieved as a result of the intervention (Table 3).

The lower serum zinc level at baseline can be explained by an increased level of copper. Both copper and zinc appear to bind to the same metallothionein, a sulfhydryl-rich protein. However, copper has a higher affinity for metallothionein than zinc and displaces the zinc that is attached to the metallothionein [22]. Inhibition of intestinal copper absorption by zinc at the brush border of the lumen may demonstrate competition between these two metals [23].

Phenolic compounds exhibit a chelating effect when they are deprotonated [24]. Pomegranate juice is a prominent source of phenolic compounds, flavonoids, and minerals [25]. The chelation of pomegranate juice and its constituent punicalagin (hydrolyzable tannins) compared to EDTA (a standard metal chelating agent) has been documented. Both these components exhibit ferrous chelating ability but significantly lower than EDTA [26]. Metal

chelating activity of pomegranate juice and its constituents punicalagin might be responsible for decrease in serum copper and lead.

In experimental rats, Pomegranate Pith and Carpellary Membrane (PPCM) extract were reported to decrease the accumulation of lead in blood and tissues in a dose-dependence manner [27]. The above activities may be attributed to ellagic acid, a similar constituent present in flower as well as juice [28].

Pomegranate extract has free radical scavenging activity against anions (superoxide) and radicals [29]. *Triphala* is also an excellent scavenger of hydroxyl radicals and superoxide radicals. The free radical scavenging ability of *Triphala* through the use of non-biological and stable free radicals has been calculated [30] as moderate free-radical scavenging components that often have strong metal chelation capacity [31].

Dadima Svarasa and *Triphala Churna* produced significant improvement in gastrointestinal symptoms like nausea, anorexia, epigastric pain, abdominal distention as well as generalized weakness and body ache in the case of the musculoskeletal system. The relief in symptoms may be explained by decreased serum level of copper and lead and an increase in serum level of zinc. An alternative explanation for the relief in the symptoms may be due to selected interventions.

Triphala has been reported to exhibit antibacterial, anti-inflammatory, and anti-diarrheal activity [32] as well as the ability to regulate of gastrointestinal movements which are crucial for heavy metal elimination and have the potential to cure heavy metal toxicity [33].

Dadima (pomegranate) is gastroprotective. An aqueous extract of pomegranate fruit peel was tested against ethanol-induced gastric lesions in rats. Oral administration resulted in a substantial reduction in the gastric lesion. Protection was more relevant when the test drug was administered at the same time [34]. Gastroprotective function of the pomegranate juice is probably due to the presence of polyphenols and chemical constituents such as punicalagin and punicalin in both juice and peel [35]. Significant relief in general weakness and body ache can be explained by the analgesic activity of pomegranate flower extracts which has a significant effect equivalent to the standard drug morphine sulfate [36]. Analgesic activity of pomegranate flower extract may be due to its constituents ellagic acid, mentioned earlier. Analgesic, antipyretic, and ulcerogenic activities of *Triphala* have been compared with the non-steroidal anti-inflammatory drugs on experimental models. *Triphala* produced excellent analgesic and antipyretic effects without any gastric changes [37].

Table 3

Effect of the intervention on workers of brass industries (n = 16).

Body Systems	Complaints	Reported in participants (n)	Baseline score [median (IQR)]	EOT score [median (IQR)]	p
Gastrointestinal system	Nausea	12	2 (1.25–2)	1 (1–1)	0.005 ^a
	Anorexia	16	3 (3–3)	1 (1–1.75)	<0.001 ^a
	Epigastric pain	11	2 (2–2)	1 (1–1)	0.001 ^a
	Abdominal distention	6	2 (2–2)	1 (1–1)	0.014 ^a
Musculoskeletal system	Weakness	16	2 (2–2)	1 (1–1)	0.005 ^a
	Bodyache	15	2 (1–2)	1 (1–1)	0.005 ^a
	Lumber Pain	2	1 (1–1)	1 (1–1)	1.000
Neurological system	Headache	6	1.5 (1–2)	1 (1–1)	0.083
	Dizziness	11	1 (1–1)	1 (1–1)	0.157
	Numbness of hands	7	2 (1–2)	1 (1–2)	0.083
Respiratory system	Chest pain	11	1 (1–2)	1 (1–1)	0.083
	Dyspnea	5	1 (1–1)	1 (1–1)	1.000
	Cough	2	1.5 (1–0)	1 (1–1)	0.317
Local complaint	Occupational Dermatitis	11	2 (2–2)	2 (1–2)	0.083
	Nose irritation	3	1 (1–1)	1 (1–1)	1.000
	Eye irritation	6	1 (1–1)	1 (1–1)	1.000

EOT- End of treatment, IQR- Inter Quartile range.

^a p < 0.05 (Level of significance).

Table 4
Effect of the intervention on biochemical and hematological parameters (n = 16).

Parameters	BT	AT	p ^a
	Mean ± S.D	Mean ± S.D	
Hb	14.25 ± 1.24	14.00 ± 1.38	0.090
FBS	98.43 ± 33.86	100.06 ± 38.64	0.795
SGPT	26.62 ± 9.21	24.75 ± 8.91	0.375
SGOT	22.31 ± 4.28	20.12 ± 4.48	0.06
Serum creatinine	1.11 ± 0.12	1.16 ± 0.10	0.240

^a Paired t-test, p < 0.05 (level of significance).

4.1. Limitations of the study

This single-arm clinical trial study design has some limitations. Without control, it is difficult to assess an the impact of interventions. The sample size of the present study was small and included workers from casting and machining units only. Hence, these results may not be generalized to the workers of other units of brass industry and other industries. Confounding effect of the precautionary measures was also not analyzed due to the absence of control group. Furthermore, a single-center trial may not be generalized to another geographical location and cultural groups. The generalizability of the study finding is therefore, limited to the selected characteristics of participants.

Based on the encouraging result of the present study, future randomized control trials may be planned adopting classical guidelines considering the specific *Aushadha Sevana Kala* (time of administration) referred by *Anupana Manjari*, freshly prepared pomegranate juice, and *Triphala Churna* prepared from different ratios of the three ingredients quoted by different Ayurveda texts.

5. Conclusion

This single-arm pilot study supports the traditional use of *Dadima Phala Svarasa* (juice of ripe pomegranate) and *Triphala Churna* for heavy metal toxicity. Both drugs appeared safe for internal administration and in combination, can be considered as the alternative option for the management of heavy metal toxicity of brass. However, the finding needs further confirmation through good design randomized controlled trials.

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Conflict of interest

None.

Author contributions

Vivek Kumar Patel: Methodology, Formal analysis, Investigation, Writing- original draft preparation. **Kalpesh Panara:** Methodology, Formal analysis, Supervision, Writing - reviewing and editing. **Rabinarayan Acharya:** Conceptualization, Supervision, Funding acquisition, Writing - reviewing and editing.

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