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Original Research Article (Experimental)

Tissue distribution of mercury and copper after *Aarogyavardhini Vati* treatment in rat model of CCl_4 induced chronic hepatotoxicity

Shrirang Jamadagni [a, *], Pallavi Jamadagni [a], Binita Angom [b], Dhirendranath Mondal [b], Sachchidanand Upadhyay [b], Sudesh Gaidhani [c], Jayram Hazra [b]

[a] *Regional Ayurveda Institute for Fundamental Research, Nehru Garden, Gandhi Bhawan Road, Kothrud, Pune, 411038, India*
[b] *Central Ayurveda Research Institute of Drug Development, 4CN, Sector -5, Bidhannagar, Kolkata, 700091, India*
[c] *Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India, 61-65, Institutional Area, Opposite D Block, Janakpuri, New Delhi, 110058, India*

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ABSTRACT

Background: *Aarogyavardhini Vati* is a classical Ayurvedic herbomineral formulation. It contains mercury and copper compounds as principal minerals along with other minerals and herbal ingredients. *Aarogyavardhini Vati* is indicated in chronic liver ailments. However, safety concerns are often raised regarding the use of mercury containing ayurvedic drugs in disease conditions due to the risk of mercury and copper toxicity.
Objective: This study was performed to address the safety concerns regarding mercury and copper toxicity from Ayurvedic herbomineral formulations by investigating accumulation of these minerals in tissues and subsequent toxicity in chronic hepatotoxicity rat model.
Materials and methods: Quantification of mercury and copper in *Aarogyavardhini Vati* was done. Chronic hepatotoxicity was induced in the Wistar rats by repeated administration of CCl_4 for 8 weeks. Animals were treated with *Aarogyavardhini Vati* for various durations. Post treatment of 8 weeks, serum biochemical marker estimations was done. Estimation of mercury and copper from the liver, kidney and brain tissues was done after animal sacrifice. Histopathology evaluation of visceral organs was also performed.
Results: Treatment with *Aarogyavardhini Vati* exhibited significant accumulation of mercury in the kidney but not in the brain and liver. Similarly, no significant accumulation of copper was observed in liver, kidney, and brain due to the treatment of *Aarogyavardhini Vati*. Serum biochemical and histopathological changes were not affected by the treatment with *Aarogyavardhini Vati*.
Conclusion: *Aarogyavardhini Vati* did not show any biologically significant potential to cause toxicity due to its mercury and copper content when administered for prolonged duration to rats with chronic hepatotoxicity.

1. Introduction

Aarogyavardhini Vati is an ayurvedic formulation classified under the category of *Rasa Yoga*, in which minerals are the main ingredients. Its oldest authentic reference dates back to 11th century, which is a classical ayurvedic treatise of *Rasaratnasamuchchaya, Visarpadichikitsa; Adhyaya* 20 written by the legendary scholar and Ayurvedic physician Vagbhatta [1]. This herbomineral formulation contains processed mercury, sulfur, copper, iron, mica, pericarps of *Terminalia chebula*, *Terminalia bellirica*, *Emblica officinalis*, stolon and root of *Picrorhiza kurroa*, the resin of *Commiphora wightii*, leaves of *Azadirachta indica, shilajit* and roots of *Ricinus communis* as ingredients. It is used extensively in Ayurveda as a drug for treating disorders of liver, chronic fever, disorders of adipose tissue and diseases of the skin [1]. A double-blind clinical trial of *Aarogyavardhini Vati* has revealed its significant hepatoprotective effect for the treatment of acute viral hepatitis [2]. *Aarogyavardhini Vati* has also exhibited hypolipidemic activity in experimental

* Corresponding author.
E-mail: shrirangb@gmail.com
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animals [3]. However, safety issues are often raised on the use of mercury containing Ayurvedic and other traditional drugs in disease condition due to the risk of mercury toxicity [4–7]. Kumar et al. [8]. conducted a safety study of *Aarogyavardhini Vati* in the healthy rat model in which the efforts were also taken to study tissue distribution of mercury.

The lethal effects of excess ingestion of mercury and copper on the liver have already been reported, moreover, *Aarogayavardhini Vati*, comprising of mercury and copper is used to treat liver ailments in Ayurvedic system of medicine [1,8,9]. Hence, there is unmet need to obtain more relevant data of *Aarogayavardhini Vati* pertaining to its safety profile and its use for prolonged duration in patients with hepatotoxicity.Therefore, the present study was performed in chronic hepatotoxicity model in rats for a period of two months.

2. Materials and methods

2.1. Test drug

Aarogyavardhini Vati was procured from Dabur India Ltd. 22, Site IV, Sahibabad, Uttar Pradesh, India −201010 (Batch No. SB0165, Mfd on 05/15). The formulation was prepared as per the method mentioned in established Ayurvedic standards [1,10]. The ingredients of the *Aarogyavardhini Vati* are as given in Table 1. The test formulation was analyzed as per the Ayurvedic Pharmacopoeia of India [11] standards at a laboratory accredited by the National Accreditation Board for Testing and Calibration of Laboratories (NABL). The physicochemical parameters evaluated were ash value, water-soluble extractive, alcohol soluble extractive, loss on drying, pH value, pesticides residue analysis, heavy metals analysis for lead, arsenic, mercury, cadmium, and for copper alongwith microbial contamination analysis by microbial count for specific pathogenic microorganisms like *Escherichia coli, Salmonella* spp., *Pseudomonas* spp. *Staphylococcus aureus* and aflatoxins were also carried out.

2.1.1. Preparation of Aarogyavardhini Vati

All the ingredients were collected and weighed in the required quantity as per their ratio in the formulation. The flow chart of the method of preparation of the *Aarogyavardhini Vati* is as follows (Fig. 1).

Step 1: The dried plant parts *viz. T. chebula* (pericarp), *T. bellirica* (pericarp), *E. officinalis* (pericarp), *R. communis* (root) and *P. kurroa* (stolon and root) were subjected to grinding and passed through sieve no. 44. *A. indica* leaves were separately collected and passed through sieve no. 16 to obtain a coarse powder.

Step 2: Purified mercury was prepared by triturating the equal quantity of raw mercury and lime powder together for three days, added an equal part of Garlic (*Allium sativum*) and rock salt and again triturated till the paste of garlic turned black [10]. Purified sulfur was prepared by mixing small pieces of raw sulfur in an iron pan with an equal quantity of cow ghee, further heated till the melting of sulfur and then poured into a pot containing cow milk (q.s.).Sulfur was collected after cooling by decanting the milk and subjected to washing with hot water. The process was repeated seven times. At the end of the process, sulfur was washed and dried [10]. Finally, *kajjali* was prepared by triturating an equal quantity of purified mercury and purified sulfur in edge runner for sufficient time till it became smooth black powder without any shining [10].

Step 3: *A. indica* leaves powder *kwatha* (decoction) was prepared by boiling the powder in water (8 times of the quantity of the powder taken) in a stainless steel pot till the volume of water reduces to 1/4th. *Kwatha* was filtered through nylon cloth number 60 and collected in a suitable stainless steel vessel and allowed to cool.

Step 4: Lauh Bhasma [1], Abhraka Bhasma [1], Tamra Bhasma [1], Shuddha Shilajit [10], Shuddha Guggulu [10] and powder of herbs were added to *Kajjali* in the suitable edge runner and triturated well till a homogenous blend was formed. Then *A. indica* leaves *kwatha* was added to the blend in sufficient quantity to form a smooth homogenous semi-solid bulk. Small boluses of the bulk were dried in a tray-dryer at a temperature not exceeding 60 °C and subjected to granule preparation in a mixer. The granules were passed through the multi mill to give the desired weight of 500 mg.

2.2. Experimental animals

Animal experimentation study was performed in male Wistar rats bred in the Institute's animal house. The breeding stock of the rats was obtained from the National Center for Laboratory Animal Sciences, Indian Council for Medical Research- National Institute of Nutrition, Hyderabad. The study protocol was approved by Institutional Animal Ethics Committee (approval number IAEC/2015/01 dated 17th April 2015). Forty male adult rats were selected based on the body weight and randomly distributed into five groups (n = 8) in such a way that means of groups were the same and body weight variation was bot more than ±20% of the mean body weight. The body weights and age of the rats ranged from 110 to 150 g and 6–7 weeks respectively. Rats were acclimatized for 7 days. Temperature and relative humidity were maintained at 25 ± 1°C, and 40–70%

Table 1
Ingredients of the *Aarogyavardhini Vati*.

Sr. No	Ingredients Ayurvedic name	Scientific Name/description	Part used	Form used	Ratio of quantity to be used	Absolute quantity required per 100 g formulation
1	*Rasa (Paarada)- shuddha*	Purified Mercury	–	–	1 Part (2.2% w/w)	2.2 g
2	*Gandhaka - shuddha*	Purified Sulfur	–	Powder	1 Part (2.2% w/w)	2.2 g
3	*Lauha (Lauha Bhasma)*	Calcined Iron	–	Bhasma powder	1 Part (2.2% w/w)	2.2 g
4	*Abhra (Abhraka bhasma)*	Calcined Biotite mica	–	Bhasma powder	1 Part (2.2% w/w)	2.2 g
5	*Sulva (Taamra bhasma)*	Calcined Copper	–	Bhasma powder	1 Part (2.2% w/w)	2.2 g
6	*Triphala*					
	a. *Haritaki*	*Terminalia chebula*	Pericarp	Powder	2 Parts (4.5% w/w)	4.5 g
	b. *Bibhitaka*	*Terminalia bellirica*	Pericarp	Powder	2 Parts (4.5% w/w)	4.5 g
	c. *Amalaki*	*Emblica officinalis*	Pericarp	Powder	2 Parts (4.5% w/w)	4.5 g
7	*Shilajit- shuddha*	–	Ooze[a]		3 Parts (6.8% w/w)	6.8 g
8	*Pura (Guggulu) – shuddha*	*Commiphora wightii*	Oleo-gum Resin		4 Parts (9% w/w)	9 g
9	*Chitra (Eraṇḍa) moola*	*Ricinus communis*	Root	Powder	4 Parts (9% w/w)	9 g
10	*Tikta (Katuka)*	*Picrorhiza kurroa*	Stolon & Root	Powder	22 Parts (50% w/w)	50 g
11	*Nimba vruksha dalaambha (Nimba)*	*Azadirachta indica*	Leaf	Svarasa-juice	Q.S.[b]	Q.S.

[a] Ooze of decayed vegetable matter from rock clefts.
[b] Quantum satis for mixing for two days.

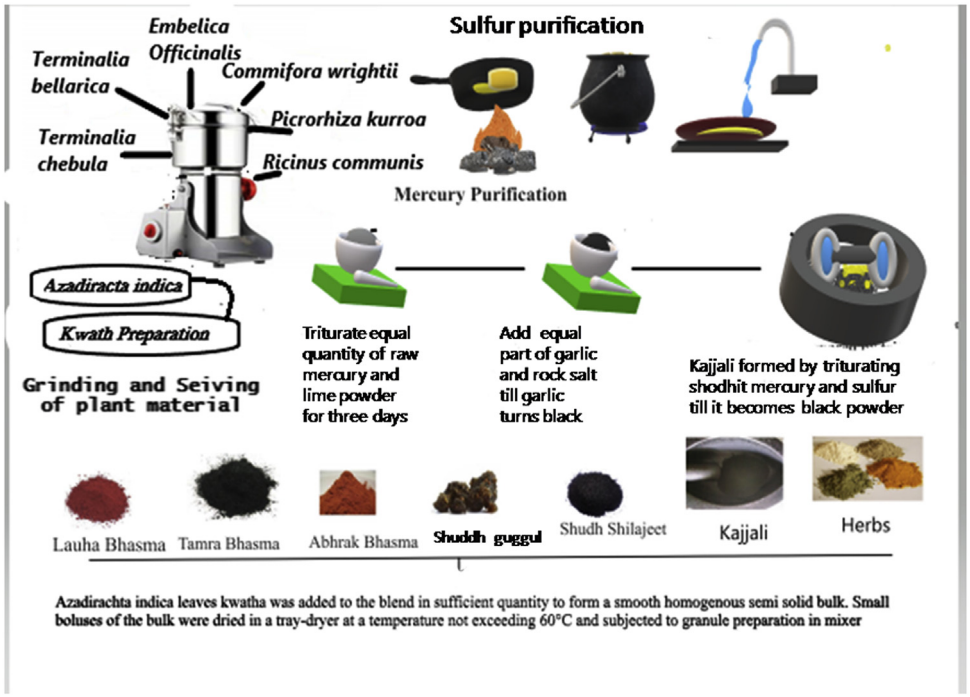

Fig. 1. Flow chart for preparation of the *Aarogyavardhini Vati.*

respectively, and illumination was controlled to give approximately a sequence of 12 h light and 12 h dark. All rats were individually housed in polypropylene cages (27 cm × 19 cm × 14 cm) with lids and rice husk bedding. Pelleted rodent feed obtained from the National Institute of Nutrition, Hyderabad, was provided along with de-ionized water using plastic nozzle bottles *ad libitum*. The chemical analysis report of the rodent feed for copper and mercury levels was obtained from the feed manufacturer.

2.3. Experimental design and pathology

Since carbon tetrachloride (CCl_4) is known to cause hepatotoxicity, it was used to induce chronic hepatotoxicity in the form of fatty changes in the hepatocytes and subsequent induction of fibrosis in the rats [12].

The dose of *Aarogyavardhini Vati* was 300 mg/kg/day, which was selected based on the published literature with due consideration to the toxicity and efficacy of the drug [8]. The dose for Silymarin (Micro Labs Ltd, Mumbai, India) was 200 mg/kg body weight/day which was selected based on available literature [13,37]. The volume of the dose administered by oral gavage was calculated at a rate of 10 mL/kg of body weight.

2.3.1. Animal groups

2.3.1.1. Disease Control group (DC). Animals received CCl_4 (Merck India Ltd.) through subcutaneous injection thrice weekly at the dose of 2 mL/kg (50% v/v in mineral oil) for 8 consecutive weeks.

2.3.1.2. Positive Control group (PC). Animals received CCl_4 through subcutaneous injection thrice weekly at the dose of 2 mL/kg (50% v/v in mineral oil) for 8 consecutive weeks alongwith Silymarin at the dose of 200 mg/kg/day/animal from week 5 onwards till sacrifice after 8 weeks; Treatment group (TG): Animals received CCl_4 through subcutaneous injection thrice weekly at the dose of 2 mL/kg (50% v/v in mineral oil) for 8 consecutive weeks alongwith *Aarogyavardhini Vati* at the dose of 300 mg/kg/day from week 5 onwards till sacrifice after 8 weeks (28 days).

2.3.1.3. Preventive Treatment Group (PT). Animals received CCl_4 (Merck India Ltd.) through subcutaneous injection thrice weekly at the dose of 2 mL/kg (50% v/v in mineral oil) for 8 consecutive weeks alongwith *Aarogyavardhini Vati* at the dose of 300 mg/kg/day from day 1 onwards till sacrifice after 8 weeks; Normal Control group (NC): Healthy male rats of the same age without any treatment as concurrent controls.

2.4. Dose preparation

Each animal's body weight at the beginning of every week was considered for calculating the drug weight and volume to be administered. The doses were prepared every day before drug administration. Weighed quantity of *Aarogyavardhini Vati* and Silymarin tablet were crushed in distilled water using mortar and pestle and kept on vortex mixer so as to make the *Aarogyavardhini Vati* suspension with a concentration of 30 mg/mL and Silymarin suspension with a concentration of 20 mg/mL.

2.5. Clinical observations

All animals were observed for morbidity and mortality twice daily. General clinical observations and neurological observations in the open field were made twice a day at the same time throughout the study. Body weight and feed consumption of each animal were recorded at the start of the study and thereafter at weekly intervals.

2.6. Serum biochemical estimation

Blood collection was carried out to assess the changes in blood biochemical parameters of animals after the 8th week. Capillary tubes were used for blood collection through retro-orbital plexus of

the rats. Serum biochemical analysis was done for glucose, total protein, total bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin and blood urea nitrogen (BUN). Biochemical estimation was done using Autopak diagnostic kit method in Robonik Semi-Auto Biochemistry Analyzer (Thane, India).

2.7. Histopathology

At the end of the study after 8 weeks, all animals were weighed and euthanized by deep CO_2 asphyxiation and subjected to detailed gross pathological examination. The liver, kidney, spleen, pancreas, stomach, and intestine were collected for histopathology evaluation. As the entire brain was required for mercury and copper estimation, it could not be subjected to histopathology evaluation. Organs were fixed in 10 % neutral buffered formalin fixative. Fixed tissues were processed i.e. dehydrated in graded isopropyl alcohol (Merck India Ltd) and cleared in xylene (Merck India Ltd) and embedded at 58^0–60 °C in paraffin wax (Merck India Ltd). The sections of the tissue blocks were taken at 4.5–5 μm thickness, stained with hematoxylin and eosin and finally mounted using DPX. These tissue sections were then examined under light microscope for histopathology evaluation.

2.8. Mercury and copper level estimation in tissues

Mercury and copper levels in *Aarogyavardhini Vati* and animal feed were estimated to calculate their actual amount of ingestion [8,14,15]. Post sacrifice liver, brain and kidney tissues of animals from DC, TG, and PT group were weighed and minimum 1 g of each tissue sample was collected in labeled test tubes, kept at 2–8 °C. The samples were subsequently transported in a container with an ice-pack bag to analytical laboratory within 3–4 h after collection. Mercury and copper levels were estimated using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) and values were expressed in μg/g-wet tissue [8].

2.9. Statistical analysis

SPSS Statistics 17.0 software was used for statistical analysis. In-life phase observations were recorded and analyzed statistically by one-way analysis of variance (ANOVA), followed by Tukey test. Wilcoxon/Kruskal–Wallis test was used for tissue mercury and copper levels data analysis.

3. Results

3.1. Analysis of Aarogyavardhini Vati

The results of physicochemical and microbiological parameters tested as per the Ayurvedic Pharmacopoeia of India are depicted in Table 2.

3.2. Clinical sign observations and serum biochemical analysis

Effects of the CCl_4 induced hepatotoxicity were evident from clinical signs of rats from DC, PC, TG and PT group which showed rough body coat, reduction in body weight gain, and aggressive behavior from the second week onwards. The hepatotoxicity was also reflected in serum biochemistry parameters as increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) when compared to the NC group (Table 3). No *Aarogyavardhini Vati* treatment-related changes in serum biochemical parameters and clinical signs were observed when compared to the disease control group.

Table 2
Physicochemical and microbiological analysis of *Aarogyavardhini Vati*.

Sr. No.	Test Parameter	Result
1	Ash value	18.41% w/w
2	Water soluble extractive	10.96% w/w
3	Alcohol soluble extractive	4.61% w/w
4	Loss on drying	1.19%
5	pH value	2.98
6	Pesticides	Not detected
7	Aflatoxins	Not detected
8	Specific pathogenic microorganisms like *Salmonella* spp, *Escherichia coli*, *Staphylococcus aureus Pseudomonas aeruginosa*	Not detected
9	Lead	3.75 μg/g
10	Arsenic	0.58 μg/g
11	Cadmium	0.12 μg/g
12	Mercury	3494.05 μg/g
13	Copper	5300 μg/g

3.3. Histopathology

Gross examination of liver revealed, rounding of borders, mottling and pale pink to yellowish discoloration in all animals treated with CCl_4. No gross abnormality was found in any other organ. The kidney, spleen, pancreas and intestinal tract of all the animals of all groups did not reveal any histopathological changes. The liver tissues from all CCl_4 treated animals showed marked diffused centrilobular vacuolar changes. No *Aarogyavardhini Vati* and Silymarin treatment-related alteration in histopathological changes in liver was found.

3.4. Mercury and copper level estimation

a) Mercury levels recorded in *Aarogyavardhini Vati* and feed were 3494.05 μg/g and 0.0161 μg/g respectively. Mercury levels in tissues showed no significant accumulation of mercury in the liver and brain from *Aarogyavardhini Vati* treated groups i.e. TG and PT when compared to DC group. However, the mercury levels in the kidney from *Aarogyavardhini Vati* treated groups i.e. TG and PT were significantly increased when compared to the DC group (Table 4).

b) Copper levels in *Aarogyavardhini Vati* and in feed were 5300 μg/g and 12.3 μg/g respectively. Copper levels in tissues showed no significant accumulation of copper in the liver, brain, and kidney from *Aarogyavardhini Vati* treated groups i.e. TG and PT when compared to DC group (Table 5).

4. Discussion

The Ayurvedic Pharmacopoeia of India prescribes the working standards for ayurvedic drugs [1]. Although *Aarogyavardhini Vati* is a commonly used ayurvedic formulation, the perusal of the literature revealed that there is a scarcity of literature regarding its physicochemical standardization with special reference to mercury and copper level in the formulation. Moreover, various reports expressing the risk of mercury toxicity due to ayurvedic and other traditional drugs containing mercury have necessitated the generation of standard data pertaining to physicochemical properties and safety profile of *Aarogyavardhini Vati* [4–7]. The ash value 18.41%, one of the prescribed parameters for working standards of the drug sample, was found to be high due to the significant quantity of herbs used in the preparation of the drug. Further, the lead, arsenic, and cadmium levels detected in the drug sample were within their permissible limits as specified in the Ayurvedic Pharmacopoeia of India [11]. Since *Arogyavardhini Vati* is a *Rasa Yoga*

Table 3
Effect of Arogyavardhini Vati on serum biochemical parameters.

Group	Total protein g/dL	Albumin g/dL	Total bilirubin mg/dL	ALT Units/Liter	AST Units/Liter	BUN mg/dL	Creatinine mg/dL	Glucose mg/dL
DC	7.55 ± 0.48	3.30 ± 0.63	0.48 ± 0.09	191.55 ± 30.39 *	307.30 ± 51.69*	19.67 ± 0.60*	0.66 ± 0.07	103.29 ± 9.01
PC	7.05 ± 0.93	2.57 ± 0.28	0.73 ± 0.13	112.86 ± 13.48*	229.01 ± 14.28*	17.00 ± 1.07	0.54 ± 0.06	95.62 ± 8.26*
TG	7.10 ± 1.20	2.41 ± 0.17	0.66 ± 0.06	114.27 ± 13.03*	231.71 ± 20.42*	19.44 ± 1.83*	0.64 ± 0.06	85.82 ± 6.81*
PT	9.80 ± 1.39	2.79 ± 0.09	0.61 ± 0.14	120.15 ± 10.46*	271.63 ± 13.71*	17.86 ± 0.71*	0.61 ± 0.05	114.36 ± 9.35
NC	8.78 ± 0.39	3.37 ± 0.04	0.74 ± 0.15	48.18 ± 1.34	109.26 ± 5.16	13.65 ± 0.41	0.55 ± 0.05	128.40 ± 3.02

Values are expressed as mean ± SEM. * mean difference is significant with $p < 0.05$ as compared to normal control. g/dL: gram/deciliter, mg/dL: milligrams/decilitre; DC: Disease Control, PC: Positive Control,TG: Treatment Group, PT: Preventive Treatment, NC: Normal Control.

Table 4
Effect of *Aarogyavardhini Vati* on mercury levels in the liver, kidney, and brain (μg/g wet-tissue).

Group	Liver	Kidney	Brain
DC	0.078 ± 0.054	0.324 ± 0.211	0.180 ± 0.112
TG	0.088 ± 0.026	3.240* ± 0.966	0.042 ± 0.009
PT	0.105 ± 0.043	3.928* ± 1.642	0.028 ± 0.002

Values are expressed as Mean ± SEM.
* Mean difference is significantly higher with $p < 0.05$ as compared to the disease control group.

Table 5
Effect of *Aarogyavardhini Vati* on copper levels in liver, kidney, and brain (μg/g wet-tissue).

Group	Liver	Kidney	Brain
DC	4.121 ± 0.449	34.136 ± 5.438	3.466 ± 0.213
TG	4.695 ± 0.536	31.443 ± 2.333	4.350 ± 0.795
PT	5.156 ± 0.265	31.343 ± 3.080	3.378 ± 0.282

Values are expressed as Mean ± SEM.

formulation, minerals like mercury and copper are intentionally added to it [1]. *Arogyavardhini Vati* used in the present study had remarkably high mercury concentration of 3494.05 μg/g which could be directly correlated to the addition of *kajjali* while preparing the drug. Further, the mercury concentration value was significantly higher than earlier reported value of 1841 μg/g [8]. Similarly, the high copper concentration of 5300 μg/g was also attributed to the addition of *Tamra bhasma* while preparing the drug. The perusal of literature did not show any report of copper concentration measurement in the *Aarogyavardhini Vati*. The remarkable difference in the mercury concentrations from two different samples of *Aarogyavardhini Vati* and scarcity of data pertaining to copper concentration underscores the need for more studies on mercury and copper levels estimation in the drug. However, the findings of the present study should serve as baseline data for future studies.

Aarogyavardhini Vati is prescribed for 8–12 weeks to treat chronic liver ailments such as nonalcoholic fatty liver disease [16,17]. Mercury is a known toxic element in its organic forms such as methyl mercury as well as inorganic forms such as mercuric chloride, mercurous chloride, and mercurous oxide, with brain, kidney, and liver being the target organs [14,18–20]. Kang-Yum and Oransky [5] highlighted potential hazards associated with the mercury containing Chinese traditional medicines based on the reported cases of mercury poisoning related to the use of Chinese patent medicines in the United States. Saper et al. [4] indicated that the users of Ayurvedic drugs might be at risk for toxicity due to the heavy metals like mercury and lead added in them, and further emphasized on mandatory testing of the drugs for the heavy metal toxicity (4). Therefore, concerns regarding the safety of the mercury and copper containing drugs such as *Aarogyavardhini Vati* are reasonable especially when they are used in treating the liver ailments [4–7]. Mercury in the form of *kajjali* is used in *Aarogyavardhini Vati*. Mercuric chloride at the dose level of 1000 μg/kg body weight administered for 4 weeks in rats is well known to cause toxicity and accumulation of mercury in liver, brain, and kidney [8]. The calculated daily dose of mercury in *Aarogyavardhini Vati* treated animals in the present study was 1048 μg/kg body weight up to 8 weeks which was higher than earlier reported study by Kumar et al. [8]. which was 522.4 μg/kg body weight for 4 weeks. It was interesting to note that, in spite of the higher ingestion of mercury, its levels were significantly increased only in kidneys from *Aarogyavardhini Vati* treated groups as compared to disease control group without inducing any changes in histopathological and serum biochemical markers. The finding could be attributed to the altered physicochemical properties of the mercury during the preparation of the *kajjali.* According to Singh et al. [21], the macro particle size of the mercury in the *kajjali* matches well with the colloidal size. When attached to the human intestine provides a large surface area thereby increasing the absorption of other nutrients and drug ingredients but does not get absorbed itself. Our findings are also in accordance with the observations recorded by Ramanan et al. [22], based on X-ray Absorption Near-Edge Structure (XANES) based analysis of *Rasa Sindoor* and reported that chemical form, rather than the content of mercury, was the proper parameter for evaluating its toxicity in the drugs. A similar finding was reported in a study on *Samagandhaka Kajjali* a mercury-containing drug [23]. The findings of the present study are also in line with the outcome of the study on cinnabar which is a mineral used in Chinese traditional medicine. Cinnabar contains mercury in the form of HgS compound. According to Liu et al. [24], mercury in the compound form was poorly absorbed from the gastrointestinal tract when cinnabar was orally administered to mice and whatever minute quantity of mercury was absorbed; got deposited in the kidney.

According to Kumar et al. [8], 28 days *Aarogyavardhinin Vati* treatment in normal Wistar rats lead to a significant accumulation of mercury not only in the kidney but also in the liver and brain. The accumulation of mercury only in the kidney as observed in the present study could be attributed to chronic hepatotoxicity in rats. Further studies are required to investigate the cause of lesser accumulation of mercury in various tissues of the rats with chronic hepatotoxicity when compared to the accumulation in the normal rats.

Liver is the target organ for chronic copper ingestion-induced toxicity and its accumulation in humans [14,15,25]. The chronic toxicity of copper sulfate in the rats showed liver as the most vulnerable organ of toxicity and site of accumulation followed by the kidney and brain [26]. The calculated daily dose of copper was 1590 μg/kg body weight in the PT and TG group. However, there was no significant increase in copper levels in liver, kidney, and brain from *Aarogyavardhini Vati* treated animals as compared to the non-treated animals. Copper in the form of *Tamra bhasma* was used for the preparation of the *Aarogyavardhini Vati* in the present study. According to Chaudhari et al. [27], *Tamra bhasma* did not

produce any toxicity when administered at a dose of 27.5 mg/kg body weight for 28 consecutive days in Wistar rats and attributed it to the specific process of *Amritkarana* used during the preparation. Therefore the probable reason for low tissue accumulation of copper could be the specific method of preparation of calcined copper and its further mixing with other animal and plant origin compounds [1]. The finding is also in line with Chaudhari et al. [28]. who concluded that, when copper was processed as per ayurvedic texts to prepare *Tamra Bhasma* and subsequently consumed as per the ayurvedic texts, did not possess any toxic potential.

Our finding regarding tissue accumulation of mercury and copper strongly implies that, the ayurvedic method employed for the preparation of the drug may have resulted in alteration in the properties of mercury and copper *in vivo* leading to the minimal or negligible accumulation in various tissues.

Carbon tetrachloride is a known hepatotoxic agent, which leads to marked diffused centrilobular fatty degeneration and necrosis of the hepatocytes, increased serum ALT and AST levels and impaired liver functions leading to improper metabolism of food consumed [29]. A significant decrease in body weight gain of animals from the DC, PT and TG group can be correlated with CCl_4 treatment. Further, a significant increase in serum AST and ALT values in animals from DC, PT and TG group can be attributed to CCl_4 treatment for a prolonged duration of 8 weeks [12,29,30]. According to Choi et al. [31] and Lee et al. [32] in a long term observational study in human patients, chronic ingestion of mercury leads to decline in the liver function and increase in blood levels of enzymes such as ALT, and GGT and AST. Prolonged experimental mercury administration in rats is reported to induce histopathological changes such as degeneration and necrosis of hepatocytes along with an increase in plasma levels of liver injury markers such as ALT and AST [33,34]. Copper can accumulate in the liver due to chronic liver diseases such as primary biliary cirrhosis or chronic hepatitis in humans as well as animals [26,35]. The copper accumulation in the liver of the animals further induces histopathological changes such as foci of hepatocellular degeneration and necrosis with the scattered inflammatory response [36]. However, it was remarkable to note that, *Aarogyavardhini Vati* treatment in the present study did not attenuate or deteriorate the histopathological and serum biochemical changes of the hepatotoxicity [29,30]. The drug treatment did not induce any histopathological change in other organs. The findings can also be correlated with the nonsignificant or negligible accumulation of mercury and copper in the tissues.

The finding of the study clearly implies that the forms of mercury and copper present in the *Aarogyavardhini Vati* do not possess any potential to cause toxicity *in vivo*. Our findings can help in allaying the concerns regarding the risk of mercury and copper toxicity due to the use of *Arogyavardhini Vati* in disease conditions.

5. Conclusion

Aarogyavardhini Vati did not exhibit any biologically significant potential to cause toxicity due to its mercury and copper content when administered for prolonged duration in rats with induced chronic hepatotoxicity. These findings clearly imply that the use of *Aarogyavardhini Vati* prepared by traditional methods, at recommended dose and duration does not possess any potential risk of either mercury or copper toxicity in human patients having liver ailments. The present study also provides basic physicochemical data of the drug along with the quantification of mercury and copper which will serve as a baseline for future studies.

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Conflict on interest

None

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