Tissue distribution of mercury and copper after Aarogyavardhini Vati treatment in rat model of CCl₄ 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

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 CCl₄ 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.

© 2019 The Authors. Published by Elsevier B.V. on behalf of Institute of Transdisciplinary Health Sciences and Technology and World Ayurveda Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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, *Aarogyavardhini 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 *Aarogyavardhini 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 along with 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 not 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% RH.

### Table 1

| 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 (Paaraada)-shuddha | Purified Mercury | — | — | 1 Part (2.2% w/w) | 2.2 g |
| 2      | Gandhika - shuddha | Purified Sulfur | — | Powder | 1 Part (2.2% w/w) | 2.2 g |
| 3      | Lusha (Lusha Bhasma) | Calcined Iron | — | — | 1 Part (2.2% w/w) | 2.2 g |
| 4      | Abhra (Abhraaka bhasma) | Calcined Biotite mica | — | — | 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 | — | — | — | 1 Part (2.2% w/w) | 2.2 g |
| 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 | — | — | Oozea | 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 (Eranja) moola | Ricinus communis | Root | Powder | 4 Parts (9% w/w) | 9 g |
| 10     | Tikta (Katuksa) | Pongonorhiza kurroa | Stolon & Root | Powder | 22 Parts (50% w/w) | 50 g |
| 11     | Nimbo vruksa dalamba (Nimba) | Azadirachta indica | Leaf | Svarasa-juice | Q.S. | Q.S. |

---

a) Ooze of decayed vegetable matter from rock clefs.
b) Quantum satix for mixing for two days.
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 (CCl₄) 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 CCl₄ (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 CCl₄ through subcutaneous injection thrice weekly at the dose of 2 mL/kg (50% v/v in mineral oil) for 8 consecutive weeks along with Silymarin at the dose of 200 mg/kg/day/animal from week 5 onwards till sacrifice after 8 weeks; Treatment group (TG): Animals received CCl₄ through subcutaneous injection thrice weekly at the dose of 2 mL/kg (50% v/v in mineral oil) for 8 consecutive weeks along with 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 CCl₄ (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 along with 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 CO₂ 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°C–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].

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 CCl₄ 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 biochemical 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.

3.3. Histopathology

Gross examination of liver revealed, rounding of borders, mottling and pale pink to yellowish discoloration in all animals treated with CCl₄. 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 CCl₄ 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 Aarogyavardhini Vati is a Rasa Yoga...
formulation, minerals like mercury and copper are intentionally added to it [1]. Aarogyavardhini 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 with p < 0.05 as compared to normal control. Values expressed as Mean ± SEM.

| 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 | 3.929 ± 1.642 | 0.028 ± 0.002 |

Values expressed as Mean ± SEM.

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, mercurochloride, 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 physiochemical 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.

Arogyavardhini Vati is used in the treatment of nonalcoholic fatty liver disease, diabetes mellitus, and hyperlipidemia [25–27]. The findings of the present study are in line with the outcomes 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. 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

Table 3

| 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 ± 5.81 * |
| 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 ± 0.19  | 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.

Table 4

| 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    | 0.078 ± 0.054     | 0.324 ± 0.211 | 0.180 ± 0.112        | 3.466 ± 0.213  | 34.136 ± 5.438 | 3.450 ± 0.795 | 3.378 ± 0.282 |
| TG    | 0.088 ± 0.026     | 3.240 ± 0.966 | 0.042 ± 0.009        | 3.143 ± 2.333  | 31.443 ± 3.080 | 3.378 ± 0.282 | 3.378 ± 0.282 |
| PT    | 0.105 ± 0.043     | 3.929 ± 1.642 | 0.028 ± 0.002        | 5.156 ± 0.265  | 31.343 ± 3.080 | 3.378 ± 0.282 | 3.378 ± 0.282 |

Values expressed as Mean ± SEM.

Table 5

| 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    | 4.121 ± 0.449     | 34.136 ± 5.438 | 3.466 ± 0.213        | 34.136 ± 5.438 | 3.466 ± 0.213 | 34.136 ± 5.438 | 3.466 ± 0.213 | 34.136 ± 5.438 |
| TG    | 4.695 ± 0.536     | 31.443 ± 2.333 | 3.450 ± 0.795        | 31.443 ± 2.333 | 3.450 ± 0.795 | 31.443 ± 2.333 | 3.450 ± 0.795 | 31.443 ± 2.333 |
| PT    | 5.156 ± 0.265     | 31.343 ± 3.080 | 3.378 ± 0.282        | 31.343 ± 3.080 | 3.378 ± 0.282 | 31.343 ± 3.080 | 3.378 ± 0.282 | 31.343 ± 3.080 |

Values expressed as Mean ± SEM.
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 CCl₄ treatment. Furthermore, a significant increase in serum AST and ALT values in animals from DC, PT and TG group can be attributed to CCl₄ 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 Aarogyavardhini 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.

Source(s) of funding

The study was funded by Central Council for Research in Ayurvedic Sciences, New Delhi through Intra Murai Research scheme vide project sanction letter 3–9/2014-CCRAS/Admin./IMR4/Pharmacology/(4.2.6) dated 18.06.2014.

Conflict of interest

None

Acknowledgments

The authors gratefully acknowledge the Director General, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India for financial support and Edward Food Research and Analysis Centre Limited, Barasat, Kolkata for chemical analytical work. The authors also acknowledge Dr. Nikhil Jirankalgikar, from the Pharmacopoeia Commission of Indian Medicine and Homoeopathy for his technical inputs during the writing of the manuscript.

References

[1] Anonymous. The ayurvedic Pharmacopoeia of India Part I second edition. In: The controller of publications. 2nd ed. Delhi: Civil lines; 2003. p. 43.
[2] Antarkar DS, Vaidya AB, Doshi JC, Athalave AV, Vinchoo KS, Natekar MR, et al. A double-blind clinical trial of Arogya-wardhani—an ayurvedic drug—in acute viral hepatitis [cited 2019 Jul 6] Indian J Med Res [Internet] 1980;72:588–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7014426.
[3] Kumar G, Srivastava A, Sharma SK, Gupta YK. The hypolipidemic activity of Ayurvedic medicine, Arogyavardhini vati in Triton WR-1339-induced hyperlipidemic rats: a comparison with fenofibrate. J Ayurveda Integr Med 2013;4(3):165–70. https://doi.org/10.4103/0975-9476.118707.
[4] Saper RB, Kales SN, Paquin J, Burns MJ, Eisenberg DM, Davis RB, et al. Heavy metal content of Ayurvedic herbal medicine products. J Am Med Assoc 2004;292(21):2688–73.
[5] Kang-Yum E, Oransky SH. Chinese patent medicine as a potential source of mercury poisoning. Vet Hum Toxicol [Internet] 1992;34(3):235–8. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt= Citation&ui=1609495.
[6] Lynch E, Braithwaite R. A review of the clinical and toxicological aspects of “traditional” (herbal) medicines adulterated with heavy metals. Expert Opin Drug Saf 2005;4(4):269–78. https://doi.org/10.1517/14740338.4.4.769.
[7] Cooper K, Noller B, Connell D, Yu J, Sadler R, Oslobwy H, et al. Public health risks from heavy metals and metalloids present in traditional Chinese medicines. J Toxicol Environ Health Part A Curr Issues 2007;70(19):1694–9.
[8] Kumar G, Srivastava A, Sharma SK, Gupta YK. Safety evaluation of an Ayurvedic medicine, Arogyavardhini vati on brain, liver and kidney in rats. J Ethnopharmacol [Internet] 2012;140(1):151–60. https://doi.org/10.1016/j.jep.2012.01.004.
[9] Sawaki M, Hattori A, Tuszuki N, Sugawara N, Enomoto K, Sawada N, et al. Chronic liver injury promotes hepatocarcinogenesis of the LEC rat. Carcinogenesis 1998;19(2):311–5.
[10] Anonymous. The ayurvedic Pharmacopoeia of India part - II (formulations) volume - II, appendix 6. 1st ed. Delhi: Delhi: The Controller of Publications; Civil Lines; 2008.
[11] Anonymous. The ayurvedic Pharmacopoeia of India Part I volume VIII. In: The ayurvedic Pharmacopoeia of India. 1st ed.vol. 8. Delhi: The Controller of Publications; Civil Lines; 2010. p. 171.
[12] Xu J, Song J, Chang X-M, Luo J-Y, Dong L, Hao Z-M, et al. Estrogen reduces CCL4- induced liver fibrosis in rats. World J Gastroenterol [Internet] 2002;8(5):883–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12378635.
[13] Ramadan SI, MA Shalaby NA, HE -B. Hepatoprotective and antioxidant effects of Silybum marianum plant in rats. Int J Agro Vet Med Sci (IJAVMS) 2011;01(1): 36.
[14] Liu J, Goyer RWM. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull’s toxicology - the basic science of poisons. 7th ed. McGraw Hill; 2007. p. 931–79.
[15] Copper in drinking-water background document for development of WHO guidelines for drinking-water quality. 2004.
[16] Panda AK, Das D, Dixit AK, Giri R, Hazra J. The effect of Arogyavardhini Vati and Phalatrikadi Kwatha in non alcoholic fatty liver disease – case studies. Int J Adv Case Reports 2016;3(2):59–62.
[17] Patil M, Kadam R. The efficacy of Arogyavardhini Vati in alcoholic fatty liver - a clinical study. World J Pharmaceut Sci 2017;8(2):353–7.
[18] Park JD, Zheng W. Human exposure and health effects of inorganic and elemental mercury. J Prev Med Public Heal 2012;45(6):344–52.
[19] Hong YS, Kim YM, Lee KE. Methylmercury exposure and health effects. J Prev Med Public Heal 2012;45(6):353–63.
[20] Jadhav SH, Sarkar SN, Aggarwal MTH. Induction of oxidative stress in erythrocytes of male rats subchronically exposed to a mixture of eight metals found as groundwater contaminants in different parts of India. Arch Environ Contam Toxicol 2007;52:145–51.
[21] Singh S, Chaudhary A, Rai DRS. NOPR: preparation and characterization of a mercury based Indian traditional drug — ras-sindoor welcome to NISCAIR online periodicals repository. Indian J Tradit Knowl 2009;8:346–51.
[22] Ramanan N, Lahiri D, Rajput P, Varma RC, Arun A, Muraleedharan TS, et al. Investigating structural aspects to understand the putative/claimed non-toxicity of the Hg-based Ayurvedic drug Rasasindura using XAFS. J Synchrotron Radiat 2015;22(5):1233–41.
[23] Thakur KS, Vahalia MK, Jonnalagadda VG, Rashmi K. Evaluation of structural, chemical characterisation and safety studies of samagandhak kajjali, an Indian traditional ayurvedic drug. J Pharmacogn Phytochem 2014;2(6):57–67.
[24] Liu J, Shi J-Z, Yu L-M, Goyer RA, Waalkes MP. Mercury in traditional medicines: is cinnabar toxicologically similar to common mercurials? Exp Biol Med 2008;233(7):810–7.
[25] National Research Council (US) Committee on Copper in Drinking Water. Health effects of excess copper [internet]. Copper in Drinking Water 2000:78–113. Available from: http://www.nap.edu/catalog/9782.html#Ahttp://www.nap.edu/catalog/9782.
[26] Kumar V, Kalita J, Misra UK BH. A study of dose response and organ susceptibility of copper toxicity in a rat model. J Trace Elem Med Biol 2015;29:269–74.
[27] Chaudhari SY, Nariya MB, Galib R, Prajapati PK. Acute and subchronic toxicity study of Tamra Bhasma (incinerated copper) prepared with and without Amritkaran. J Ayurveda Integr Med 2016;7(1):23–9. https://doi.org/10.1016/j.jaim.2015.11.001. Available from:.
[28] Chaudhari S, Galib R, Jagtap C, Patgiri B, Prajapati P, Bedarkar P. Review of research works done on Tamra Bhasma [incinerated copper] at Institute for post-graduate teaching and Research in Ayurveda, Jamnagar. ANV 2013;34(1):21.
[29] Lee GP, Jeong W II, Jeong DH, Do SH, Kim TH, Jeong KS. Diagnostic evaluation of carbon tetrachloride-induced rat hepatic cirrhosis model. Anticancer Res 2005;25(2 A):1025–38.
[30] Althaana T, Albokhadaim I, El-Bahr SM. Biochemical and histopathological study in rats intoxicated with carbon tetrachloride and treated with camel milk. SpringerPlus 2013;2(1):1–7.
[31] Choi J, Bae S, Lim H, Lim JA, Lee YH, Ha M, et al. Mercury exposure in association with decrease of liver function in adults: a longitudinal study. J Prev Med Public Heal 2017;50(6):377–85.
[32] Lee MR, Lim YH, Lee BE, Hong YC. Blood mercury concentrations are associated with decline in liver function in an elderly population: a panel study. Environ Heal A Glob Access Sci Source 2017;16(1):1–8.
[33] Hazelloff MH, Torres AM. Gender differences in mercury-induced hepatotoxicity: potential mechanisms. Chemosphere 2018;202:330–8. https://doi.org/10.1016/j.chemosphere.2018.03.106.
[34] Dardouri K, Haouem S, Gharbi I, Srira B, Haouas Z, Hani A El, et al. Combined effects of Cd and Hg on liver and kidney histology and function in wistar rats. J Agric Chem Environ 2016;05(04):159–69.
[35] Fuentealba IC, Aburto EM. Comparative Hepatology Animal models of copper-associated liver disease. Comp Hepatol [Internet] 2003;2(2):1–12. Available from: http://www.comparative-hepatology.com/content/2/1/5.
[36] Fuentealba C, Guest S, Haywood S, Horney B. Chronic hepatitis: a retrospective study in 34 dogs. Can Vet J 1997;38(6):365–73.
[37] Wang L, Huang QH, Li YX, Huang YF, Xie JH, Xu LQ, et al. Protective effects of Silymarin on triptolide-induced acute hepatotoxicity in rats. Mol Med Rep 2018 Jan;117(1):789–800.