

## Safety evaluation of a traditional knowledge based copper device for microbial purification of drinking water at home

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Copper as a trace element plays an important role in several physiological functions. Earlier studies have reported an affordable copper based device causing microbial decontamination of drinking water by leaching of ~120 ppb of copper, when left in contact with water for 16 hrs. This study tested the oral toxicity of copper device treated water over a period of 90 days in Sprague Dawley rats following the OECD (Organisation for Economic Co-operation and Development) test guideline 408. Two groups of animals were used (12 rats in each test and control group) wherein the control group animals were fed with rodent diet with normal drinking water and test group animals fed with the same diet but supplemented with water containing 100-120 ppb of copper. After 90 days of exposure, clinical chemistry, histopathology, tissue enzymes of liver and kidney and serum copper levels were studied. There was no significant difference observed in the clinical chemistry of serum samples ( $p > 0.05$ ) and histopathology of various organs examined. A gender-related difference in the serum copper levels in female rats was found to be statistically significant,  $p < 0.05$ . Overall, copper in drinking water at levels of 100-120 ppb was found to be non toxic in SD rats in this study.

**Keywords:** *Ayurveda*, Drinking water, Copper, Safety evaluation, Sub chronic exposure, SD rats

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Copper is one of the important trace elements required for various physiological functions. It is well known for its beneficial effects since antiquity and scientifically proven in this recent era for its various beneficial physiological functions including antimicrobial property and therefore in the application at different fields<sup>1,2</sup>. The ancient texts of *Ayurveda* recommend the use of metals such as gold, silver and copper for water purification. Traditionally in India, it is believed that drinking water that is stored overnight in copper vessels imparts good health<sup>3</sup>. Our previous studies indicated that copper pot kills major diarrhoea causing bacteria and virus including *Vibrio cholerae*, *Salmonella typhi*, *E. coli*, *Shigella flexneri*<sup>4,5</sup> and *Rotavirus*<sup>6</sup> in distilled and/ or tap water. Since copper pots are expensive, a device was designed guided by the surface area to volume ratio used in the copper vessel experiments to provide a cost effective solution for microbially safe drinking water. The copper device was made out of 99.9 % pure copper

and does not contain lead. Copper levels leached into water about 100-120 ppb after overnight storage (~16 hrs). These copper levels are well within the permissible limits of WHO (2000 ppb)<sup>7</sup> and EPA (1300 ppb)<sup>8</sup>. WHO safety guidelines based on meta-analysis of research papers report different copper salts as test compounds such as Copper (II) acetate monohydrate, Copper (II) chloride, Copper (II) nitrate, Copper (II) oxide and Copper (II) sulfate<sup>7</sup> and not for copper treated water per se. It was known that the toxic effect of copper differs with the type of copper salts used<sup>9</sup>. And the toxicity of copper salt dependent on its solubility profile<sup>10</sup> and therefore, exhibit differently in the metabolic process such as absorption, distribution and storage. It was reported that the toxicity is partially dependent on copper salts when tested comparative action of copper chloride and copper sulfate. It was found that tribasic copper chloride was safer and more available than copper sulfate in broiler chickens<sup>11</sup>. In another study, the trace amount of copper as copper sulfate at concentration 120 ppb was found to be toxic when

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administered through water in cholesterol fed rabbits<sup>12</sup> and Watanabe rabbits<sup>13</sup> though it was one tenth of the permissible level. The recent research article emphasized the need of reassessing the safe level of copper in drinking water in the normal animals with no genetic defects<sup>14</sup>. Therefore, it was our interest to test the sub chronic toxicity of the copper leaching into water from the copper device. The objective of this study was to determine the effect of copper leached from the copper device on the health status in Sprague-Dawley rats. The important serum biochemical parameters, tissue antioxidant enzyme levels, body weight of the animals, histopathological parameters and serum copper levels were evaluated after 90 days of *ad libitum* oral route of exposure.

## Materials and methods

### Fabrication of copper device

Copper device was prepared as per the method given by Sudha *et al.*,<sup>4</sup> with some modification. Copper cable was obtained from Pawan Metal Industries, Bangalore, India and tested for purity (> 99 %; lead content not detected) at a commercial laboratory (Essen & Co, Bangalore, India, a UKAS, NABL & ISO 9001:2008 certified laboratory).

### Copper device treated water (CDTW)

The CDTW was prepared by leaving the copper device in tap water filtered in Aquaguard<sup>TM</sup> (10 L) for 16 hrs. Copper content of the CDTW was measured using copper kit (Spectroquant<sup>TM</sup>, Merck, Cat no: 1.14767.0001, Darmstadt, Germany) according to manufacturer's instructions.

### Animals and conditions

A study protocol was submitted to and approval obtained (IAEC/ABMRCP/2013-14/18) from the Institutional Animal Ethics Committee (IAEC) of Acharya & BM. Reddy College of Pharmacy, Bangalore. The study was conducted on SD (Sprague Dawley rats (~150 gm) at Acharya & BM. Reddy College of Pharmacy, Bangalore following the OECD guideline 408. Twelve animals (6 male and 6 female) were assigned in each of the control and test groups. The rats were fed on a rodent diet *ad libitum*. CDTW was freshly administered *ad libitum* on a daily basis to the test group, throughout the study period of three months. The control group received tap water filtered in Aquaguard<sup>TM</sup>. After collecting blood samples, the rats were sacrificed by cervical dislocation.

### Body weight measurement, clinical Chemistry and Histopathology

Body weight of the rats in test and control groups were measured at day 0, 20, 30, 40, 60 and 90 to find if there are any changes upon copper water treatment. At the conclusion of the 90 days experiment, the food was withheld for 24 hrs, rats were anesthetized and blood was drawn in heparinized vacutainers and analyzed for renal and hepatic biochemical parameters as below. The serum sample was analysed for SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamic-pyruvic transaminase), GGT (Gamma-glutamyl transferase), LDH (Lactate dehydrogenase), CHO (Cholesterol), HDL (High-density lipoprotein), GLU (glucose), TP (Total protein), BUN (Blood urea nitrogen), Na (sodium), K (potassium), Cl (Chloride), TG (Triglyceride) and A/G (ratio of albumin to globulin) using a biochemical blood analyzer (Mispa excel, Agappe diagnostic ltd, India and Electrolyte analyzer, 9180, Roche, Germany). Homogenized liver and kidney tissues were also analysed for enzyme levels such as MDA (Malondialdehyde) as per method described<sup>15</sup>, SOD (Superoxide dismutase)<sup>16</sup>, MPO (Myeloperoxidase)<sup>17</sup> and CAT (Catalase)<sup>18</sup> using spectrophotometer (Shimadzu, 1700, Japan). Organs including brain, heart, spleen, lung, testis, ovary, intestine, thymus, kidney and liver were removed carefully and fixed in 10% formalin containing neutral phosphate-buffered saline. All the tissues were processed by routine paraffin embedding technique and 5 micron thick sections were cut and subjected for H&E (Heamatoxylin Eosin) staining<sup>19,20</sup> and examined under light microscopy.

### Statistical analysis

The data were expressed as mean  $\pm$  standard deviation (SD). The statistical significance of the difference between control and treatment group was evaluated by Student's t-test. A significant difference was set at  $p < 0.05$ .

### Results

Consumption of copper device treated drinking water for 90 days did not induce any obvious symptom of toxicity in rats of both sexes. The *ad libitum* water consumption in both test and control groups was observed to be normal. No death of animals was observed in any of the groups throughout the experimental period. Test group rats did not produce any significant changes in body weight when compared to control group rats (Fig.1). It is apparent

Table 1 — Serum biochemical values of SD rats after 90 days oral administration of copper treated drinking water

Parameters	Male control	Male test	Female control	Female test
SGOT U/L	163.91±4.06	145.23±27.48	111.41±38.89	111.41±34.78
SGPT U/L	85.65±10.04	68.26±15.60	88.49±20.11	64±28.47
GGT U/L	0.7±0.41	0.95±0.75	0.97±0.73	0.88±0.51
Total protein g/L	5.71±0.31	6.01±0.19	6.01±0.30	6.47±0.24
Albumin g/L	2.78±0.14	2.47±0.27	2.47±0.19	2.73±0.24
Globulin g/L	2.92±0.38	3.53±0.39	3.54±0.11	3.74±0.27
AG ratio g/L	0.96±0.17	0.71±0.14	0.69±0.03	0.73±0.11
Glucose mg/ dL	67.71±21.25	62.83±10.70	56.04±21.83	42.16±14.26
Electrolyte Na+ (mmol/L)	103.33±15.56	108.33±15.16	93.66±26.53	92±16.48
Electrolyte K+ (mmol/L)	3.76±0.35	4.43±0.64	3.86±1.10	4.16±0.96
Electrolyte Cl- (mmol/L)	74.66±10.06	81.33±10.03	70.66±17.03	68.33±11.03
BUN (mg/dl)	14.26±2.03	12.99±1.19	16.62±1.01	13.86±1.89
LDH U/L	1937.87±562.70	1853.60±411.65	1666.77±572.95	1246.03±484.96
Cholesterol mg/ dL	65.18±13.38	68.68±9.11	60.72±7.78	75.90±13.30
Triglycerides mg/ dL	82.69±4.68	88.04±6.66	99.07±24.99	100.13±12.12
HDL mg/ dL	24.53±10.78	22.56±7.32	22.51±6.51	28.32±5.70
Serum Copper ug/ dL	79.86±3.70	95.85±11.90	88.36±11.49	174.45±46.96*

Values are expressed as Mean ± SD ( $n = 12$  for each group).  $p$  values < 0.05 were considered significant. Asterisks (\*) denote significant difference compared to control.

from Table 1 that, biochemical parameters were also found normal. Test group rats had no significant effect on the serum electrolytes, such as Sodium, Potassium, and Chloride. The kidney function parameters such as BUN, LDH and liver function parameters such as SGOT, SGPT and GGT did not have any significant changes. Additionally, no significant changes in Glucose, Cholesterol, total protein, globulin and albumin levels were noted. But, significant difference was observed in serum copper in female rats of test group. Furthermore, levels of enzymes such as CAT, MDA, SOD and MPO of liver and kidney tissues were also not altered significantly (Table 2). The light microscopy examinations of the transverse section of organs of the test and control group rats are shown in Fig. 2. Histopathological examination of the control group and test group rats showed normal structure and absence of any pathological lesion in all the studied organs such as brain, heart, spleen, lung, testis, ovary, intestine, thymus, kidney and liver.

## Discussion

Ancient texts of *Ayurveda* recommend the use of metals like gold, silver and copper for 'purification' of water. The water that is stored in clean, copper pots overnight and consumed the next morning is believed to impart health benefits<sup>3</sup>. Earlier we have demonstrated that the traditional Indian practice of storing drinking water overnight in copper vessels

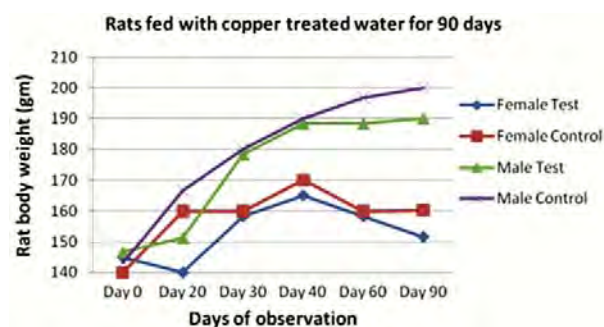


Fig. 1 — Body weight of SD rat at 90 days study duration: Statistically no significant changes in body weight ( $p > 0.05$ )

is a good way to kill water borne pathogens<sup>4,5,6</sup>. In order to provide a cost-effective solution for microbial purification of water, we subsequently developed and tested a low-cost copper based device that was found to be as effective as the copper vessels in decontaminating drinking water from diarrheagenic pathogens.

The permissible limit of copper in drinking water is 2000 ppb as per WHO standard. The background research evidence from published literature is based on different copper salts in drinking water<sup>7</sup>. There are studies that report that the effect of copper varies based on copper salts and its solubility<sup>7</sup>. This study was undertaken to evaluate the safety of CDTW on Sprague Dawley rats as per OECD guidelines. Copper leached into water from the device was 100-120 ppb, after overnight storage in water at room temperature.

Table 2 — Tissue enzyme (U/g) levels of SD rats after 90 days oral administration of copper treated drinking water - No significant changes observed

Target organ	Parameter	Male Control	Male Test	Female Control	Female Test
Liver	CAT	1960.25±704.07	2562.81±179.89	2511.32±257.12	3102.64±488.07
	MDA	172.47±6.72	181.41±8.42	166.51±9.09	179.91±8.05
	SOD	293.97±58.97	349.80±38.22	449.85±49.42	444.43±27.56
	MPO	4.08±0.27	3.81±0.28	4.33±0.59	4.19±0.21
Kidney	CAT	3610.78±548.73	3160.17±404.31	2952.75±739.97	3048.97±530.95
	MDA	189.49±3.82	197.67±6.51	185.65±4.24	193.90±9.80
	SOD	221.06±37.13	253.10±8.73	258.84±29.46	268.57±42.70
	MPO	4.01±0.28	3.97±0.30	4.39±0.47	4.07±0.15

Values are expressed as Mean ± SD (n = 12 for each group). *p* values < 0.05 were considered significant. Asterisks (\*) denote significant difference compared to control.

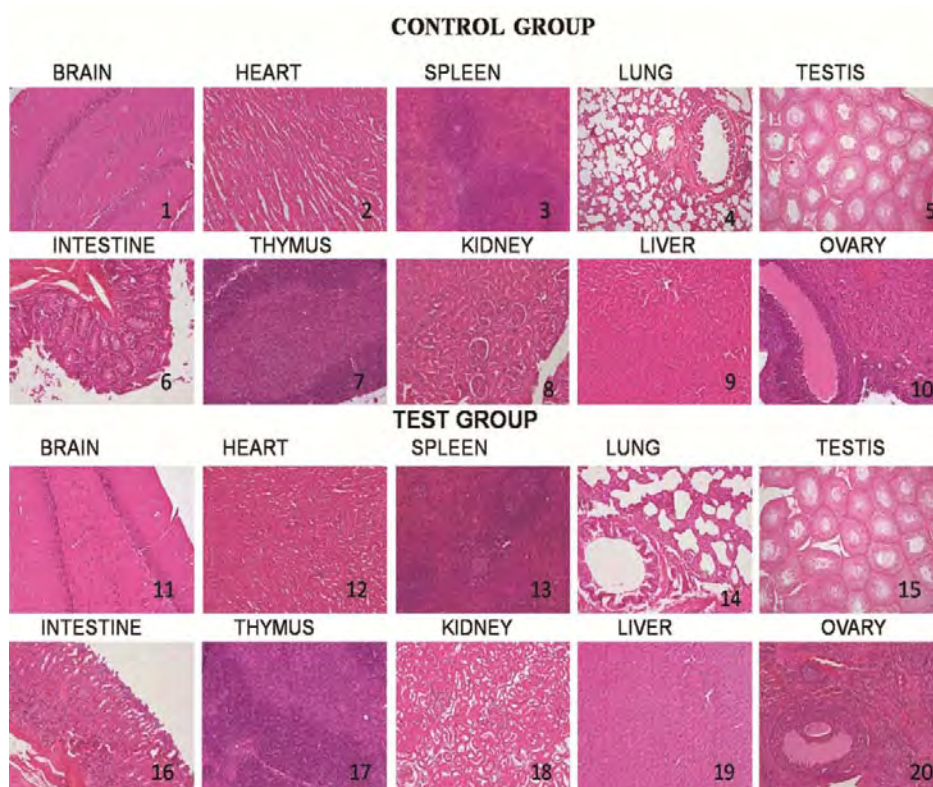


Fig. 2 — H & E (Heamatoxylin Eosin) staining of different tissues: Figures in control group 1-10 showing normal architecture, H&E X 100 Figures in Test group 11-20 Showing No alteration in architecture, H&E X100

There was no difference observed in renal- hepatic biochemical parameters among the test and control groups. No changes were also observed for CAT, MDA, SOD and MPO from liver and kidney tissues. Significant difference ( $p < 0.05$ ) was observed in serum copper levels in female rats (Table 1), as compared to male rats. Our observation is supported by earlier studies that it is safe for the serum copper level to increase in normal female rats as compared to males and that it is related to the hormones

particular to female sex and age of the animal<sup>21,22</sup>. Histopathological parameters such as degeneration, necrosis and infiltration of inflammatory cells were similar among control and test groups. All the organs examined appeared normal in the morphological architecture.

### Conclusion

These results indicate that the CDTW at the specified levels (100-120 ppb) is non toxicant in SD rats at sub-chronic exposure.

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