Effect of a mercurial drug of Siddha medicine on hematological, biochemical and antioxidant status in rats

B Ilango1, K Vinoth Kumar2, R Rajkumar3 & E Sukumar4*

1Department of Pediatrics, Division of Hematology/ Oncology/ BMT, University of Colorado, Aurora-80045, USA;  
2Department of Research, Meenakshi Academy of Higher Education and Research (Deemed University), Chennai-600 078, India;  
3Department of Quality Assurance, Strides Shasun Limited, Kudikadu, Cuddalore-607 005, India;  
4Department of Research, Saveetha Institute of Medical and Technical Sciences (Deemed University), Thandalam, Chennai-602 105, India  
E-mail: drsuku3@gmail.com  
Received 11 July 2017, revised 12 March 2018

Metallic drugs are used in traditional medicines to treat several chronic diseases. But apprehensions are raised on their toxicity and adverse effects on vital organs of the body. Traditional practitioners claim that if these drugs are prepared as per ancient texts and used rationally, they do not cause deleterious effects but can be effective in treating diseases. A mercurial drug of Siddha medicine, Gowri Chinthamani Chendooram (GCC) has been screened for its effect on blood and tissues of rats. Three doses of GCC (5, 10 and 20 mg/kg) were administered for 28 consecutive days and observed for various changes. On day 29, the animals were sacrificed and the blood was studied for hematology, biochemistry, lipid peroxidation and reduced glutathione while the tissues were observed for histopathological alterations, if any. No changes in anatomical and behavioral profiles as well as body and organ weights were seen. Dose-dependent increase in hematology and insignificant change in total protein besides dose-dependent decrease in total cholesterol, triglycerides, VLDL, LDL and increase in HDL level were observed. Mercury content in the serum and tissues of liver and kidney revealed a mild increase (in microgram level) while LPO in plasma and erythrocytes exhibited a significant decrease. GSH in plasma and erythrocytes were found to increase when compared to control animals. No histopathological changes were noticed. The drug showed significant hypolipidemic and antioxidant activities supporting the claim of traditional medical practitioners.

Keywords: Siddha medicine, Mercurial drug, Gowri Chinthamani Chendooram, Biochemistry, Antioxidant activity

IPC Int. Cl.: C09K 15/00, C40B, A61P 25/16, A61P 3/00, A61P

The objective of this study is to screen GCC for safety profile by investigating its effect on hematological, biochemical and antioxidant parameters along with histopathology of liver and kidney tissues of Wistar rats. Earlier studies on mercurial drugs served as the basis for this work4-6.

Methodology

Analysis of GCC

GCC prepared as per the procedure of traditional Siddha medical literature, has been procured from the Indian Medical Practitioners’ Co-operative Pharmacy and Stores (IMCOPS), Chennai, India. Determination of elements present in the drug was carried out using S4 Pioneer wavelength Dispersion X-ray Fluorescence Spectrometer (WDXRFS, Bruker-AXS, Germany).

Animals

Wistar male albino rats (150-170 g) obtained from Tamil Nadu University of Veterinary and Animal Sciences, Chennai (India) were maintained in the Animal House in the temperature range of 25-27 °C.
with 12 h light-dark cycle. Necessary permission was obtained from the Institutional Animal Ethics Committee and experiments were conducted as per the guidelines of CPCSEA and principles prescribed for laboratory animal use. The animals were fed with pelleted diet supplied by Poultry Research Station, Chennai (India) and water was provided ad libitum.

Treatment

Four groups of rats (n = 6) were selected; the first group served as control and to the other three groups, GCC was orally administered in doses of 5, 10 and 20 mg/kg body weight, as a suspension in 1% carboxymethyl cellulose by intubation for 28 consecutive days. These doses were selected on the basis of reported study. On day 29, over-night fasted animals were sacrificed by cervical decapitation; the blood was collected, centrifuged, serum separated and stored for further studies. Liver and kidney were separated from adhering tissues, washed with cold saline and used for histopathological investigations.

Body weight changes

The animals were weighed for any changes in body weight on 0, 14th and 28th days.

Hematological analysis

Blood samples were collected in EDTA anticoagulant tubes and taken to laboratory for analysis. All hematological parameters (WBC, RBC, Hb and haematocrit) were analyzed using Beckmann Coulter Auto Analyzer to check the reproducibility of results.

Biochemical analysis

From serum, total protein (TP), total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), very low density lipoproteins (VLDL) and high density lipoprotein (HDL) were estimated by standard methods.

Lipid peroxidation and antioxidant studies

The levels of lipid peroxide (LPO) and reduced glutathione (GSH) were estimated in plasma and erythrocytes.

Estimation of mercury accumulation

The accumulation of mercury in serum, liver and kidney of the normal and GCC treated animals were studied with ICP-OES Spectrometer (5300 DV, Germany).

Histopathological studies

For histopathology, the tissues of liver and kidney alone were considered as they are well known detoxifying organs. The tissues of the control and GCC treated animals were fixed in neutral buffered 10% formalin. 3-5 μm paraffin sections were mounted and stained with haematoxylin and eosin. The slides were examined under light microscopy.

Statistical analysis

The data obtained from haematogical and biochemical studies were subjected to statistical analysis using Student’s t-test and the values are expressed as mean ± SD of six observations. Values with p < 0.05, p < 0.01 and p < 0.001 were considered significant. For estimating mercury content in serum and tissues, One way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) has been used.

Results

GCC analyzed by X-ray Fluorescence Spectrometric (XRFS) method was found to contain 29.90% of mercury, 7.51% of sulphur and trace amounts of phosphorus, iron and calcium (Table 1).

Table 1 — Analysis of Gowri Chintamani Chendooram

<table>
<thead>
<tr>
<th>S.No</th>
<th>Element</th>
<th>Amount present (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mercury</td>
<td>29.90</td>
</tr>
<tr>
<td>2.</td>
<td>Sulphur</td>
<td>7.51</td>
</tr>
<tr>
<td>3.</td>
<td>Phosphorous</td>
<td>0.19</td>
</tr>
<tr>
<td>4.</td>
<td>Iron</td>
<td>0.15</td>
</tr>
<tr>
<td>5.</td>
<td>Calcium</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Fig. 1 — Effect of GCC on hematological parameters WBC-White blood cells (10³/μL); RBC-Red blood cells (10⁶/μL); Hb-Haemoglobin (g/dL); PCV-Packed cell volume. Values represent mean ± SD of six animals. *p < 0.05, when compared to control animals; NS -Non-significant.
been observed in the higher dose group. Lipid parameters such as TC, TG, LDL and VLDL in serum of experimental animals were found to decrease dose-dependently when compared to control group while the HDL level showed a significant increase (Fig. 2). Also, the drug treatment resulted in a mild but significant increase (in μg levels) of mercury in serum, liver and kidney (Fig. 3). The increase was low and within the accepted range; it could possibly get eliminated from the system through excretion. In case of LPO in plasma and erythrocytes, the values were found to decrease in a dose-dependent manner and GSH slightly decreased indicating no significant changes in 30 days treatment (Fig. 4). The liver and kidney showed normal histology and no obvious changes in the tissue architecture were noted in the doses studied.

**Discussion**

The toxicity of mercury depends upon its chemical form. In the toxicological studies with metallic drugs, an understanding of the deposition of metals in the vital organs is the important aspect. In toxico-kinetics, the absorption, distribution, transformation, storage and excretion are the important steps involved. Usually excess intake of metals either through environmental pollution or by the indiscriminate use of metal-containing drugs leads to metal deposition in vital organs such as liver, kidney, heart, testis, lung, brain and intestines of the mammals.
Pure metallic form of mercury and many other metals produce nephrotoxicity and hepatotoxicity. Absorption, distribution, metabolism and excretion of mercury are dependent upon its form and oxidation state. Inorganic form of mercury exhibits considerable toxic effect on most vital organs in the rats treated with 2 mg/kg of body weight. The toxic effect of inorganic mercury is due to its ability to adhere or form link with cell enzymes of the respiratory chain and proteins that alter the metabolism (mostly TCA cycle) of target cells in organs participating in its elimination. In chronic condition, its deposition has been observed predominantly in hard tissues and after reaching a critical condition, gets eliminated through excretory organs. These adverse reactions were not observed in the present study with GCC administration.

Analysis of GCC showed an appreciable amount of sulphur (7.51 %) besides mercury (29.9 %) suggesting that the metal is present in the drug as its sulphide (HgS). Cinnabar, the ore of mercury, has the metal as sulphide that has been shown earlier to be chemically inert and low toxic when taken orally. Also it has been reported to possess less toxicity when compared to other forms of mercury. It has been shown that mercuric sulphide in its compound form is also non-toxic. Probably these factors might be the reason for non-toxic nature of GCC.

In Siddha medicine, toxic metals that exert adverse effects on living organisms even in small doses are purified through specialized traditional techniques such as repeated trituration with herbal juices and ashing them in sealed earthenware containers. These processes not only free the final product from toxicity but also change their properties rendering them therapeutically useful products. Earlier investigations on mercurial drugs such as Linga Chendooram, Ayurvedic preparations Makaradhvaja, Rasasindura and Sidh Makhhardhwaj that are widely used in the therapeutic management did not show any adverse toxic effects.

The results obtained in the present study showed non-toxic nature of GCC in doses studied which is in line with reported findings. No change in total protein level might be construed as the synthetic function of liver remaining unaffected by the metallic preparation. Significant elevation in HDL strengthens the study and gives an indication to take up further investigation on its hypocholesterolemic activity. No appreciable changes in LPO and GSH levels in plasma and erythrocytes indicated drug’s ability in controlling or possibly preventing the free radical reactions which are beneficial from clinical point of view.
Modern researches have proved the significant role of metals and trace elements in the growth and maintenance of human body in the same way as that of proteins, vitamins and essential micronutrients. Also they possess therapeutic efficacy, which the ancient Siddhars have realized many centuries ago while developing this system of medicine and making use of metals and minerals in formulating drugs to treat chronic and baffling diseases. However, more studies in future can lend further support in the development of traditional medicines, which are serving the society for so many years in several parts of the world.

Acknowledgement
The authors thank the Management and Principal of The New College, Chennai for the facilities to carry out the work. They also thank the Sophisticated Analytical Instrumentation Facility (SAIF), I.I.T., Madras for XRFS studies.

References