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Invited Article

Researches on mercurial preparations: The prime requirement for their acceptance in medical world

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Abstract

Ayurvedic and Siddha medicinal preparations containing mercury have been used over centuries in India. The recent WHO guidelines on the use of mercurials as well as actions by other international organizations into eliminating mercury in all forms have put the people practicing Rasa Shastra in a quandary. Active research in the mechanism of curative actions of mercurials is very much essential, to have widespread acceptance of the ancient practice. The toxicity of a substance depends on its bio-availability; the chemical form in which it is present and the biochemical reactions it participates. Mercury is usually administered as mercuric sulfide (*Rasasindura* or *Linga Chendooram*) which has a KSP value of 10^{-54}. Despite this extreme insolubility, how mercury becomes bio-available under enzymatic conditions needs to be studied. Its bioaccumulation in critical organs and excretory pathways are to be ascertained. Research is also needed to establish whether *Rasasindura* or equivalent medicines induce the (excess) synthesis of sulfur containing biomolecules in human systems, which act as cell protectors against free radical-induced cell damage. The antioxidants themselves could be the curative agents; mercury being just a catalyst. It may also be possible that the exposure to mercury, even in very small amounts, could lead to the synthesis of specific metallothioneins in the human system, helping to detoxify the mercury exposure. The author is of the opinion that Ayurvedic practitioners/researchers should carry out long-term follow-up studies on human patients. The superiority of mercury based Ayurvedic preparations, as against modern allopathic medicines, in providing rapid and long lasting cure for specific diseases needs to document and published. In the absence of such supportive research literature, the use of mercury will become untenable, even for medicinal purposes.

Key words: *Kajjali*, *Makaradwaja*, mercurial preparation, mercury, *Parpati*, *Rasasindura*

Introduction

Ayurvedic and Siddha medicinal preparations containing mercury have been prepared and used over centuries in India. Similar preparations exist in China and Japan too. The preparatory methods are based on various classical texts and administered to patients along with various adjuvants depending on the type of disease. The types of diseases treated with mercurials range from tuberculosis to diarrhea. The mercury-containing preparations are many: Ayurveda has *Kajjali*, *Parpati*, *Rasasindura*, and *Makaradhwaja*, (which are essentially a combination of mercury and sulfur) and some medicines like *Garbhapala Rasa* which have lower mercury contents. Similar mercury medicines like *Linga Chendooram* exist in Siddha traditions.

All forms of mercury (vapor, inorganic salts, and organic forms) are considered as toxic to human beings and in-depth reports exist detailing the deleterious effects of these various forms both in animal studies and in humans inadvertently exposed to mercury. The Minamata episode in Japan and the poisonous effects on children born to mothers who had consumed organic mercury contaminated wheat in Iraq are well documented and

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the recent outcry in the USA on the use of Thimerosal as a preservative of vaccines and the suspected link to autism in children, is the latest in the campaign against the use of mercury in any form and at any dose levels for medicinal purposes. The WHO mentions that exposure to mercury – even small amounts – may cause serious health problems, and is a threat to the development of the child *in utero* and early in life; mercury may have toxic effects on the nervous, digestive and immune systems, and on lungs, kidneys, skin, and eyes; and mercury is considered by WHO as one of the top 10 chemicals or groups of chemicals of major public health concern (WHO fact sheet No. 261, updated September 2013). Studies have indicated at least in animal experiments that mercury affects the nervous system and has been shown to accumulate in kidneys leading to atrophy and failure. The WHO/JECFA has set a limit of 4 μg/kg bw for inorganic mercury.[1] This limit has been arrived at based on experiments using mercuric chloride ($HgCl_2$), a soluble salt of mercury. The PTWI for methyl mercury it is 1.6 μg/kg bw. The literature is also replete with toxicity suffered by patients who have consumed Ayurvedic preparations containing heavy metals particularly, mercury and lead. It is to be noted; however, that majority of these cases are due to self-medication by the people and not under conditions of supervision under an Ayurvedic doctor.

It is in this background, the claim of Ayurvedic and Siddha practitioners on the safety of their preparations containing mercury under therapeutic doses is viewed with suspicion by modern medicine. However, many journal publications in recent times have appeared, based on animals (mice, rats, and dogs), reporting on the nontoxic effects of Ayurvedic and Siddha mercurial preparations and changes in biochemical parameters. These studies can help evolve therapeutic doses for humans.

The Need for Complete Chemical and Physical Characterization of the Mercurials

Much of the fear induced on the use of mercury is due to the inability of the most common analytical methods, which report on the total elemental concentration,[2] (X-ray fluorescence, atomic absorption spectroscopy [AAS], inductively coupled plasma atomic emission spectroscopy [ICP-AES]), and not on the exact chemical form in which mercury is present. The exact chemical form in which mercury is present has also to be analyzed by X-ray diffraction (XRD), which provides the information on crystalline structure and X-Ray photoelectron Spectroscopy (XPS), which provides information on the oxidation state of the material, (especially where mercuric sulfide [HgS] is a major constituent) and infrared spectroscopy/fourier transform infrared spectroscopy for the presence of organics. The presence of free mercury/free sulfur should also be ascertained in the preparations. Toxicity perception based total concentration levels can be misleading because the toxicity of a substance depends on its bio-availability; the chemical form in which it is present, the biochemical reactions it participates, and the dose combined with age and other medical conditions of the patient.

Bio-availability of Mercury

The ancients brilliantly overcame the problem of mercury toxicity by severely reducing its bioavailability through the use of sulfur. They purified the raw mercury and sulfur through many steps using plants and salts and standardized the administered form of mercury as HgS, one of the least soluble substances. The KSP of HgS is 1×10^{-54}. Thus, the quantum of mercury ions that would be available on the administration of mercury as sulfide can be much below the threshold of toxic limit (the use of arsenic, a toxic substance, again used as a highly insoluble sulfide, *Rasamanikya*, is illustrative of the concern of the ancient sages over the detoxification efforts needed, before declaring a substance as a medicine). However, HgS may be more soluble in the gastrointestinal (GI) tract due to the action of digestive enzymes, changing pH conditions and complexation with other biomolecules present in the food. This has to be determined upon by experimentation.

Experiments on the bio-availability of various forms of mercury indicated the following percentages of absorption: Cinnabar <0.2% in GI, mercury vapor 80% in lungs and <0.01% in GI, $HgCl_2$ 7–15% in GI and methyl mercury >95% in GI. The mercury thus adsorbed is found distributed to liver, kidney, and spleen while mercury vapor and methyl mercury result in accumulation in the brain.[3] Autopsy studies on diseased humans in Greenland, exposed to mercury through food, have shown accumulation in kidney, spleen and liver, with kidney exhibiting highest accumulation.[4] Neurotoxicity induced by cinnabar in guinea pigs has also been reported.[5]

However, in the Indian context, all the animal studies have invariably reported that *Rasasindura* has not been found toxic under therapeutic doses;[6] even though no analytical studies on the accumulation of mercury in different organs (in animal studies) have been published from major Indian institutions. Reviews on beneficial applications of *Rasasindura*, on human patients,[7] as well as types of diseases it has been used have been published.[8]

This dichotomous situation needs to be thoroughly examined to establish the safety associated with the Indian practices.

Standardization of the Preparation and Composition

Assay of purified mercury

Standard texts uniformly mention some 8 stages of purification or mercury.[9] These steps are meant to remove natural impurities in mercury and make it "potent" but no high-quality assay on the purity (stoichiometrically) obtained after the 8 steps has been reported. Some authors surmise that while the inorganic metallic impurities are removed many organic entities get bound to the mercury up to even 4% by weight. There is also a mention that mercury purified by destructive distillation of Cinnabar after some specific treatment with plant juices can be used for medicinal preparations without the 8 steps. It is very essential to assay the purity of the mercury meant for medicinal preparation including the presences of other organics derived from treatment with plant extracts. Boiling point investigation and chromatographic techniques will help.

Purification steps have also been prescribed for sulfur which is more or less standard among all practitioners.

Understanding the Process of Preparation of *Kajjali*

Intimate mixing of purified mercury and sulfur to prepare the *Kajjali* seems to be the first step in the preparation of many mercury-based preparations, except for the use of mercurous chloride (*Rasapushpa*) and Hg_2Cl_2 (*Rasakarpura*) in very small amounts in certain cases. The compound of mercury and sulfur is prepared in many ways, but one always finds the use of excess sulfur in the preparation of a form called *Kajjali*, more than required for the stoichiometric preparation of HgS (approximately sulfur at $1/6^{th}$ of the weight of mercury). Classical texts have recommended the use of 1:1, 1:6 or even 1:16 of mercury and sulfur by weight. Why this emphasis on excess sulfur? The *Kajjali* itself is used as a simple medicine or forms the basis of preparations such as *Parpati, Rasa Sindura, Makaradwaja* (the most celebrated of all Ayurvedic medicines). Similar Siddha preparations are named as *Linga Chendooram* and *Poorna Chandrodayam*. Many recent reviews on the preparations and uses of these are found in literature.

In a study related immobilization and disposal of mercury, detailed observations on the process of mixing mercury and sulfur have been reported, using XRD and electron microscopy, as a function of grinding time.[10] The grinding process slowly forms meta-cinnabar (black HgS) and up to 60 min of grinding, globules of mercury could be found to be present, using electron microscopy. After 90 min, free mercury could not be found but the most important observation is the formation of particles containing 2–15 weight% of mercuric oxide after 120 min of grinding. It is also found that the HgS particles are surrounded by sulfur particles. Aqueous extracts of this sample yielded <5 μg/L leachable mercury.

The study above used Hg: S in 1:1 weight proportion but in Ayurvedic literature one finds the mention of 1:6 and higher ratios of mercury and sulfur in the preparation of *Kajjali*. The idea could be to prevent oxidation as well as to make available more sulfur. Studies similar to the above should be done in so as to establish the desirable/optimum quantum of grinding and prevention of oxidation of the mercury due to over grinding under exposure to oxygen. It may possible to use mechanical grinders under an inert atmosphere.

Mercury Speciation Studies in Body Fluids and Tissues

The major species of mercury one usually encounters are the elemental (vapor), inorganic (Hg[I] and Hg[II]) and organic forms (methyl and ethyl mercury). Mercury vapor can be very easily determined by using cold vapor AAS (CVAAS) at nanogram levels and when combined with ICP-mass spectrometry (ICP-MS), can be determined at parts per trillion levels.[11] Inorganic mercury is reduced to elemental mercury and then determined using CVAAS or ICP-MS. Methods are available for the online separation of inorganic and organic (methyl) mercury and sequential determination of both in a single experimental step at parts per billion levels.[12] All the methods have been standardized and are routine in many advanced analytical laboratories.

The uptake and distribution of mercury through the use of mercurial can be easily determined through the analysis of body whole blood and serum samples. The excretion can also be studied through the analysis of urine and fecal matter which can kind of give a mass balance of the dose administered and the mercury retained in the system. Such studies can go a long way in determining the variation in the biological sorption of mercury when administered as an insoluble sulfide (*Rasasindura/Makaradwaja/Linga Chendooram*) or as ionic salts (chlorides of mercury). The enhanced sensitivity of the modern analytical techniques enable the determination of mercury in biopsy samples of kidney, liver, etc., without having to sacrifice the animals in such experiments.

In humans, it is possible to analyze head hair before, during and after the stoppage of the mercury-based medicines, to follow-up qualitatively, the sorption and excretion of mercury.

Analysis of Mercurials Prepared with Sulfur

For over a decade, our laboratory, the National Centre for Compositional Characterisation of Materials, BARC, Hyderabad, has been involved in the development of a standardized analytical procedure for many mineral and metallic Ayurvedic medicines, as part of our association with CCRAS in this activity. Mercurials such as *Rasasindura, Makaradwaja* and *Rasa Gandhi Mezhugu* (a Siddha preparation) have been analyzed for the total content of mercury, sulfur and trace elements based on solution-based techniques such as ICP-AES and AAS. Scanning electron microscopy/EDAX has been used for rapid screening of the major element contents. XRD and XPS have been utilized to find out about the major crystalline phases and the oxidation states of the major elements, respectively. These standardization efforts have been shared with the concerned agencies. *Rasasindura* samples from many institutions were found to contain close to 81–83% of mercury and 14–16% of sulfur. The use of similar multi-technique approaches for the analytical characterization of Siddha medicines have been reported,[13] which is very encouraging.

However, there exist in literature, compounds named as *Rasasindoor* with only 9% HgS and having a lot of organic matters still intact.[14] How the organics could survive the extremely high temperatures used in the synthesis is a mystery! A study has reported that *Siddha Makradwaja* has only mercury and sulfur but does not contain even traces of any other element.[15] However, *Makaradwaja* samples received from Gujarat Ayurved University, analyzed in our lab, have been found to contain gold at 20–270 ppm [Table 1]. It has been explained that the variation of gold content depends on the type of gold used[16] or the extent sulfur used.[17] Some preparations even report *Poorna Chandrodayam* (a preparation similar to that of *Makaradwaja*) having close to 9% gold![18]

Table 1: Analysis of mercury and gold in *Makaradwaja* (ICP-AES)

Sample (from GAU)	Mercury (%)	Gold (ppm)
Makaradwaja 1	82.9	131
Makaradwaja 2	80.1	268
Makaradwaja 3	81.6	19.8

ICP-AES: Inductively coupled plasma atomic emission spectroscopy

It is essential standard methods for preparations and standard nomenclatures should be arrived at, well documented and be adopted in pharmaceutical literature, for specific medicines, pertaining to the discipline.

Analysis of Free Mercury and Sulfur in the Medicines

It has been our observation, in the analysis of *Rasasindura* and *Makaradhwaja*, that the contents of the major constituents do not add up to 100%. The qualitative analysis did not identify any extra constituents. This leads to the suspicion that there could be free sulfur embedded onto the matrix of the HgS. In a study mentioned earlier,[13] based on EDAX it is found that the *Linga Chendooram* sample could have up to 5.8% excess sulfur than calculated for the total mercury being in HgS form. Methods exist to analyze free sulfur[19] which, when used will help investigate the thoroughly the *Kupipakwa* procedure, used by many for HgS based preparations. Free sulfur, if found, can help explain the nontoxic nature of the mercurial as prepared and used by Indian practitioners, as can be surmised in the subsequent sections of this paper.

Further Research Studies Needed to Make Mercurials Acceptable on Par with Modern Medicines

The following suggestions are put forward for the consideration of people working in biochemistry, pharmacology, and pharmacovigilance.

The curative action of mercurials will have to be explained on molecular action basis. Certain actions like anti-bacterial activities are explained based on cell wall damage or suppression of antioxidant activity when soluble mercurials are used. However, when nominally insoluble sulfide is used, it is very essential to quantify the dissolution and bioavailability in the GI track in human studies. That orally administered *Rasasindura* or *Makaradhwaja* is therapeutically active indicates that mercury is absorbed in the GI tract and reaches the target organ/tissue. The mechanism of such transfer needs to be understood first.

The Role of Adjuvant *(Anupana)*

Each mercurial preparation is administered along with a specific adjuvant, depending upon the disease.[8] Current knowledge enables us to understand that many of these adjuvants, themselves have many anti-oxidant molecular entities. Why the ancients chose a specific *Anupana* needs to be researched and explained, in medical parlance.

Role of Metallothioneins in Reducing/ Eliminating Mercury Toxicity

Metallothioneins are small molecular weight peptides containing close to 20 and above cysteine amino acid units and are considered to play a central role in the physiology of detoxification of heavy metals.[20] The SH group very strongly complexes mercury. Heavy metals induce the synthesis of phytochelatines (cysteine containing peptides) in plants; similarly it may be possible that the exposure to mercury, even in very small amounts, could lead to the synthesis of specific metallothioneins in the human system, helping to detoxify the mercury exposure. Metallothionein has been shown to be responsible for binding most of the mercury in rat kidney when $HgCl_2$ was administered.[21] Further research in the mechanism of mercury detoxification through metallothioneins is very much essential.

Whether Antioxidant Synthesis is Induced by Consuming Mercurials

As mentioned earlier, some mercurials like *Kajjali* have excess sulfur, and even *Linga Chendooram* is reported to contain free sulfur, perhaps trapped in the crystal lattice of HgS. It is well known that sulfur is a very important nutrient and many biomolecules such as methionine, cysteine, cystin, taurine, and antioxidant enzymes such as glutathione (GSH) and many more, contain sulfur.[22,23] Thus, research is needed to establish whether *Rasasindura* or equivalent medicines induce the (excess) synthesis of these sulfur-containing biomolecules in human systems. Antioxidants are the cell protectors against free radical-induced cell damage and it is quite possible that the rejuvenating effects and the reversal of aging effects, alluded to *Makaradhwaja* could be due to sulfur consumed along with. Detailed biochemical analyses will be the key to answer these questions. The antioxidants themselves could be the curative agents while mercury could serve as a transient catalyst. Enhanced production of GSH production in the kidney due to $HgCl_2$ orally given than when HgS (mercuric salts of very different solubility) was administered, has been observed.[24]

A Brief Summary of Works Carried out at National Centre for Compositional Characterisation of Materials, Hyderabad

- Toxicity of *Rasasindura* (HgS) and *Rasamanikya* (arsenic trisulfide) were evaluated through standard bacterial study procedures. While *Rasasindura* was found not to show any bactericidal effects, inorganic mercury (Hg^{2+}) species exhibited

high toxicity. At 5 ppm of Hg^{+2} completely inhibited the growth of *Pseudomonas aurgenosa* and yeast and at 1 ppm, a twofold and 4 fold decrease in the viability of *P. aurgenosa* and yeast respectively, have been observed. *Rasamanikya* did not show any toxicity at 10 and 20 μg but showed slight toxicity at 30–50 μg levels, but much lower than the toxicity exhibited by gentamycin (5 μg) [Table 2]

- A study in our laboratory on the anti-oxidant status of mice fed with *Rasamanikya* has shown that the superoxide dismutase, GSH, and glutathione peroxidase (GPx) levels had shown an increase in the liver and showed a significant decrease in liver TBARS thus ensuring cell protection. In the case of the kidney, the GPx levels were much reduced at the double dose level as well as elevation in the kidney TBARS levels indicated that at double dose levels the kidneys could be impaired. Such assessments are planned for *Rasasindura*, but other research groups have reported on its nontoxic nature based on biochemical parameters and histopathology observations [Figure 1].

Table 2: Toxicity studies on three bacterial species w.r.t. *Rasamanikya* (As_2S_3)

Well number	*Rasamanikya* concentration (μg)	Zone of inhibition (mm)		
		PA 27853	SA 25923	EC 25922
1	10	0	0	0
2	20	0	0	0
3	30	13	5	11
4	40	14	10	13
5	50	16	12	15
Center	Gentamycin (5 μg)	25	21	22

PA ATCC 27853 EC ATCC 35218 SA ATCC 25923. PA: *Pseudomonas aurgenosa*, SA: *Staphylococcus aureus*, EC: *Escherichia coli*

These preliminary studies clearly indicate that the synthesis of antioxidant molecules to get triggered on the exposure to toxic elements but the response in liver and kidney are different. Much more detailed studies are the need of the day.

Conclusions

Controlled experimentation with complete records as well as follow-up of patients over a long period is essential when mercurials have been used to treat specific diseases. The use of modern analytical techniques is a must to characterize the drug, its interaction with specific body tissues/organs and to provide a molecular basis for the curative aspects. In the task of bringing into the mainstream, the unutilized potential of the ancient science of healing, a close interaction of practitioners of traditional Indian Systems of Medicine (ISM) and Allopathy and involvement of national institutions is a must. Regional institutions with a suite of sophisticated analytical instruments catering to the needs of ISM would go a long way in supporting generating validated data.

The author is of the opinion that Ayurvedic practitioners/researchers should carry out long-term follow-up studies on human patients, and publish them in standard medical literature, rather than in in-house or obscure journals. Therapeutic efficiencies of mercury based preparations and modern Allopathic medicines in providing rapid and long lasting cure for specific diseases need to be compared, documented and published.

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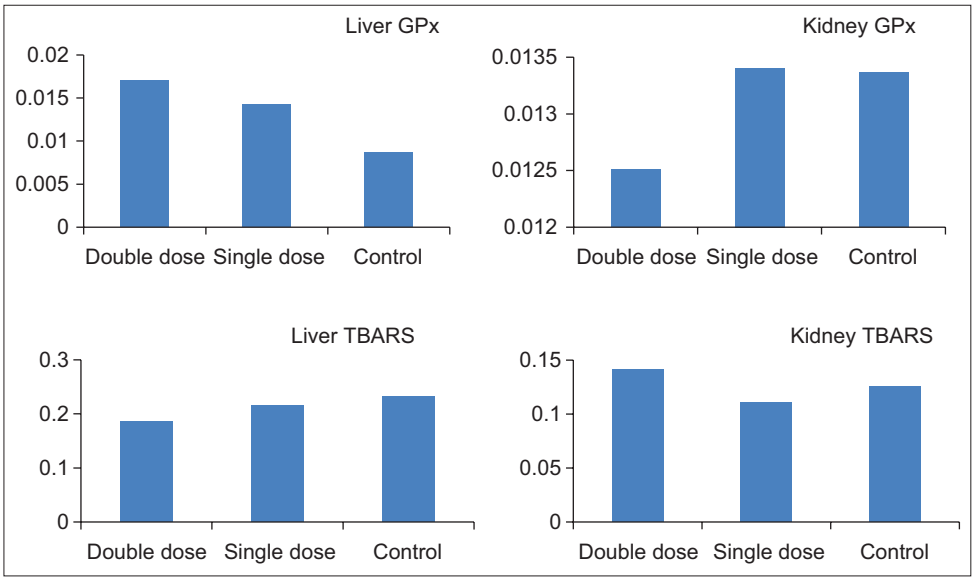

Figure 1: Effect of *Rasamanikya* on antioxidant status of mice: An *in vivo* study

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हिन्दी सारांश

चिकित्सा की दुनिया में पारद युक्त औषधियों पर अनुसंधान की परम आवश्यकता

जे. अरुणाचलम

आयुर्वेद एवं सिद्ध भारत में प्राचीन काल से ही पारद युक्त औषधियों का प्रयोग करते आ रहे है। विश्व स्वास्थ्य संगठन के ताजा मार्गदर्शी सिद्धांत पर पारद के उपयोग के साथ-साथ अंतर्राष्ट्रीय संगठनो द्वारा पारद के विभिन्न रूपों का निष्कर्षन पर प्रतिबंध से रसशास्त्र का पेशा करने वाले वैद्य पेशोपेश में पड़ गये है। पारद की चिकित्सात्मक क्रियाविधि पर अनुसंधान अति आवश्यक हो गया है, जिससे प्राचीन काल से इसके प्रयोग की सार्थकता सम्पूर्ण विश्व में फैल सके। किसी द्रव्य की विषाक्तता उसकी बायोएवेलॅबिलिटी पर निर्भर होती है अर्थात द्रव्य में रसायनिक तत्व किस अवस्था में है और वह शरीर पर किस प्रकार से प्रतिक्रिया करती है। पारद का चिकित्सीय उपयोग मुख्यतः मरक्यूरिक सल्फाइड (रससिन्दूर या लिंग चन्द्ररम) रुप में होता है जिसकी घडि मुख्य १०-५४ है इस के साथ अति अघुलनशीलनता के कारण पारद एन्जाइम की उपाधि में किस प्रकार क्रियाशील होता है उसके अध्ययन की आवश्यकता है। संवेदनशील अंगो में इसका जमा होना तथा शरीर से बाहर निकलने का रास्ता की अनिश्चितता पर भी अनुसंधान आवश्यक है। इस सिंदूर या इनके समान अन्य औषधियां शरीर के विभिन्न संस्थानों मे सल्फर युक्त जैवतत्वों के निर्माण को प्रेरित करता है जो कोशिकाओं को फ्री रेडिकल होने वाली क्षति के विरुद्ध कार्य करता है। एन्टीओक्सिडेन्टस की क्रिया को पारद प्रेरित करता है। यह भी मान सकते है कि पारद की सुक्ष्म मात्रा में प्रयोग शरीर के संस्थानो में मेटेलो पाइनिन्स के निर्माण से पारद के विषैले प्रभाव को नष्ट करने में सहायक है। लेखक की सलाह है कि आयुर्वेद चिकित्सक या अनुसंधान कर्ता मनुष्य पर लम्बे समय तक अनुपरीक्षण अनुसंधान करें। विशिष्ट व्याधियों में पारदयुक्त उच्च आयुर्वेद औषधियों का एलोपेथी औषधियों के सापेक्ष में तीव्र एवं दीर्घकालिक परिणामों को प्रलेख तथा प्रकाशित करने की आवश्यकता है। इस सहायक अनुसंधान सामग्री के अभाव में औषधि प्रयोग के लिए भी पारद अनुपलब्ध हो जायेगा।