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RESEARCH ARTICLE

Assessment of Heavy Metal Induced Organ Toxicity in marketed Ayurvedhic Formulation and Report its LD50 value with Brine Shrimp Lethality Assay

Saranya A, Nithya S*
Department of Pharmacology, School of Pharmaceutical Sciences, Vels University (VISTAS), Chennai, Tamil Nadu, India
*Corresponding Author E-mail: **sermugapandian.nithya@gmail.com**

ABSTRACT:
Ayurvedhic system of medicine is an ancient and traditional medicine of India. These preparations use combination of remedies to alleviate diseases. Heavy metal poisoning in ayurvedhic preparation contribute to 0.6% of global burden for diseases. Children consuming this estimated to contribute about 6, 00,000 of newer intellectual disabilities every year. Previously the ayurvedhic preparations were available as bhasmas and they are incorporated with adjuvant heavy metals to increase the potency. Heavy metals often found are lead, arsenic and mercury. Lead interferes with many bodily function affecting brain, lungs, liver and kidney. Arsenic causes keratosis, gangrene; cancer in skin, kidney, bladder and lungs after 20 yrs. Mercury is a neurodevelopmental poison affecting cell migration causing cell degeneration and death. In modern formulations their presence may be excessive because of poor quality control, contamination, adulteration and improper purification. Therefore users of ayurvedhic formulation are at risk of developing heavy metal poisoning leading to organ toxicity. In this study a selective herbal formulation were subjected to estimate for their heavy metal content and its lethality value is found by carrying out with brine shrimp lethality assay.

KEYWORDS: Ayurvedha, Lead, Arsenic, Mercury, Lethality Assay.

INTRODUCTION:
Ayurveda is one of the traditional systems in India. Ayurvedic formulation is the system of healing that originated in ancient period in india .the ancient hindu science of health and medicine, based on maintaining balance among the five elements earth, air, fire and ether [1].Ayurvedha is derived from theSanskrit words “Ayur” means “living or life” and “veda” means “science or knowledge” that defined as the “knowledge of living” or “the science of longevity”[1,2].

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Ayurveda is a stream of the knowledge passed on from generation to generation from teachers to their students. Samhitas - the authentic text books of Ayurveda are a compendium of experiences by ancient doctors and discussions by teachers and disciples. Aim of Ayurveda is to prevent diseases in a healthy person and to cure the diseased one.World Health Organization estimated that 80% of the world inhabitants still rely mainly on traditional medicines for their health care[3]. Ayurvedic medicines containing detoxified, toxic material, poisonous substances, heavy metals should be taken under medical supervision[4].

Demerits of Ayurvedic Medicine:

- Lack of dosage instructions.
- Poison risk associated with wild herbs.
- Medication interactions.
- Lack of regulation[5].

Heavy metals:

Heavy metals are the natural components of the earth crusts[6]. Heavy metals refer to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentration[7,8].Heavy metal poisoning in ayurvedic preparation contribute to 0.6% of global burden for diseases. Children consuming this estimated to contribute about6, 00,000 of newer intellectual disabilities every year.[9]

Mercury (Hg), Copper (cu), Arsenic (As), Lead (Pd), Cadmium (Cd), Chromium (Cr) this is some of the example of heavy metal. Mercury is a heavy, silvery – white metal and hence it is commonly known as quick silver and was formerly named hydrargyrum .Compared to other metals, it is a poor conductor of heat, but a fair conductor of electricity[6,7] .

A Normal mercury level is less than 10µg/l or 0.47µg/kg/day it is according to world health organization.Mercury has a freezing point of -38.83°C and a boiling point of 356.73°C, both exceptionally low for a metal. In addition, mercury's boiling point of 629.88K (356.73°C) is the lowest of any metal. Mercury is a chemical element with symbol Hg and atomic number80.[10,11]

Mercury is a prominent environment contaminant that causes detrimental effect to human health[12,13.] Although the liver has been known to be a main target organ, there is limited information on in-vivo molecular mechanism of mercury –induced toxicity in the liver. Mercury (Hg) is not only a threat to public health but also a growth risk factor to plants,as it is readily accumulated by higher plants.[10]

Mercury (Hg) is deadly toxic to humans and ecosystems,which is considered as a global pollutant because it is highly toxic or mobile and extremely persistent in the environment .It is not only a threat to public health but also a growth risk factor to plants, as it is readily accumulated by higher plants[14,15].Hence forth the preparations made from plant extracts have the possibility to have traces of heavy metals in it and thereby leading to organ toxicity.[16,17]

Organ toxicity is the degree to which a substance can damage an organism. Toxicity can refers to the effect on a whole organism, such as an animals, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell or an organ such as the liver toxicity.[18,19]

Copper is a chemical element with symbol Cu and atomic number 29. It is a soft, malleable and ductile metal with very high thermal and electrical conductivity. A freshly exposed surface of pure copper has a reddish-orange color[18]. Symbol: Cu, Melting point: 1,085 °c; Molar mass: 63.546 g/mol; atomic number: 29

Copper Health Benefits and Recommended Intake. Copper is an essential trace mineral necessary for survival. Most of the copper in the body is found in the liver, brain, heart, kidneys and skeletal muscle.It is a ductile metal with very high thermal and electrical conductivity. Pure copper is soft and malleable; a freshly exposed surface has a reddish-orange color. It is used as a conductor of heat and electricity, a building material, and a constituent of various metal alloys[20,21].

Copper is a mineral that's found throughout the body. It helps your body make red blood cells and keeps nerve cells and your immune system healthy. It also helps form collagen, a key part of bones and connective tissue.[22,23]

Copper is incorporated into a variety of proteins and metallicenzymes which perform essential metabolic functions; the micronutrient is necessary for the proper growth, development, and maintenance of bone, connective tissue, brain, heart, and many other body organs[24,25].

All US coins are now copper alloys, and gun metals also contain copper. Most copper is used in electrical equipment such as wiring and motors. This is because it conducts both heat and electricity very well, and can be drawn into wires.[14]

Used in electrical switches, fluorescent light bulbs, thermometer ect. Emission from mercury containing product are batteries, thermometers. Mercury is also used in dental filling as amalgam[26,27].

Signs and symptoms of metal toxicity:

As an example of the scope of a heavy metal's toxicity, lead can affect the nervous system, gastrointestinal system, cardiovascular system, blood production, kidney, and reproductive system [28].Symptoms of heavy metal toxicity include mental confusion, pain in muscles and joints, headaches, short term memory loss, allergies, vision problems, chronic fatigue and others[24,29].

The symptoms are so vague that it is difficult to diagnose based on symptoms alone. Mercury toxicity has been linked to, among other things, mercury dental fillings, particularly when people have a large number of them. Symptoms include a metallic taste in the mouth, excess salivation, gingivitis, tremors, stomach and kidney troubles. Mental symptoms include shyness, irritability, apathy and depression, psychosis, mental deterioration and anorexia[10,14,17].

Treatment:
The first step in treating any heavy metal toxicity is to identify the toxic elements and begin the removal process. The easiest screening process is a Hair analysis. For many patients intravenous Vitamin C and replacement mineral infusion are also recommended to support the body through the metal removal process[29,30].

Symptoms will often begin to improve within weeks or even days of commencing treatment. Therapy may last from 6 months to 2 years.[31]

Brine Shrimp:
Artemia is a genus of aquatic crustaceans known as Brine Shrimp. Brine shrimp is a small fairy which lives in a pool and salt water like oceans and is used as food for aquarium fish. Sea monkeys are the brand name for brine shrimp[32].In their first stage of development, Artemia do not feed but consume their own energy reserves stored in the cyst. Wild brine shrimp eat microscopic plank tonic algae[33].

Scientific classification:
- Family : Artemiidae.
- Kingdom : Animalia.
- Order : Anostraca.
- Species : Artemia saline.

Structure of Brine Shrimp:
The body of the Artemia is divided into head, thorax, and abdomen. The entire body is converted with a thin, flexible exoskeleton of chitin to which muscles are attached internally and shed periodically. The adult has 3 eyes and 11 pairs of legs and can grow to 15 millimeter in size. Their blood contains the pigment Hemoglobin, which is also found in vertebrate[34].Artemia do not feed but consume their own energy reserves stored in the cyst. Wild brine shrimp eat microscopic plank tonic algae. Cultured brine shrimp can also be fed particulate foods including yeast, wheat flour, and soybean powder or egg yolk[35].

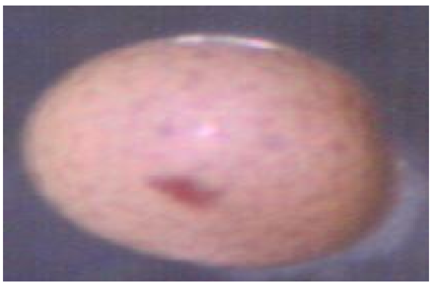

Figure No -1 Brine Shrimp egg.

Figure No-2-Structure of Brine Shrimp under Compound Microscope

Uses:
- They are soft and easily digestible and contain enzymes that help fish to better utilize other feeds.
- They are high in protein, ranging from 55% to 60% protein by dry weight, supporting rapid weight gain in young fish[36].
- They can be feed to both marine and freshwater fish, surviving and swimming for hours –even in fresh water.
- They can grow quickly, multiplying in weight 500 fold in three to four weeks and increasing in size from 450 microns to 1.5 centimeters in length[37].

Lethal Dose 50:
LD stands for "Lethal Dose". LD_{50} is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD_{50} is one way to measure the short-term poisoning potential (acute toxicity) of a material[38].

LD 50 (Lethal Dose 50) =
The apparent least lethal dose –[a×b]/N.

Where;
N = Number of animals in each groups.
a = Dose difference.
b = Mean mortality.

Brine Shrimp Lethality Assay:
The brine shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity .It has also been suggested for screening pharmacological activities in ayurvedic formulation[39,40].

MATERIALS AND METHODS:

Collection of ayurvedic medicine:
Marked Ayurvedic drug were collected from the ayurvedic retail shop in guindy,(Chennai) for the project or research work. The collected ayurvedic medicines are NityanandaRas, Vata gajankush Ras, Prabhakara Vati. These drugs were selected because of their uses and it is easily available in market but only get with the prescription.The drugs that selected is in tablet form so it is easy to carry .Nityananda Ras is used to treat Gout, Tumor, Piles, Elephantiasis, Obesity etc . It has potential

in the treatment of cancer .It can taken once or twice along with cold water. And it having the doses of 250-500 mg. Vata gajankush Ras is used to treat Neuro muscular disease, Paralysis, Arthritis, Cramps. Vata gajankush Ras is taken 1-3 time in the morning and evening. It is advised along with long pepper, honey. In this drug the dosage are125-259 mg once or twice a day. Prabhakara Vati is used in the treatment of Heart disease. It contains mineral origin ingredients and hence should only be taken under a doctor's prescription. The ayurvedic doctor should advise toget the dosage of 125mg- 250 mg once or twice a day.

Preparation of drug solution:

The collected of three marketed ayurvedic drug were taken each separately and crushed it thoroughly until it converted into a fine powder. Then the ayurvedic drug (each 100g) were individually defatted with warm water (100ml) and then filtered and subjected for the activity studies[40].

Table No 1- The Number of Shrimp nauplii that survived after treating with the three Ayurvedic Drug Solution and their percentage mortality

Drug Solution	Concentration (ppm or mg/l)	Number of surviving nauplii after 24 hours			Total number of surviving nauplii (live)	% Mortality
		T_1	T_2	T_3		
NITYANANDA RAS	1	10	09	08	27	10%
	10	07	08	06	21	30%
	100	06	05	04	15	50%
	1000	00	00	00	00	100%
VATAGAJANKUSH RAS	1	07	07	08	22	27%
	10	04	06	05	15	50%
	100	00	00	00	00	100%
	1000	00	00	00	00	100%
PRABHAKARA VATI	1	07	08	08	23	23%
	10	05	06	05	16	47%
	100	04	05	05	14	53%
	1000	00	00	00	00	100%

$$\% \text{ Mortality} = \frac{\text{Number of animal dead in each groups}}{\text{\% of Nauplii surviving in control}} \times 100$$

Heavy metal report:

The ayurvedic drug is given for the test to identify the heavy metal amount or the quantity present in it. The test is given in SGS India private limited in Ambattur Industrial state, Ambattur ,Chennai -600058.

Table No -2 Heavy Metal Report

Ayurvedic drug Solution	Test for heavy metal	Methods used	Result (in units)
NITYANANDHA RAS	Mercury	SO-IN-MUL-TE-063 BY ICP-MS	1.04g/ 100 g
	Copper	SO-IN-MUL-TE-063 BY ICPOES	1.42g/ 100 g
VATA GAJANKUSH RAS	Mercury	SO-IN-MUL-TE-063 BY ICP-MS	1.33g/ 100 g
	Copper	SO-IN-MUL-TE-063 BY ICPOES	0.02g/ 100 g
PRABHAKARA VATI	Mercury	SO-IN-MUL-TE-063 BY ICP-MS	0.20g/ 100 g
	Copper	SO-IN-MUL-TE-063 BY ICPOES	0.02g/ 100 g

Brine Shrimp Lethality Assay (BSLA)[39,40]:

Brine shrimp eggswere obtained from the Aquamarine world in guindy. Filtered, artificial seawater was prepared sea salt 38 gms in 1 liter of distilled water for hatching the shrimp eggs. The initial PH should be between 7.5 and 8. The pH is likely to fall during the culture period and can be adjusted upward with the addition of baking soda or $NaHCO_3$. Monitor P_h regularly and adjust as needed. The seawater was put in a small plastic container (hatchingchamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae are ready, 4 ml of the artificial seawater was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. Then the volume was adjusted with artificial seawater up to 5 ml per test tube. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 hours.

RESULTS AND DISCUSSION:

The ayurvedic drug solution of the powdered drug tested shows good brine shrimp activity. The lethality dose of Nityananda Ras, Vatagajankush Ras, and Prabhakara Vati solution were 1ppm (µl/ml), 10ppm,100ppm, and 1000ppmrespectively (Table-2). The degree of lethality was directly proportional to the concentration of the solution. Maximum mortality 100% were observed at a concentration of 1000ppm in Nityananda Ras,and Prabhakara Vatisolution while that of Vata gajankush Ras was at 100 and 1000ppm.

In result, the brine shrimp lethality of the ayurvedic drugs were found to be concentration will be dependent .The observed lethality of the drug solution indicated the presence of potent activity. According to ayurvedic drug solution is toxic(active) if it has an LD50 value of less than 1000µg/ml while non-toxic (inactive) if it is greater than 1000µg/ml.

The results showed that the drug solution of the three selected ayurvedic drug were potent or active against brine shrimp where Vata gajankush Ras was the most active at 10 ppm. In the study conducted by drug solution Vata gajankush Ras was observed as having an neuromuscular activity and it is more potent even at small amount.

Prabhakara Vati ranks as the second potent drug against brine shrimp at 1-10ppm in this presence study. And it is used to treat heart diseases and it is also act as cardiac tonic. The result on the lethality of Nityananda Rason brine shrimps is less than the other drugs of Vata gajankush Ras and Prabhakara Vati. Thus, some useful drugs of therapeutic importance may develop out of the research work.

In the other hand, some studies have shown that Nityananda Ras solution exhibited selective cytotoxicity against several cancer cell lines. Thus,the result on Nityananda Ras supports its use in traditional ayurvedic medicine as well as that of Prabhakara Vati and Vata gajankush Ras.

The tested ayurvedic drugs contain heavy metal of mercury and copper at the amount of non-toxic level at below 1000ppm.Theayurvedic drug solution is toxic (active) if it has an LD50 value of more than 1000µg/ml while non-toxic (inactive) if it is less than 1000µg/ml.The Nityananda Ras contain heavy metal of mercury is 1.04g/100 and copper is1.42g/100 g. The Vata gajankush Ras contain 1.33g/100 g in mercury and copper contain 0.02g/100 g. The Prabhakara Vati contains mercury at the level of 0.20g/100 g and copper is the lesser amount of 0.02g/100 g.

CONCLUSION:

The ayurvedic extract of Nityananda Ras, Vatagajankush Ras, Prabhakara Vati,are the drug exhibited for brine shrimp toxicity against the brine shrimp and considered as containing active or potent components. This is because their LD50 values are less than 1000 ppm or 1g/l. It is very useful for any future in vivo or clinical study of the drug solutions. However, further toxicity studies are needed to determine the effect on this ayurvedic drug on chronic toxicity, on animal fetus, pregnant animals, and their reproductive capacity,to complete the safety profile of this solution. Thus some useful drugs of therapeutic importance may develop out of the research work.

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