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ESTIMATION OF HEAVY METALS FROM SHANKH-VATI TABLET

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ABSTRACT

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. Eight common heavy metals are discussed in this brief: arsenic, barium, cadmium, chromium, lead, mercury, selenium, and silver. Even these metals do not have any biological role, they remain present in our body harms human body and its functioning. As all ayurvedic formulation contains large amount of heavy metals due to the herbal ingredients used for preparation. One of the ayurvedic formulation from among formulation i.e. Shankh-vati tablet. We have studied the amount of heavy metals present in shankh-vati tablet. Shankh-vati is and ayurvedic classical formulation available in tablet form which is used to manage the digestive disorders. It is also used to resolve the various problems like anorexia, vomiting, gastritis etc. Natural ingredients used in this medicine help to balance the tridoshas in body. Shankh-vati totally contains 15ingredients. Mainly parad (mercury) is mostly used in ayurvedic formulation. Mercury is present in large amount in ayurvedic formulation. Along with determination of heavy metals, limit test for iron and sulfate is also done. Sulfate occur as microscopic particles(aerosols) resulting from fossil fuel and biomass combustion. They increase the acidity of the atmosphere and form acid rain. The limit test has passed which confirms that heavy metals in shankh-vati tablet is in limit. Also other metals like iron and sulfate are in limit.

KEYWORDS: Shankh-vati Tablet, Heavy Metals, Toxicity, Mechanisms of Heavy Metals, Limit tests.

INTRODUCTION

Heavy Metals

A metal of relatively high density (specific gravity greater than about 5 g/cm^3) or of high relative atomic weight is defined as a heavy metal. The term "Heavy metals" is used to describe more than a dozen elements that are metals or metalloids ex; chromium, arsenic, cadmium, lead, mercury, manganese, etc. Heavy metals are natural constituents of the Earth's crust. Heavy metals cannot be destroyed nor degraded. In small amounts, they enter the human body via food, drinking water and air. Living organisms require varying amounts of "heavy metals". Iron, cobalt, copper, manganese, molybdenum, and zinc are required by humans. Therefore, heavy metals can be described as any metallic element that has a relatively high density and is toxic or poisonous at low concentrations. Human activities affect the natural geological and biological distribution of heavy metals through pollution of air, water, and soil. Heavy metals which are toxic are realsed by humans only. Bioaccumulation refers to an increase in the concentration o f a metal in a biological organism over time, compared to the normal concentration in the environment.[25] Some heavy metals like mercury and lead are toxic metals that have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of animals can cause serious illness of harmful effects. Certain elements that are normally toxic are, for certain organisms or under certain conditions, may be beneficial. Therefore, they tend to accumulate in the soil, seawater, freshwater, and sediments. In small quantities, certain heavy metals are nutritionally essential for a healthy life (e.g., iron, copper, manganese, and zinc). Some of these are referred to as the trace elements. These elements, or some form of them, are commonly found naturally in foodstuffs, in fruitsand vegetables, and in commercially available multivitamin products.As some ayurvedic formulation also includes needed essential heavy metals. The ayurvedic formulations contain required amount of the essential heavy metals which are useful for lives. There are many types of different ayurvedic formulations in which all of them contains heavy metals. Although it is acknowledged that heavy metals have many adverse health effects and last for a long period of time, heavy metal exposure continues is increasing in many parts of the world. Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The most commonly found heavy metals in waste water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risks for human health and the environment. Various sources of heavy metals include soil erosion, natural weathering of the earth’s crust, mining, industrial

effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others.[24] These metals bind with protein sites which are not made for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity. Previous research has found that oxidative deterioration of biological macromolecules is primarily due to binding of heavy metals to the DNA and the nuclear proteins.[11]

Shankh-vati is an Ayurvedic formulation or medicine available in tablet form which is used to manage the digestive disorders. Calmative action of shankh-vati helps to provide relief in the burning sensation of stomach, also helps to improve the body's ability to absorbs the nutrition in a natural way. It is mainly prepared by triturating the fine powder of ingredients with lemon juice to prepare a paste. From which paste, tablets are prepared, dried and stored.

The toxic effects of these metals, even though they do not have any biological role, remain present in some or the other form harmful for the human body and its proper functioning. They sometimes act as a pseudo element of the body while at certain times they may even interfere with metabolic processes.

Various public health measures have been undertaken to control, prevent and treat metal toxicity occurring at various levels, such as occupational exposure, accidents and environmental factors.

Physiological roles of heavy metals in humans

- Iron – hemoglobin, myoglobin
- Cobalt – coenzyme
- Copper – co-factor in enzymes
- Zinc – in enzymes
- Selenium – in enzymes
- Chromium – Cr3+ in enzyme

Uses of heavy metals

- Mercury is found in batteries, dental amalgam, vacuum pumps and valves. Airborne mercurycomes from the combustion of diesel, jet fuel and heating oil.

- Arsenic is high in seafood and may also be found as a contaminant in animal feeds. It is also present in wood preservatives, herbicides, corrosion inhibitors, in lead and copper alloys.
- Cadmium is used industrially as an anti-friction agent, as a rust-proofer, in plastics manufacture, in alloys and in alkaline storage batteries.
- Chromium is found in fresh foods, copy machine toner and nickel in coins, kitchen utensils and milk.
- Copper is essential to all living organisms and has a wide range of effects depending on concentration and chemical formulation. It is used in the electrical industry in alloys such as brass, in chemical catalysts and in wood-preservatives.
- Lead has been used in batteries, electronic equipment's, in petrol, toys, paint, etc. Lead has been used as fuel additive in many countries for several years, although this practice has since stopped in most of the countries of the world, because of the health implications of lead.
- Manganese compounds are used in manufacturing of products such as batteries, steel and unleaded petrol. Manganese dioxide is commonly used in the production of dry-cellbatteries, matches, fireworks, porcelain and glass-bonding materials. It is also used as thestarting material for the production of other manganese compounds.
- Manganese chloride is a precursor of other manganese compounds. It is used as a catalyst inthe chlorination of organic compounds, in animal feed to supply essential trace minerals andin dry-cell batteries. Manganese sulfate is used as a fertilizer, livestock nutritional supplement and in ceramics.

Effects of heavy metals on human

Humans are always exposed to the natural levels of trace elements. Under normal circumstances; the body is able to control some of these amounts. However, continuous exposure to elevated levels of metals could cause serious illness or death. Increased exposure is due to contaminated soil and other industrial wastes which affects the skin.

There are 35 metals that are of concern for us because of residential or occupational exposure, out of which 23 are heavy metals: antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc. These heavy metals are

commonly found in the environment and diet. In small amount they are required for maintaining good health but in larger amounts they can become toxic or dangerous.

The health hazards presented by heavy metals depend on the level and the length of exposure. In some cases, the health effects are immediately apparent; in others, the effects are delayed.

High levels of toxic metals deposited in body tissues and subsequently in the brain, may cause significant developmental and neurological damage, including depression,increased irritability, anxiety, insomnia, hallucination, memory loss, aggression and many other disorders. Heavy metal toxicity can lower energy levels and damage the functioning of the brain, lungs, kidney, liver, blood composition and other important organs.

MATERIALS AND METHODS

Shankh-Vati

Shankh-vati, an ayurvedic medicine available in tablet form which is used to manage the digestive disorders. This remedy is quite effective to improve the digestive fire in the body and also helps to strengthen the digestive system. It is quite good to resolve the various problems like anorexia, vomiting, gastritis etc. For anorexia, it helps to improve the digestion and appetite as well. Natural ingredients, used in this medicine help to balance the tridoshas in body. Works well in the problem of excessive gas, flatulence, burping etc. Calmative action of Shankh-vati helps to provide relief in the burning sensation of stomach, also helps to improve the body's ability to absorb the nutrition in a natural way.

It contains heavy metals ingredients and hence should only be used under strict medical supervision. 15 varieties of shankh-vati were found available across different across different Ayurveda texts. Shankha bhasma, Hingu, Vatsanabha, Trikatu, Kshara and Lavana are the ingredients common to most varieties of Shankha Vati.Shankh-vati is a classical Ayurvedic formulation that is widely used by the practitioners of Ayurveda, contains a heavy metal i.e. Parad(mercury) and a poisonous herbal drug i.e. Vatsanabha (Aconitum chasmantum Staffex Holmes) along with other ingredients. There are certain apprehensions on this formulation, mainly due to the presence of its ingredients Vatsanabha Shankha Vati(Bhaishajya Ratnavali 10/186-187, also in Ayurvedic Formulary of India part 1), is one of the formulations in which Vatsanabha is used without its usual coingredient and antidote Tankana (Borax).

Fine powder of each of

Panchalavana\Ingredients

1. Saindhava Lavana- Rock salt
2. Sauvarchala Lavana- Sochal salt
3. Samudra Lavana- Common salt
4. Vida Lavana- Vida salt
5. Nimbu swarasa- Lemon juice- Quantity Sufficient
6. Shankha Bhasma- Bhasma of Conch Shell
7. Hingu- Asafoetida
8. Shunti- Ginger Rhizome – *Zingiber officinalis*
9. Maricha- Black pepper- *Piper nigrum*
10. Pippali- Long pepper fruit- *Piper longum*
11. Purified Mercury- Parad
12. Vatsanabha- Aconitum ferox
13. Shuddha Gandhaka
14. Krishna Lavana

Uses

1. Dyspepsia
2. Low digestion power
3. Anorexia
4. Peptic diseases
5. Emaciation
6. Improper digestion
7. Excessive gas
8. Gulma
9. Burning sensation in stomach

Dosage

1-2 tablets (250-500 mg) once or twice in a day, before or after food or as directed by ayurvedic doctor.

Precautions[9]

1. This medicine should be strictly taken under the medical supervision.
2. It should be avoided by pregnant, lactating mothers and children.

3. Over dosage may cause slight burning sensation in stomach.
4. For high BP patients this medicine should be taken with care as it contains salt as an ingredient.
5. Keep out of reach and sight of children.
6. Store in a cool dry place.

Mercury

The metallic mercury is a naturally occurring metal which is a shiny silver-white, odorless liquid and becomes colorless and odorless gas when heated. Mercury is very toxic and exceedingly bio accumulative. Major sources of mercury pollution include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater.[10]

Mercury exists mainly in three forms

1. Metallic elements
2. Inorganic salt
3. Organic compounds

Each of which possesses different toxicity and bioavailability. These forms of mercury are present widely in water resources such as lakes, rivers and oceans where they are taken up by microorganisms and get transformed into methyl mercury within the microorganism, eventually undergoing bio magnifications causing significant disturbance to aquatic lives. Consumption of this contaminated aquatic animal is the major route of human exposure to methyl mercury. Mercury is extensively used in thermometers, barometers etc. Mercury poisoning symptoms include blindness, deafness, brain damage, digestive problems, kidney damage, lack of coordination and mental retardation. One of the most famous cases of mercury poisoning resulting from chronic exposure was the disaster that occurred in Minamata, Japan, where methylmercury was discharged from a plastics manufacturing plant into the waters of Minamata Bay in the 50s and 60s. This is reflected in its abundance in the earth's crust as oxides, carbonates, silicates with iron, magnesium and as sulphides, arsenide's and telurides. Nickel salts are soluble and can occur as a leachate from nickel bearing rocks.[20] It is also a known 'carcinogen'.

Mercury is considered the most toxic heavy metal in the environment. Mercury poisoning is referred to as acrodynia or pink disease. Mercury is released into the environment by the

activities of various industries such as pharmaceutical, papers and pulp preservatives, agriculture industry, and chlorine and caustic soda production industry. Mercury has ability to combine with other elements and form organic and inorganic mercury. Exposure to elevated levels of metallic, organic, and inorganic mercury can damage brain, kidneys and the developing fetus. Mercury is present in most foods and beverages in the range <1 to 50ug/kg. In marine foods it is often seen at higher levels.

Micro-organisms convert the mercury present in soil and water into methyl mercury, a toxin which can accumulate with fish age and with increasing trophic levels. EPA has declared mercuric chloride and methyl mercury to be highly carcinogenic. The nervous system is very sensitive to all types of mercury. Increased exposure of mercury can alter brain functions and lead to shyness, tremors, memory problems, irritability, and changes in vision or hearing.

Exposure to metallic mercury vapors at higher levels for shorter periods of time can lead to

Damage to lungs, Vomiting, Diarrhea, Nausea, Skin rashes, Increased heart rate or blood pressure.

Symptoms of organic mercury poisoning include

1. Depression
2. Memory problems
3. Tremors
4. Fatigue
5. Headache
6. Hair loss etc.

Since these symptoms are common also in other conditions, it may be difficult to diagnose such cases. Due to the excess health effects associated with exposure to mercury, the present standard for drinking water has been set at lower levels of 0.002 mg/L and 0.001mg/L by the Environmental Protection Act and World Health Organization.

Mechanism of mercury toxicity

The brain remains the target organ for mercury, yet it can impair any organ and lead to malfunctioning of nerves, kidneys and muscles. It can cause disruption to the membrane potential and interrupt with intracellular calcium homeostasis. Mercury binds to freely

available thiols as the stability constants are high. Mercury vapours can cause bronchitis, asthma and temporary respiratory problems. Mercury plays a key role in damaging the tertiary and quaternary protein structure and alters the cellular function by attaching to the selenohydryl and sulfhydryl groups which undergo reaction with methyl mercury and hammer the cellular structure. It also intervenes with the process of transcription and translation resulting in the disappearance of ribosomes and eradication of endoplasmic reticulum and the activity of natural killer cells. The cellular integrity is also affected causing free radical formation. The basis for heavy metal chelation is that even though the mercury sulfhydryl bond is stable and divided to surrounding sulfhydryl consisting ligands, it also contributes free sulfhydryl groups to promote metal mobility within the ligands.[4]

Procedure[2]

1. Take powder 0.5gmof tablet, treat it with 7 ml of concentrated nitric acid, add 15ml concentrated sulphuric acid in kjeldahl flask.
2. Heat the mixture under reflux gently strongly for 30min.
3. Cool the mixture and add 50ml of concentrated nitric acid to remove brown fumes.
4. Continue the addition of concentrated nitric acid and boiling until liquid is colourless. Cool, wash the condenser with 100ml of water.
5. Remove flask add 1.0% potassium permanganate solution dropwise until pink colour persist.
6. Decolorize it by adding 6.0% hydrogen peroxide dropwise to remove excess potassium permanganate followed by 3.0ml concentrated nitric acid.
7. Titrate with 0.1N ammonium thiocyanate solution using ferric alum as indicator.

Each ml of 0.1N ammonium thiocyanate solution is equivalent to 0.01003gm mercury.

Observation table

0.1N Ammonium thiocyanate as titrant	
Burette reading	**Mean(ml)**
2.5ml	2.5ml
2.5ml	
2.5ml	

Factor: - As each ml of 0.1N ammonium thiocyanate solution is equivalent to 0.01003gm of mercury.

2.5ml of 0.1N ammonium thiocyanate contains 0.025015gm of mercury.

Iron

Iron is the second most abundant metal on the earth's crust. Iron is the most abundant transition metal in the earth's crust. Biologically it is the most important nutrient for most living creatures as it is the cofactor for many vital proteins and enzymes. Iron occupies the 26^{th} elemental position in the periodic table. Iron is a most crucial element for growth and survival of almost all living organism. It is one of the vital components of organisms like algae and of enzymes such as cytochromes and catalase, as well as of oxygen transporting proteins, such as hemoglobin and myoglobin. Iron is an attractive transition metal for various biological redox processes due to its inter-conversion between ferrous and ferric ions. The source of iron in surface water is anthropogenic and is related to mining activities.

Iron occurs as particulate ferric hydroxide or in the form of organometallic compound in natural system. Fe (III) oxide and Fe (II) are ubiquitous in anoxic environments and they affect the distribution, transport, and biogeochemistry of chemical contaminants by absorption onto Fe(III) oxides and by control of oxidation and reduction reactions. Iron is regarded as one of the essential elements for humans. Approximately 3000 to5000 mg of iron exists in the human body. Therefore, as long as the quantity of iron in the environment is not too large, it may not be harmful to the human body.[1]

However, iron can cause undesirable problems in industrial processes or ecosystems if itsconcentration in water is not managed properly. WHO has recommended a value of 0.3mg/L as permissible limit for drinking water. For fresh Chronic excessive intake of iron may lead to hemosioderosis or hemochromatosis. Iron constitutes about 4.7 % of the earth's crust. It is the second most importantmetallic element in the terrestrial environment. Iron is extremely useful, but can also behighly toxic to cellular constituents when present in excess. Iron is an important part of theplant's oxidation- reduction reactions. Iron is a structural component of cytochromes, hemes, and numerous otherelectron-transfer systems, including nitrogenase enzymes necessary for the fixation ofdinitrogen gas. The major problem with iron availability is how to keep iron sufficientlysoluble for plants to absorb enough of it. In strongly acidic solutions (pH < 5), iron becomesincreasingly soluble, and is rarely deficient It is essential for the physiological processes ofall living organisms.

The production of sulphuric acid and the discharge of ferrous takes place due oxidation of iron pyrites that are common in coal seams. The concentration of dissolved iron in the deep ocean is normally 0.6nM or 33.5x10-9 mg/L. In freshwater the concentration is very low with

a detection level of 5 ug/L-ICP, whereas in groundwater the concentration of dissolved iron is very high with 20 mg/L.A study of iron toxicity on aquatic plants, particularly rice, reported that the growth of species of aquatic reed was found to be inhibited by concentration of 1 mg/L total iron. Acid soils restrict rice production and together with Zn deficiency cause a macronutrient disorder in wetland rice. The production of lowland rice was greatly affected by high concentrations of reduced iron in the flooded soils. The features of iron toxicity in rice include high uptake of fe2+ by roots, acropetal translocation into leaves, bronzing of rice leaves and yield loss.[20]

Children's are highly susceptible to iron toxicity as they are exposed to a maximum of iron-containing products. Iron toxicosis occurs in four stages;

- First stage: This stage occurs after 6 hours of iron overdose is marked by gastrointestinal effects such as gastrointestinal bleeding, vomiting and diarrhea.
- Second stage: The second stage progresses within 2 to 4hrs of overdose and it is considered as the latent period, a period of apparent medical recovery.
- Third stage: The third stage occurs between 2 to 24 hrs. After the onset of certain clinical symptoms. This stage is characterized by shocks, hypotension, lethargy, tachycardia, hepatic necrosis, metabolic acidosis and sometimes death.
- Fourth stage: The fourth stage occurs within 12 to 24hrs of iron overdose. This stage is marked by the formation of gastrointestinal ulcerations and development of strictures.
- Fifth stage: These stage occurs within 1 to 7weeks.

Mechanism of iron toxicity

A wide range of harmful free radicals are formed when the absorbed iron fails to bind to the protein, which in turn severely affects the concentration of iron in mammalian cells and biological fluids. This circulating unbound iron results in corrosive effect of the gastrointestinal tract and biological fluids. An extremely higher level of iron enters into the body crossing the rate-limiting absorption step becomes saturated. These free irons penetrate into cells of the heart, liver and brain. Due to the disruption of oxidative phosphorylation by free iron, the ferrous iron is converted to ferric iron that releases hydrogen ions, thus increasing metabolic acidity. The free iron can also lead to lipid peroxidation, which results in severe damage to mitochondria, microsomes and other cellular organelles. The toxicity of iron on cells has led to iron mediated tissue damage involving cellular oxidizing and reducing mechanisms and their toxicity towards intracellular organelles such as mitochondria and

lysosomes. A wide range of free radicals that are believed to cause potential cellular damage are produced by excess intake of iron. The iron produced hydrogen free radicals attack DNA, resulting in cellular damage, mutation and malignant transformations which in turn cause an array of diseases.[1]

Procedure[31]

Standard

1. Take 2.0ml of iron standard solution (20 ppm fe) in labeled Nessler's cylinder (S).
2. Add 2ml of a 20%w/v solution of iron free citric acid.
3. And add 0.1ml of thioglycollic acid, mix well, make alkaline with iron free ammonia solution.
4. Dilute to 50ml with water and allow to stand for 5 minutes.

Test

1. Weigh accurately 2.5g of zinc sulphate and dissolve in sufficient carbon dioxide free water to produce 50ml in a beaker. Take 2.0ml of solution and diluted to 10ml with water in a labeled Nessler's cylinder(T).
2. Add 2ml of a 20%w/v solution of iron-free citric acid.
3. And add 0.1ml of thioglycollic acid, mix well and make alkaline with iron free ammonia solution.
4. Dilute to 50ml with water and allow to stand for 5minute.
5. View the colour intensity against white background and compare with that of standard.

Observation: The colour intensity produced in test solution is **same** as the colour intensity produced in standard solution.

Inference: -The given sample of shankh-vati tablet complies the limit for iron.

Lead

Lead is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Lead is a bright silvery metal, slightly bluish in a dry atmosphere. It begins to tarnish on contact with air, thereby forming a complex mixture of compounds, depending on the given conditions. The sources of lead exposure include mainly industrial processes, food and smoking, drinking water and domestic sources. The sources of lead were gasoline and house paint, which has been extended to lead bullets, plumbing pipes, pewter pitchers, storage batteries, toys and faucets. In the US, more

than 100 to 200,000 tons of lead per year is being released from vehicle exhausts. Some is taken up by plants, fixation to soil and flow into water bodies, hence human exposure of lead in the general population is either due to food or drinking water.

Lead is extremely toxic heavy metal that disturbs various plant physiological processes and unlike other metals, such as zinc, copper and manganese, it does not play any biological functions. A plant with high lead concentration fastens the production of reactive oxygen species(ROS), causing lipid membrane damage of chlorophyll and photosynthetic processes and suppresses the overall growth of the plant. Metallic lead does not dissolve in water and does not bum, however, lead can combine with other chemicals to form lead compounds or lead salts. Some lead salts dissolve in water better than others. Although lead itself cannot be broken down, lead compounds in water may combine with different chemicals depending on the acidity and temperature of the water.

Most of the lead used by industry comes from mined ores (primary) or from recycled scrap metal or batteries (secondary). The main sources of lead pollution are lead smelters, battery manufacturers, paper and pulp industries, boat and ship fuels and ammunition industries. In addition, the production of television picture tubes, pigments, petroleum fuels, printing, glass industries, photographic materials, etc., also adds Pb(II) to the environment. People living near hazardous waste sites may be exposed to lead by breathing air, drinking water, eating foods, or swallowing or touching dust or dirt that contains lead.

Cigarette smoke also contains small amounts of lead. Lead may enter foods if they are put into improperly glazed pottery or ceramic dishes and from leaded-crystal glassware.

Hypertension has also been associated with lead exposure in the general population. At the typical levels to which individuals are exposed, lead can cross the placenta and damage developing fatal nervous systems. High level exposure to lead may cause miscarriage in pregnant woman and can also damage the organs responsible for sperm production in male.

The most severe neurological effect of lead in adults is lead encephalopathy, which is a general term to describe various diseases that affect brain function. Lead exposure may cause weakness in fingers, wrists, or ankles.

Children are more sensitive to the effects of lead than adults. A child who swallows large amounts of lead may develop blood anemia, kidney damage, severe stomachache, muscle

weakness, and brain damage. The lower IQ levels and other neuropsychological deficiencies among the children exposed to higher lead levels have been well documented. Lead acetate and lead phosphate have been shown to be potential carcinogens based on studies in animals.

Bioavailability is defined as the fraction of the element from an ingested matrix such as soil, water or food that can be absorbed by an organism. The bioavailability and environmental mobility of the metals are dependent upon the form in which the metal is associated with the soil.

Lead is used for production of batteries, cosmetics, metal products such as ammunitions, solder and pipes. Lead is highly toxic and hence its use in various products, such as paints, gasoline has been considerably reduced nowadays.[8]

The main sources of lead exposure are- Lead based paints, Gasoline, Cosmetics, Toys, Household, dust, Contaminated, soil, Industrial emissions etc.

Lead poisoning was considered to be a classic disease and the signs that were seen in children and adults were mainly pertaining to the central nervous system and the gastrointestinal tract. Lead poisoning can also occur from drinking water. The pipes that carry the water may be made of lead and its compounds which can contaminate the water. According to the Environmental Protection Agency (EPA), lead is considered a carcinogen. Lead has major effects on different parts of the body. Lead distribution in the body initially depends on the blood flow into various tissues and almost 95% of lead is deposited in the form of insoluble phosphate in skeletal bones.

Toxicity of lead, also called poisoning, can be either acute or chronic. Acute exposure can cause: Loss of appetite, Headache, Hypertension, Abdominal pain, Renal Dysfunction, Fatigue, Sleeplessness, Arthritis, Hallucinations and Vertigo etc.

Chronic exposure of lead can result into; Mental Retardation, Birth defects, Psychosis, Autism, Allergies, Dyslexia, Weight loss, Hyperactivity, Paralysis, Muscular Weakness, Brain Damage, Kidney damage and may even cause death.

Mechanism of lead toxicity

Lead metal causes toxicity in living cells by following ionic mechanism and that of oxidative stress. Oxidative stress in living cells is caused by the imbalance between the production of

free radicals and the generation of antioxidants to detoxify the reactive intermediates or to repair the resulting damage. Antioxidants, as e.g.; glutathione, present in the cell protect it from free radicals such as hydrogen peroxide. Under the influence of lead, however, the level of the ROS increase and the level of antioxidants decreases. Since glutathione exists both in reduced (GSH) and oxidized(GSSG) state, the reduced form of glutathione gives its reducing equivalents from its thiol groups of cysteine to ROS in order to make them stable. In the presence of the enzyme glutathione peroxidase, reduced glutathione readily binds with another molecule of glutathione after donating the electron and forms glutathione disulfide(GSSG). The reduced from (GSH) of glutathione accounts for 90% of the total glutathione content and the oxidized from(GSSG) accounts for 10% under normal conditions. Yet under the condition of oxidative stress, the concentration of GSSG exceeds the concentration of GSH. Another biomarker for oxidative stress is lipid peroxidation, since the free radical collects electron from lipid molecules present inside the cell membrane, which eventually causes lipid peroxidation. At very high concentrations, ROS may cause structural damage to cells, proteins, nucleic acid, membranes and lipids, resulting in a stressed situation at cellular level.

The ionic mechanism of lead toxicity occurs mainly due to the ability of lead metal ions to replace other bivalent cations like Ca, Mg, Fe and monovalent cations like Na, which ultimately disturbs the biological metabolism of the cell. The ionic mechanism of lead toxicity causes significant changes in various biological processes such as cell adhesion, intra and inter cellular signaling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters. Lead can substitute calcium even in picomolar concentration affecting protein kinase C, which regulates neural excitation and memory storage.[14]

Procedure[31]

Standard

1. In a 50ml labeled Nessler's cylinder(S) Pippete 1.0ml of lead standard solution (20 ppm Pb).
2. Dilute with distilled water to 25ml.
3. Adjust the pH with dilute acetic acid or dilute ammonia solution in between 3.0 to 4.0.
4. Dilute with distilled water about 35ml and mix with glass rod.
5. Add 10ml of freshly prepared hydrogen sulphide solution.

6. Mix and dilute to 50ml with water.
7. Allow to stand for 5 minutes.

Test

1. Accurately weigh 4g of sodium chloride and add to labeled Nessler's cylinder(t).
2. Add 2ml of dilute acetic acid and mix well, then add sufficient water to produce 25ml.
3. Adjust the p^H with dilute acetic acid or dilute ammonia solution in between3.0 to 4.0.
4. Dilute with water to 35ml and mix well.
5. Add 10ml of freshly prepared hydrogen sulphide solution.
6. Mix and dilute to 50ml with water.
7. Allow to stand for 5 minutes.
8. View downwards over a white surface and compare with that of standard.

Observation: -The colour intensity produced in test solution is **less** than the colour intensity produced in standard solution.

Inference: -The given sample of Shankh-vati tablet complies the limit for heavy metals as per the Indian Pharmacopeia.

Sulfate

- White vitriol is zinc, sulfate heptahydrate. Some sulphates were known to alchemists. The vitriol salts, from the Latin vitreolum, glassy, were so-called because they were some of the first transparent crystals known.
- Green vitriol is iron, sulfate heptahydrate.
- Blue vitriol is copper, sulfate pentahydrate.
- Alum, a double sulfate of potassium and aluminium.

The sulfate anion consists of a central sulfur atom surrounded by four equivalent oxygen atoms in a tetrahedral arrangement. The symmetry is the same as that of methane. The sulfur atom is in the +6 oxidation state while the four oxygen atoms are each in the -2 state. The sulfate ion carries an overall charge of -2 and it is the conjugate base of the bisulfate ion, which is in turn the conjugate base of sulfuric acid. Organic sulfate esters, such as dimethylsulfate, are covalent compounds and esters of sulfuric acid. The tetrahedral molecular geometry of the sulfate ion is as predicted by VSEPR theory.

Sulfates are widely used by industries. Major compounds include;

- Gypsum, the natural mineral form of hydrated calcium sulfate, is used to produce plaster. About 100 million tons per year are used by the construction industry.
- Copper sulfate, a common algaecide, the more stable form is used for galvanic cells as electrolyte.
- Iron sulfate, a common form of iron in mineral supplements for humans, animals, and soil for plants.
- Magnesium sulfate(commonly known as Epsom salts), used in therapeutic baths.
- Lead sulfate, produced on both plates during the discharge of a lead-acid battery.
- Sodium Laureth Sulfate, or SLES, a common detergent in shampoo formulation.[26]

Procedure[31]

Standard

1. Take 1ml of 25%w/v solution of barium chloride in the Nessler's cylinder(S).
2. Add 1.5ml of ethanolic sulphate standard solution (10 ppm SO_4)mix and allow to stand for 1minute.
3. Add 0.15ml of 5M acetic acid.
4. Add sufficient water to produce 50ml, stir immediately with glass rod.
5. Allow to stand for 5 minutes.

Test

1. Take 1ml of 25%w/v solution of barium chloride in the labeled Nessler's cylinder (T).
2. Add 1.5ml of ethanolic sulphate standard solution (10 ppm SO_4) mix and allow to stand for 1minute.
3. Weigh accurately 1.0g of sodium bicarbonate and add to a labeled Nessler's cylinder.
4. Add 10ml distilled water, neutralize with hydrochloric acid and dilute to 15ml with distilled water.
5. Add 0.15ml of 5M acetic acid.
6. Add sufficient water to produce 50ml, stir immediately with glass rod and allow to stand for 5 minutes.
7. View transversely against a black background.
8. Compare opalescence with that of standard solution.

Observation: -The colour intensity produced in test solution is **less** as the colour intensity produced in standard solution.

Inference: -The given sample of Shankh-vati tablet complies the limit for sulphate as per the Indian Pharmacopeia.

RESULTS

1. 0.5gm of drug contains 0.025015gm of mercury, which is within limit.
2. The given sample of shankh-vati tablet complies the limit for iron.
3. The given sample of shankh-vati tablet complies the limit for heavy metals as per Indian pharmacopeia.
4. The given sample of shankh-vati tablet complies the limit for sulfate as per Indian pharmacopeia.

DISCUSSION

Shankh-vati Tablets were collected as a gift sample from Ayurvedeeya Arkashala Ltd. Satara, to determine whether the heavy metals like mercury i.e. also known as "Parad" in Ayurveda and lead is in limit or not. As we know many patients take ayurvedic medications which contains heavy metals, due to herbal plants used for preparations. We know, heavy metals have toxic and dangerous effects on human health. They produce threat to life and effects the body functions.

Determination of mercury is done by the procedure mentioned in ayurvedic pharmacopeia. Other metals are detected by limit test for heavy metals. As by results we are able to know that heavy metals are within limit in shankh-vati tablet. As all limit tests complies with standard solution.

CONCLUSION

By overall study, we concluded that Shankh-vati tablet an ayurvedic formulation contains heavy metals(mercury and lead) and other metals like (Iron and Sulfate) within limit. So, it cannot produce any toxic effects on consumer.

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