INTRODUCTION

Metal pollution is a major public health problem in developing countries. Metal pollution affects the physical and mental development of an individual leading to decreased work capacity that in turn affects the development of a country (Singh *et al*., 2007). Heavy metals are described as common transition metals that have the potential to cause harm to the environment and most of the heavy metal exposure to humans occurs through occupational settings (Agency for Toxic Substances and Disease Registry, 1990).

Silver occurs naturally in its pure form. It has been valued as a precious metal used to make ornaments, table wares, utensils and currency coins (Chen and Schluesener, 2007; Panyala *et al*., 2008). It is also used industrially in electrical conductors, mirrors and catalysis of chemical reactions. Its compounds are used in photographic film and are also used as dental alloys, disinfectants and microbiocides (Drake and Hazelwood, 2005). It has been known that silver based compounds are useful in a wide range of bactericidal applications (Nomiya *et al.*, 2004).

Silver is being used extensively since the ancient period to control infections and spoilages. Hippocrates, the “Father of Medicine”, taught that silver heals wounds and controls diseases. Moreover before the discovery of antibiotics, silver was used as an antiseptic particularly to clean burns and open wounds (Larese *et al*., 2009). The popularity of medicinal silver arose from 702 A.D through 980 A.D. It was widely used for blood purification and control of halitosis. Pioneers of Americans used silver and copper coins to purify drinking water to combat with bacteria and algae. In 1884, the German obstetrician C.S.F Crede, introduced a prophylactic silver nitrate (1%) eye solution for newborn for the protection of ophthalmia neonatorum (Feng *et al.*, 2000; Dallas *et al*., 2011). Many of the silver salts and silver derivatives are available commercially as antimicrobial agents, but their use may result in unwanted absorption of ions such as in epidermal cells and sweat glands (Silver, 2003).

Recent studies confirm that silver in its metallic state is inert but it gets ionized when it reacts with moisture and body fluids. The ionized silver is highly reactive, as it binds to tissue proteins and brings structural changes in the bacterial cell wall and

nuclear membrane leading to cell distortion and death. Silver ions react rapidly with prokaryotic cell walls, whereas the membranes of eukaryotic cells strongly resist the effect of silver (Lansdown, 2002; Castellano *et al.,* 2007). But some of the recent studies also indicated that silver and silver compounds show unacceptable toxic effect on human health and the environment. The chronic exposure to silver causes adverse effects such as permanent bluish-grey discoloration of the skin (argyria) and eyes (argyrosis) (*Gulbranson et al., 2000)*. Exposure to soluble silver compounds may produce other toxic effects like liver and kidney damage, irritation of the eyes, skin, respiratory and intestinal tract and changes to blood cells (Panyala *et al*., 2008). The normal concentrations of silver in human tissues are very low. If there is an overexposure, it can be accumulated in mucous membranes, corneas, nails and spleen (Sue *et al*., 2001).

The general population exposed to silver higher than background level includes people who consume drinking water or food containing elevated levels of silver. Sources of elevated dietary silver include seafood from areas near sewage outfalls or industrial sources and crops grown in areas with high ambient levels of silver in the air and soil. Important sources of atmospheric silver from human activities include steel refining, fossil fuel combustion, cement manufactures, processing of ores, cloud seeding and municipal waste incineration (http.//*www.atsdr.cdc.gov/toxprofiles/tp 146-c5.pdf).*

Jewellery making is one of the world's oldest manufacturing sectors that involves some hazardous processes. Alloys are added during the manufacturing process of gold which help to give hardness, to make the gold jewellery stronger and would change the colour. Most often, pure gold is alloyed with copper, zinc and silver in varying proportions to produce the wide range of karat gold. Goldsmiths are also known to use potent toxic chemicals like amile nitrates (polishing compounds), ammonium chloride, aniline dyes, cadmium, cadmium bicarbonate, copper sulphate, mercury, potassium cyanide, potassium hydroxide, potassium nitrate, silver, silver nitrate and nitric acid. These chemicals are used for melting, refining, welding and polishing the jewellery.

Silver is classified as a xenobiotic metal having less known physiologic function in the human body which is used in higher concentration during processing of gold. NIOSH (National Institute for Occupational Safety and Health) reports that people are potentially exposed to silver in workplace environments. According to industrial applications, jewellery, silverware and the photographic industries are the largest consumers of silver using 40, 31, 22%, respectively (Gold Fields Minerals Services, 2004).

During the manufacturing processes of gold, formation of fumes and dusts which consist of metals and hazardous compounds is common. Many of these substances are potentially harmful to human health. Workers in these units inhale, ingest and absorb large amounts of these substances daily over extended periods of time. The major route of silver entry into the body is gastrointestinal tract (ingestion) (Silver, 2003), lungs (inhalation) (Lee *et al.*, 2010) and dermal absorption (Larese *et al.*, 2009). In occupational exposure, respiratory absorption is usually greater than intestinal absorption, but ingestion and dermal absorption are also routes for the entry of silver into the body (Drake and Hazelwood, 2005).

Liver and kidneys are important organs of detoxification, metabolism, storage of important metabolites and excretion of xenobiotics. Silver is not extensively metabolized in mammalian species and this may contribute to its low degree of toxicity in animals and humans. Silver is associated with low absorption, although the presence of silver binding protein and the solubility of the silver species are important modifiers of absorption (http//*images. library. wisc. edu/econatres. argentumv03. jubergreview. pdf).* Once absorbed, it passes through the liver and spleen where it binds to metallothionein, albumins and macroglobulins. This silver protein complex is metabolized in liver and mostly excreted via urine and feces *(*Baldi *et al.,* 1988*).* Elimination of silver from the body is primarily (90 %) through fecal excretion and minor quantity via urine (Juberg and Hearne, 2001) But some of the unmetabolized or biologically active ions (Ag^{+}) are deposited in tissues or circulated in the biological system which becomes toxic factors that cause oxidative damage to the cells (Lansdown, 2010; Lansdown, 2007; Manoj and

Padhy, 2013). The half life of silver in the lungs and liver are approximately 1 day and 50 days respectively.

Various studies have shown that metals are capable of producing the oxygen free radicals. Toxicities of metal ions may be related to differences in solubility, absorbability, transport and chemical reactivity that are formed within the body. Radicals and other reactive species are formed constantly in human body and are removed by the enzymic and non - enzymic antioxidant defense systems (Rakesh *et al*., 2010).

Overproduction of reactive oxygen species (ROS) results in oxidative stress and is a deleterious process that can be an important mediator of damage to cell structures, including lipids, proteins and DNA. Antioxidants are compounds that either delay or inhibit the oxidation processes which occur under the influence of ROS (Pisoschi and Negulescu, 2011). Antioxidants exert synergistic actions in scavenging free radicals. Recent studies have suggested that metal ions may enhance the production of tumor necrosis factor alpha (TNFα) and activate protein kinase C, as well as induce the production of stress proteins (Valko *et al*., 2007).

Metallothioneins (MTs) belong to the group of intracellular, cysteine rich, non enzymic antioxidants and are closely related to stress response proteins which are involved in detoxification processes of heavy metals. Therefore it is a candidate for an index in the biological monitoring of heavy metal exposure (Jonai *et al*., 1992). They are highly conserved family of low molecular weight proteins with molecular weight from 5-16 Kilo Dalton.

MTs are free radical scavengers which bind to a number of trace metals and also save cells and tissues from heavy metal toxicity. The protein consists of 61 amino acid residues of a polypeptide chain in which 20 residues are cysteine and many lysines and arginines. It has more than 30% of cysteine residues and reduced form of cysteines direct its metal binding properties through mercaptide bonds (Hasan *et al*., 2013).

MTs can be used as biomarker in the field of industrial health due to the synthesis of MTs by heavy metals and the accumulation of these metals in the cells and the tissues (Sakulsak *et al*., 2009). Bioavailability of ionic form of silver is more toxic to organisms than any other metal. However, it is very difficult to determine the potential toxicity of silver, because its bioavailability is dependent on the physical, geochemical and biological processes that determine metal uptake by living organisms (Luoma and Rainbow, 2008).

The functional importance of MTs is still under question. The functions of mammalian MTs are hypothesized by various researchers which revealed that it is involved in homeostasis and detoxification of metals. It protects the cells and tissues from the oxidative stress, maintains the intracellular redox balance and regulates the cell proliferation (Palmiter, 1998; Miles *et al.*, 2000). Several reviews have exposed about this exclusive molecule and have expanded the inquisitiveness of researchers to study the secrecy of MTs for over five decades (Vasak and Hasler, 2000; Cherian *et al*., 2003; Cherian and Kang, 2006; Carpene *et al.*, 2007; Thirumoorthy *et al*., 2007). The metal ions have different relative order of attraction towards MTs such as Hg > Ag >> Cu > Cd > Zn reported in several *in vitro* studies (Hamer, 1986).

Metals bound to MT are considered less labile and thus less toxic, but the exact mechanism of protection against metals is complex. Indeed, the mechanism of action of MT depends on the degree of heavy metal exposure of tissues and the MT response (Bonneris *et al*., 2005).

There are several studies carried out in peripheral blood lymphocytes (PBLs) to know the expression of MTs *in vivo* and it was found that MTs can be induced appreciably with metal exposure (Hildebrand and Cram, 1979; Enger *et al.*, 1983; Harley *et al*., 1989). The heavy metal specificity of MT stimulation and the regulatory mechanism of MT production in the lymphocytes seem to be similar in the liver (Yamada and Koizumi,1991). Silver binding proteins of PBLs have not been well characterized so far, although they are expected to be a clue for understanding silver

toxicity in these immune competent cells. MTs are expressed in lymphocytes at very low level but they can be synthesized significantly with metal exposure (Lu *et al.,* 2001).

To date, information about the toxicity of silver compounds still has not been clarified. It is believed that silver compounds have no adverse health effects to humans (Wan *et al.*, 1991). Many studies have evaluated the acute toxic effects of silver nitrate ($AgNO_3$) at relatively high doses (Sriwichai, 2012; Figueira *et al*., 2012) but chronic toxicity at low doses should also be evaluated *in vivo*. To evaluate this, *in vitro* experiments were designed such that the human PBLs were exposed to $AgNO_3$ to represent the exposure to Ag^+ ions to cells and also to better understand the role of MT in metal stress.

The present study was aimed to assess the health status of workers in jewellery units. The relationship of silver, hematological and biochemical indices with MTs were examined. Experiments were carried out in cultured human lymphocytes with silver nitrate, which might provide a sound platform to know the correlation between metallothioneins and silver exposure.

Objectives of the study

The study was formulated with the following objectives:

- To assess the health status of the workers in jewellery industry with special reference to silver exposure
- To characterize the metallothioneins from peripheral blood and cultured peripheral blood lymphocytes among the selected workers
- To assess the relationship between insult to exposure to hazardous levels of silver and the metallothioneins status among the workers

The study was conducted in three phases. In phase I, workers were selected from jewellery units and evaluated for metal exposure by administering questionnaire and selected biochemical parameters were assessed. In phase II, the workers who had

high levels of serum silver and MTs were selected to isolate, determine the molecular mass and quantify the metallothioneins from peripheral blood lymphocytes. In phase III, an *in vitro* study was carried out to investigate the viability, cytotoxicity and metallothionein expression in silver nitrate exposed peripheral blood lymphocytes.