1.1. Introduction

All biota utilizes redox properties of copper to perform various biological functions. It is essential for quality of life and life itself. It is a cofactor for various vital enzymes and an important element from medical as well as engineering point of view. Various properties of copper attract technical and healthcare professionals. Technical professionals have exploited copper for its properties such as ductility, non-corrosiveness, fatigue resistance, and good conductivity of heat and electricity. Various forms of copper are used for different applications in the area of electronics, construction, manufacturing and other industries. For healthcare professionals, copper is an important nutritional component whose deficiency is associated with physiological disorders. Postmenopausal osteoporosis is physiological bone disorder of women which affects every second woman. It was found that most postmenopausal women have copper deficiency and copper supplementation could improve their physiological functions. However, increasing the bioavailability of copper offers both, opportunity and challenges. Opportunity is to improve biological functions and challenge is the copper associated toxicity. Threat of the copper toxicity is the major challenge for healthcare professionals in the way of developing a successful pharmaceutical copper based product. Only elemental copper has been approved to use as touch surface antimicrobial agents by USFDA. This chapter explains different properties, forms, applications and limitations of copper and pathophysiology of postmenopausal osteoporosis, current therapeutic regimen, their limitations and importance of copper in prevention and treatment of postmenopausal osteoporosis.

1.2. Copper

Copper is a chemical element, classified as transitional and heavy metal in the group 9 of d block (called as coinage elements) of periodic table, has atomic number 29, molecular weight 63.546, a melting point of 1083 °C and a boiling point of 2567 °C. The name ‘copper’ came from cuprum, a Latin word for the island of Cyprus where copper was mined. Pure copper has a distinct reddish-orange color and metallic luster. It is a soft, malleable and ductile metal with very high thermal and electrical conductivity. It is the 8th most abundant material on the earth. It is precious element for day to day life (https://en.wikipedia.org/wiki/Copper/14/01/2016). It is present everywhere on earth from terrestrial regions to aquatic regions. It is completely recyclable without losing any of its properties. Current market price of copper is about $6000 per ton.

Copper is essential for living organisms. It is good conductor of heat and electricity. It is noncorrosive and fatigue resistant. Two copper materials can be joined easily. Compared to other
metals, pure copper does not react easily. All these properties makes copper the most used metal in various sectors to improve quality of life. It is used for electrical appliance (60%); construction, such as roofing and plumbing (20%); industrial machinery, such as heat exchangers (15%) and alloys (5%). (http://www.gsa.org.au/resources/factites/factitesCopper.pdf/14/01/2016, Anyadike, 2002).

Copper is a precious element from engineering point of view also. Although it is an essential nutritional element, excess application of copper increases environmental copper concentration and some harmful effects on environment have been reported. However, this does not decrease its nutritional importance. It is a good electron transporter and important for various life events.

1.2.1. History of Copper

From all known metals, copper was the first metal used by mankind for various applications in large quantities. Only gold and iron were known to humans, before application of copper was understood. History of copper is ten thousand years old as earliest estimates placed it discovery around 9000 BC. First use of the copper was reported in the Mesopotamian civilization during 5000 BC to 6000 BC. So it had sixty centuries of known history. It was discovered in India and China independently. In India, artisans created copper alloy products such as icons and lamps which were used in temples for worship of God. It was formed in volcanic eruptions which are high in hot sulfuric solutions. It was discovered in the rivers as shining stone which could be converted into any shape by beating. It is found worldwide but approximately 90% of copper is distributed in four major parts viz., the Great Basin of the Western United States, Zambia, Central Canada, and the Andes regions of Peru and Chile. The whole copper history can be divided into four ages: Copper age, Bronze age, Middle age and Modern age. Copper metallurgy was probably developed in following steps: cold working with natural copper, annealing, smelting and lost wax casting method. These steps were independently invented in different parts of the world. Copper age is the start of “Age of Metal” which is also called as Chalcolithic age came in existence after “Age of Stones” (Neolithic age). It helped the humans to become master with stone, wood, bone and other substances. Bronze age came by alloying copper with other metals, mainly tin which came in existence by chance but played an important role for human civilization. It helped in casting and developing architectural ability of humans. It was middle period of the Copper and the Iron age. Middle age is referred to the Iron age. In the middle age, iron took the supreme industrial importance than copper and became the most used metal by humans after copper. This reduced
extraction of copper. However, copper remains important for cultural work. With invention of electricity, the importance of copper got established where copper had proved to be the best for good electric conductivity. This resumed extraction of copper and brought back the copper production and application in history. Ever since 10,000 years of history of copper application in human civilization, more than 95% of all copper ever mined and smelted has been extracted since 1900, and 5% was extracted in only the last 24 years. Copper was used as antimicrobial agent since ages but in modern age it is developed as touch surface antimicrobial material in construction industry to prevent spread of infection (Association, 1998, http://www.gsa.org.au/resources/factites/factitesCopper.pdf/14/01/2016, https://en.wikipedia.org/wiki/Copper/14/01/2016, Stanczak, 2005, Smith, 1965).

1.2.2. Various forms of copper

1.2.2.1. Pure copper
Pure copper is ductile metal and available in earth crust. It is a good electric conductor. But it has very poor tensile strength. It is used in electronic, manufacturing architecture and ornamental industry. It can directly be beaten to get different shape objects for different applications. Copper is the only solid material registered worldwide as antimicrobial health product for public use (Anyadike, 2002, Association, 1998).

1.2.2.2. Copper alloys
To increase tensile strength of copper, it is mixed with other metals to get its alloy which find various industrial and commercial applications. This form of copper has huge market in the world because of its tensile strength and malleability. Mostly used alloy of copper are bronze and brass where bronze is alloy of copper and tin while brass is alloy of copper and zinc. Alloy of copper and gold is called as rose gold and used in jewelry industry (Smith, 1965, Stanczak, 2005).

1.2.2.3. Copper ores
Copper ore is the natural source of copper. Copper ores are available in earth crust throughout the world. These ores are used to extract pure form of copper. Copper minerals and ores are found in both igneous and sedimentary rocks. Mining of copper ores is carried out using one of two methods. Underground mining is achieved by sinking shafts to the appropriate levels and then driving horizontal tunnels to reach the ore. Open pit mining is employed when the ores are near the surface and can be quarried after removal of the overlaying surface layer. More than 160 copper ores are known which have diverse composition, appearance and color. Many of them are very
rare; only about twelve ores are found in most of the places. Most common and brilliantly colored of them are the bright green banded malachite; bornite, which is known from its alternate name of peacock ore, and chalcopyrite, a mixed sulphide of copper and iron, which is a bright yellow crystalline mineral resembling pyrite, or ‘fool’s gold’ (http://www.icsg.org/index.php/component/jdownloads/viewdownload/16/01/2016, Smith, 1965, Stanczak, 2005).

1.2.2.4. Copper compounds

Copper has positive electron potential which is very reactive and form salts with negative electron potential elements. It has very good catalytic properties. It has four oxidation states +1, +2, +3 and +4. It forms various binary compounds with cuprous (Cu$^{+1}$) and cupric (Cu$^{+2}$). These compounds have different properties. Being a transitional element, copper forms coordination compounds with various complex forming and chelating reagents. These complexes have various properties. These complexes are useful in various chemical reactions in electron transfer. Copper forms organo-copper compounds with carbon which finds various applications in chemical reactions (https://en.wikipedia.org/wiki/Copper/14/01/2016).

1.2.2.5. Copper nanoparticles

Copper nanoparticles are atomic aggregates of copper which were stabilized at nanoscale with the help of various coating agents. Copper nanoparticles attracted considerable attention because of their catalytic, optical, and conducting properties. These nanoparticles could be natural or anthropogenic (synthetic). Natural nanoparticles are produced in many natural and biological ways. These nanoparticles are very useful in electronic industry. Anthropogenic nanoparticles are produced as byproducts of any process conducted by men at nanoscale or engineered by men for various applications. They are byproducts for normal combustion, food cooking, chemical manufacturing, grinding, welding, ore refining and smelting. Engineered copper nanoparticles are produced by various physical and chemical methods (Kim et al., 2006, Li, 2013).

1.2.2.6. Copper formulations

Copper in formulations is present in various forms such as colloidal copper, copper salts, copper peptide (Hostynek et al., 2010), copper complex (Torres Martin de Rosales et al., 2011), copper nanoparticles (Rakhmetova et al., 2010) etc. These formulations are used to supplement copper for various living beings. It has been used to improve the stability and bioavailability of drugs
Copper formulations have been developed for various route of administration like topical, oral and impregnated bandages.

1.2.3. Analytical technique for estimation of copper

1.2.3.1. UV-Visible spectrophotometer

Various sensitive methods are available to determine copper in using different color reagents. This technique is very effective in detection of copper up to parts per million (ppm) concentrations. Sodium diethyl dithiocarbamate (SDEDTC) and 2, 2'-bicinchoninic acid (BCA) are very sensitive colorimetric methods for determination of copper. SDEDTC forms a brown color complex which gives maximum absorption at 440 nm and BCA form a purple color complex with maximum absorption at 562 nm (Brenner and Harris, 1995, Cartwright et al., 1945). Copper estimation can be done in UV range by flow injection analysis method after reaction of copper with pyrophosphate at 240 nm (Haj-Hussein, 1996) and with bis (acetyl acetone) ethylenediimine at 370 nm (Chimpalee et al., 1996).

1.2.3.2. Atomic spectrometry

Molecular absorption spectroscopy, atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP)-atomic emission spectrometry (AES), and ICP-mass spectrometry (MS) flame atomic absorption spectrometry (FAAS) are the most sensitive methods for determination of copper. AAS is the most common method for determination of copper in biological samples such as blood, serum, plasma, tissue homogenate etc. Flame atomic absorption spectrometry is highly sensitive for determination of copper in food and water. Copper at nanogram and picogram level could be detected. Although these techniques are very sensitive and accurate but running and maintenance cost of the instruments are too high (Ferreira et al., 2000, Kendüzler and Türker, 2003).

1.2.3.3. Chromatography

Chromatographic techniques are used in extraction and separation of copper from complex mixture followed by detection using standard analytical techniques. Reverse phase partition chromatographic method is useful in extraction and chromatographic separation of copper from mixture of other heavy metals, copper alloys and Ayurvedic copper preparations (Phule et al., 2011, Lederer, 1949). From mixture of copper with other heavy metal, paper chromatography is useful technique for separation of copper followed by analysis using electro-deposition (Anderson and Lederer, 1951).
1.2.3.4. **Electro-analytical methods**
Copper in its ionic form can be estimated by electro-analytical methods. Potentiometry and voltammetry are used for determination of copper concentration in different solutions. Potentiometry is useful in determination of copper in running water rapidly (Jagner et al., 1993). Voltammetry was used to determine copper in complex form (Lucia et al., 1994, Krznarić et al., 2006). These techniques are beneficial in simultaneous determination of copper along with other elements.

1.2.4. **ADME of copper in human**
Copper is essential micronutrient for various vital activities. Deficiency or excess, both have effects on normal physiology. Study of pharmacokinetic parameters is essential to understand charting of copper.

1.2.4.1. **Absorption**
Copper absorption could vary from as low as 12 % to as high as 97 % but theoretical possible capacity is reported between 63-67 %. With normal diet, human gastrointestinal tract is able to absorb 30-40 % of total copper intake. Absorption of copper depends on age, gender, source of copper and route of administration. Fetus gets required copper from mother and stored, sufficient amount of copper, required for first six months of infancy. Human breast milk has highest amount of bioavailable copper than any other mammal. Young people have greater absorption than geriatric people. In typical diet, copper absorption in women (71 %) is greater than in men (64 %). Copper absorption starts from the stomach, all the way to small intestine. Copper gets absorbed through various mechanisms. Most important mechanism is divalent metal active transporter proteins on the intestinal walls. Copper isotopes have been used to understand the mechanism of its absorption in human. Absorption of copper varies from source to source. Mineral supplement can have maximum absorption but can be absorbed only as nutritional supplement as other divalent atoms inhibit its absorption and excess copper can induce vomiting. Most of the copper is absorbed in the form of organo-mineral complexes with amino acids, proteins in vegetables, grains and meat. Ascorbic acid, fructose and other divalent metal like zinc, calcium, iron reduce copper absorption in intestine. Food processing decreases copper content and its bioavailability (Johnson et al., 1992, Wapnir, 1998, Turnlund, 1998).
1.2.4.2. Distribution

Copper distribution in humans is the most complicated mechanism. Radioactive tracer isotopes had helped in proper understanding of copper distribution. After absorption through divalent metal transporter or copper transporter proteins, it is transported to liver by other proteins, mostly by other copper chaperons and albumins. Liver collects all the copper from these proteins and combined it to ceruloplasmin for transport into other body parts. 95 % of total copper in blood is found to bind with ceruloplasmin, only 5 % of copper is found in free form. Human body has about 80-120 mg total copper. Daily requirement ranges from 1-2 mg. Normal serum concentration is 0.7-1.6 µg/ml. About 50 % of total copper is distributed in skeleton system, 25 % in brain, 15 % in liver and rest of the amount is distributed in other parts of the body (Turnlund, 1998, Stern et al., 2007, Wapnir, 1998, Nevitt et al., 2012).

1.2.4.3. Metabolism

Copper is a cofactor for essential metabolic enzymes. So adequate copper should be maintained for normal metabolism. Every cell requires copper for its survival as cofactor for essential enzymes and proteins. Deficiency or excess have adverse effect on cell metabolism. Therefore, copper homeostasis is essential. Copper homeostasis is maintained by copper transporter proteins which help in the transport of copper and control copper level at adequate level. They provide desired amount of copper and remove excess amount. Apart from Ceruloplasmin, four important human copper transporter proteins are found in the body. They are known as CTR1, CTR2, ATP7A and ATP7B. Defect in these proteins lead to copper deficiency or copper toxicity. CTR1 is a high affinity transporter and CTR2 is a low affinity transporter. CTR1 is found on plasma membrane of the cells while CTR2 is intracellular protein. These two proteins along with other copper chaperons help in uptake, distribution and export of copper from cells. ATP7A and ATP7B are ATP driven proteins and take part in transport of copper from cytosol to across the membrane efflux of copper. ATP7A release into bloodstream while ATP7B releases into bile for excretion (Nevitt et al., 2012).

1.2.4.4. Excretion

Most of the copper is excreted through feces and little amount by urine and sweat. Copper excretion depends on the nutritional supply of copper. If copper absorption is more, then all the extra copper would excrete through feces along with bile from liver to intestine. During copper deficiency, excretion of copper is reduced to its lowest limit (Nevitt et al., 2012).
1.2.5. Importance of copper

1.2.5.1. Industrial Importance

Copper is crucial material in construction industry. It is important for public administration and defense services in the area of ordinance. It is essential part of computer, electronics and optical products. Each household and industry of modern era which is connected with electricity requires copper. Copper is considered as an important metal, a nation’s economy is greatly dependent on it. In context of total economic activity or gross domestic product (GDP) for a nation, copper has the status of national economic important metal and economy of the country would influence by copper production, price and application (Beylot and Villeneuve, 2015). Copper and copper alloys find broad application for household and kitchen products. Copper compounds are important chemical reagents to be utilized in the various chemical reactions such as catalyst, reactant, complex forming agent, diagnostic agent, purification and source of electron and X-ray. It is an important metal in architecture, building and plumbing industries. Marine and naval ship building industry has introduced application of copper alloys because of its excellent resistance to corrosive environment like salt-water and salt-laden. Various modes of transport like electric and bullet train, car bike, bus, truck etc. require huge amount of copper. Copper is also known as coinage metal as it has important role in making of currency coins. Copper is required for paper manufacturing, printing industry, clock and watch industry and general engineering. Copper is a very good antimicrobial agent and its compounds are used in agriculture and horticulture industries. Copper is the only solid material which is registered as touch surface antimicrobial product. Currently copper is used in various hospital setups, crowded places, infection prone area to prevent spread of any infectious disease (Association, 1998, Stanczak, 2005, Smith, 1965).

1.2.5.2. Nutritional Importance

Copper is essential micronutrient for survival of all living organisms (human, animal, plant and microorganism). It was identified as essential nutrient for humans in 1928 (Uauy et al., 1998). Copper is the critical functional component for more than twelve enzymes which catalyze important biochemical reactions that are crucial for cell physiology and function of more than 30 proteins which play important role in cell physiology (de Romaña et al., 2011). Copper, being a transitional metal, can accept and donate electrons. It is mostly found in oxidized form states, Cu\(^{+}\) and Cu\(^{++}\) which are known as Cuprous and cupric respectively. This makes copper, ideal cofactor for various enzyme and proteins. Those enzymes which require copper as cofactor are known as
cuproenzymes. These enzymes and proteins which require copper as cofactor are classified as metalloprotiens and known as cuproprotiens or copper binding proteins (de Romaña et al., 2011, Angelova et al., 2011). It essential nutritional component for energy production, connective tissue formation, iron metabolism, neurotransmission, melanin formation, antioxidant function, immune function and gene expression regulation. Various cuperoenzymes and cuproproteins are listed in Table 1.1 for their role and importance.

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Consequence of copper deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome oxidase</td>
<td>Member of electron transport chain</td>
<td>Poor energy production leads to aging</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Copper and iron transport protein</td>
<td>Anemia</td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>Cross linking of collagen and elastin</td>
<td>Bone and skin disorders</td>
</tr>
<tr>
<td>Super oxide dismutase</td>
<td>Free radical scavenging</td>
<td>Damage from free radicals and aging</td>
</tr>
<tr>
<td>Tyrosinase</td>
<td>Synthesis of melanin</td>
<td>Acceleration of graying of hair</td>
</tr>
<tr>
<td>Dopamine hydroxylase</td>
<td>Production of neurotransmitter</td>
<td>Brain disorders</td>
</tr>
<tr>
<td>Amine oxidase</td>
<td>Oxidation of biogenic amines</td>
<td>Accumulation of toxin and disturb cell metabolism</td>
</tr>
</tbody>
</table>

Environmental intake of copper is limited for human. Main source for nutritional copper is food, drinking water and copper supplements. Recommended dietary allowance (RDA) or recommended nutritional intake (RNI) of copper given by various nutritional agencies varies for gender and different age groups from 0.2 mg to 3.0 mg per day. RDA of copper for different age group and gender as per the world health organization has been shown in Table 1.2.
Table 1. Recommended dietary allowance of copper

<table>
<thead>
<tr>
<th>Life Stage</th>
<th>Age</th>
<th>Male (mg/day)</th>
<th>Female (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants</td>
<td>0-6 months</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>Infants</td>
<td>7-12 months</td>
<td>0.2-0.4</td>
<td>0.2-0.4</td>
</tr>
<tr>
<td>Children</td>
<td>1-3 years</td>
<td>0.4-1.0</td>
<td>0.4-1.0</td>
</tr>
<tr>
<td>Children</td>
<td>4-8 years</td>
<td>1-1.5</td>
<td>1.1.5</td>
</tr>
<tr>
<td>Children</td>
<td>9-13 years</td>
<td>1-2.0</td>
<td>1-2.0</td>
</tr>
<tr>
<td>Adolescents</td>
<td>14-18 years</td>
<td>1.5-2.5</td>
<td>1.5-3.0</td>
</tr>
<tr>
<td>Adults</td>
<td>19 years and older</td>
<td>1.5-2.5</td>
<td>1.5-3.0</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>all ages</td>
<td>-</td>
<td>2-3</td>
</tr>
<tr>
<td>Breast-feeding</td>
<td>all ages</td>
<td>-</td>
<td>2-3</td>
</tr>
</tbody>
</table>

Women need more amount of copper than men and during pregnancy and lactation its requirement still increases. Food contributes for most daily intake of copper. High copper rich food includes seafood, organ meat, legume, whole grains and chocolates. Drinking ground water makes 20-25% of total daily intake of copper. Cooking and processing of food material reduce copper content while storing food does not have any effect on copper (https://en.wikipedia.org/wiki/Copper_in_health/30/01/2016). It was found that western diet is deficient in copper (Klevay, 2011).

1.2.5.3. Medicinal Importance

Copper has been used for its medicinal properties since ages in various parts of the world. Since ancient times, copper has been used to sterilize water and wound, treat pulmonary diseases and to prevent spread of infectious diseases. In ancient Egypt (2000 BC), copper was used to sterilize water and wounds. Greeks in the time of Hippocrates (400 BC) prescribed copper for pulmonary diseases. Gangajal, given to Hindu devotees to drink as a blessed offering, is stored in copper utensils as it keeps the water sparkling clean. Romans were using copper cooking utensils to prevent the spread of diseases. In World War II, Japanese soldiers used copper to prevent dysentery. In 18th century, copper was used for clinical application in western world, in the treatment of lung disorders. Copper is the only material which is registered by USFDA for prevention of infections. Copper deficiency and excess both could lead to disturbance in normal physiology which leads to various medical disorders. In general populations, excess of copper is less likely to happen. However, copper deficiency is more viable situation which leads to various disorders. Copper toxicity would have acute toxic effects while copper deficiency would cause
chronic disorders. Medicinal importance of copper can be categorized into three major groups (Borkow and Gabbay, 2009).

1.2.5.3.1. Conditions associated with Infections
Copper is a member of biocidal drugs since ages. Copper, copper ion, copper complex, copper alloy and copper nanoparticles are well known for their biocidal properties. Copper ion and copper complex have been used since centuries to sterilize liquid, solid and human tissues. Currently copper is used for antibacterial, antifungal and antiviral effects. Biocidal activity of copper exerts to microorganisms and viruses by several mechanisms. It acts by interacting with surface or envelope proteins, cellular proteins, nuclear proteins and DNA. These activities of copper make it useful in prevention of infection during wound healing; in hospitals, during epidemic, during surgery and prevention of communicable diseases (Borkow and Gabbay, 2009). It has been used in hospitals in ICU, operation theatre, general wards, hospital utilities to prevent spread on infections (Salgado et al., 2013).

1.2.5.3.2. Conditions associated with copper imbalance
Copper imbalance is two sided sword and has “U” shape effects where one side lead to toxicity and other lead to chronic physiological diseases. Therefore, its deficiency and excess, both show medical complications (Klevay, 1998).

- **Anemia and iron metabolism**: Ceruloplasmin, a copper binding protein also known as ferroxidase, plays an important role in iron metabolism. It helps in absorption and transportation of iron from various parts of body to bone marrow and in redox reactions of iron. Deficiency of copper would disturb iron metabolism and leads to anemia. Copper deficiency decreases the formation of hemoglobin and red blood cells and increase accumulation of iron in different tissues (Spain et al., 2009).

- **Angiogenesis and Cancer**: Copper helps in angiogenesis by vascularization and formation of new blood vessels. Its deficiency leads to delay in healing, growth and development process. But on other hand, excess of copper helps in the pathophysiology of cancer.

- **Antioxidant and energy production**: Copper is a cofactor for superoxide dismutase and cytochrome oxidase. Copper acts as an antioxidant agent along with superoxide dismutase. However, it forms free radicals when found in free form. It helps in energy production in electron transport system by cytochrome oxidase. Therefore, deficiency of copper would lead to highly oxidant environment and fatigue (Uriu-Adams and Keen, 2005).
Arthritis and inflammation: Copper act as anti-inflammatory agent in arthritis and inflammation. Ceruloplasmin act as acute inflammation protein and reduces free radicals and superoxide to prevent the disease conditions. During infection, injury and inflammation, production and secretion of ceruloplasmin increases in liver. Copper does not have any direct role in its production and secretion from liver but required for the proper function of ceruloplasmin as cofactor. Deficiency of copper leads to proliferation and chronic arthritis and inflammation (Uriu-Adams and Keen, 2005, Iakovidis et al., 2011).

Alzheimer, Parkinson and other brain disorders: Free form of copper in brain is reported to be very hazardous to brain. It plays an important role in progression of brain disorder by induction various redox reaction. In Alzheimer disease, free copper found to have interactions with precursor protein for amyloid and aggregation of β amyloid peptide to form plaques in the neurons or these situations could arise from copper deficiency by induction of oxidative stress deficiency.

Bone disorders: Copper deficiency leads to osteoporosis, fracture ephiphyeal, separation and spur formation. Osteoporosis is known to be socially significant bone disorder; most commonly occurs in geriatric patients and occurs especially in postmenopausal women. Cross linking of collagen fiber is an important step in the development of bone architecture. Lysyl oxidase, secreted by osteoblasts, requires copper as cofactor for cross linking of collagen fibers. Deficiency of copper reduces the activity of lysyl oxidase and disturbs the formation of bone architecture. This affects the bone mineral density by reducing the deposition of calcium phosphate in incompletely cross liked collagen fiber (Angelova et al., 2011).

Cardiovascular disease: Cardiovascular system is highly susceptible to copper deficiency and cause alteration in cardiac morphology. It causes hypertrophy, abnormality in mitochondria, myofibrils, myocytes, blood pressure and other issues related to cardiovascular system. Copper deficiency affects the formation of elastin and collagen fibers which cause alteration in morphology of blood vessels. This abnormality has been reported to develop rupture in blood vessels. Copper deficiency could cause weakening of oxidant defense system of heart. Copper deficiency alters blood lipid profile and play a crucial role in progression of cardiovascular diseases (Uriu-Adams and Keen, 2005).

Immunity: Copper deficiency reduces production of antibodies and weakens the defense mechanism of a host by reducing its immunity power. In host cells, free form of copper causes
Fenton reaction and helps through passive immunity against the pathogen. Here, copper helps in activating macrophages and neutrophils, which generate free radicals for phagocytosis of pathogens. Copper plays an important role in oxidative burst by reactive oxygen species, phagosomes and other mediator of inflammation like cytokines. Deficiency of copper reduces humoral immunity of host cells (Leary and Winge, 2007).

- **Wound healing**: Copper is a crucial factor for angiogenesis. It improves pace of wound healing, by the induction of secretion of vascular endothelial growth factor (VEGF), antimicrobial activity and improving passive immunity against pathogen. Induction of VEGF by copper helps in angiogenesis. Copper is further involved in expression and stabilization of extracellular proteins such as collagen and elastin. It has been reported for up regulation of wound healing in copper impregnated dressings (Sen et al., 2002, Borkow et al., 2010).

1.2.5.3.3. **Conditions associated with genetic defects**

In humans, most of the copper associated medical reports are associated with the genetic defects in copper metabolism. These genetic defects links to copper transporting proteins (ATPase) which are responsible for transport of copper from liver to other parts of body and vice versa. These two proteins are homologous and encoded by two deferent genes known as ATP7A and ATP7B. Defect in these gene develop X linked autosomal recessive disorders known as Menkes disease and Wilson’s disease. Menkes disease is associated with defect in ATP7A gene which encodes a copper binding protein responsible for transport of copper in blood stream from tissue or cell or liver which is utilized by other cells. In Menkes disease, insufficient delivery of copper in various tissue leads to reduction in the activity of copper dependent vital enzymes for growth and development. Wilson’s disease is associated with defect in ATP7B gene which encodes protein responsible for clearance of copper from the liver to bile excreted along with feces. Wilson’s disease cause accumulation of copper in various tissues which leads to Fenton reaction and oxidative burst in various tissues (Iakovidis et al., 2011, Uriu-Adams and Keen, 2005).

1.2.6. **Copper toxicity in human**

Copper toxicity is rare event in human beings. From total body copper, 95% is in bound form and 5% in free form. Even little increase in free form of copper has harmful effects. Free form of copper starts series of free radical reactions that are harmful to proteins as well nucleic acids. But, there are various copper binding proteins present in body to bind with free form of copper. Free form of copper has been reported for hepatotoxicity, neurotoxicity and nephrotoxicity but most of
them are single case reports happened because of high intake of copper with suicide interest. As liver is the first target for excess intake of copper and high copper concentration in liver would disturb liver function to combat with excess of copper and would lead to hepatotoxicity. Brain receives most of the total body copper after bone and highly prone to copper toxicity. Free form of copper has harsh effects on brain. Normally most of the copper is excreted through feces. However, free from of copper is excreted through urine and excess will lead to nephrotoxicity. In general, copper toxicity is very rare and found only when there is any genetic defect in copper metabolism (Uriu-Adams and Keen, 2005).

1.2.7. How nature is taking care of copper for normal functioning?
Being a two sided sword, copper does not create any medical emergency easily. Because, nature had taken good care of copper for normal functioning. Various food products contain sufficient amount of copper. Drinking water is also a good source of copper. Copper deficiency has not been reported in normal population. Free form of copper is harmful in body if absorbed through stomach or intestine. Therefore, oral intake of inorganic copper induces vomiting and prevents any possibility of copper toxicity. If any excess amount of inorganic copper is absorbed from copper rich diet into blood, copper get transported to liver where it get converted in organic form. Excess of organic copper get stored in hepatocytes and get released, whenever there is a copper deficiency. Nature has made arrangements to maintain copper homeostasis for every age group. For infants copper has been stored in their liver, when they were there in their mother’s womb, which is sufficient for about six months requirement of copper until they receive other nutritional food source. Apart from this, breast milk is the richest source of biological copper.

1.2.8. Recent interest in copper science
Recent advances in molecular biology and biotechnology had improved our knowledge to understand various biochemical reactions. They had allowed us to understand the significant and specific roles of copper at molecular level. Elaborated molecular study of human genome and various known and unknown proteins and their interaction with trace element such as copper has initiated new thinking in the area of research and development. Identification of various new copper chaperons or copper binding proteins had helped to understand important role of copper. Knowledge of various pathways and catalytic properties of copper in various physiological events had helped to precisely understand the role of copper in the medical sciences. Its various physicochemical properties had made it key component of engineering and technology field. It's
interaction with nucleic acid and various vital proteins made it key component of research in medical science.

1.2.9. **Why it is not explored for therapeutics?**

Redox properties of copper are useful to perform various vital biochemical reactions and pathways. Copper deficiency leads to inactivation of these copper binding proteins. Increase in bioavailability of copper, can help to reactivate these inactive copper binding proteins which could improve various pathophysiological conditions of copper deficiency disorders. Despite of having good potential to develop as a successful pharmaceutical product, copper does not gain any desired attention in pharmaceutical industry. Major limitations in the process of development of pharmaceutical copper products are scientific myths and pharmacokinetic of copper.

In spite of various reports, on the impact of copper deficiency on biological functions, copper deficiency is not considered as a viable situation because of its easy availability in broad range of food source and drinking water. It has been considered that if a person is taking sufficient food and drink enough water he would not face copper deficiency. In mammals, copper homeostasis is well regulated process which only fails to control copper toxicity due to rare genetic defects in expression of copper binding proteins. Even though, application of copper is not appreciated for treatment purpose. Contrary to being an essential trace element, copper supplementation is compared with other hazardous heavy metal toxicity.

Pharmacokinetic limitations are associated with its ADME. Gastrointestinal absorption of ingested copper has very broad range. Therapeutic window for minimum effective concentration (0.7 mg/mL) and minimum toxic concentration (1.6 mg/mL) of copper is very narrow. So it requires a controlled release supplementation. High intake of inorganic copper causes vomiting while ingestion or toxic effect if gets absorbed. This reduced the chance of copper supplement alone and if required provided along with other therapeutic metal supplements in trace amount. But supplementing copper along with other divalent elements will further decrease its absorption. **Figure 1.1** represents various limitations and opportunities with copper for its development of successful pharmaceutical product.
1.2.10. Why it requires to be studied?

Normally copper deficiency and toxicity are very rare events. However increased intake of highly processed and fast foods, change in life style, intake of packed mineral water and increase in intake of copper antagonists had decreased the copper intake. Copper, which helps in maintaining various physiological events by easily giving and accepting electrons and its deficiency had lead us to various physiological disorders. These disorders are called as developed world diseases like cardiovascular diseases, osteoporosis, obesity, diabetes etc. Copper has become key element of engineering and technology. However, copper is known for its therapeutic role since ancient time, use of copper in medical science is not established yet. Even nature has exploited redox property of copper during ‘Great Oxidation Event’. There is no successful improvement in the development of successful pharmaceutical product of copper.
1.3. Women and Copper

Copper is considered as a feminine element. Earlier it was represented by ankh (♀), which is the universal symbol for female and associated with Friday, planet Venus, Goddess and eternal life. Softness and ductility of copper are the important features for its feminine nature. Women require more amount of copper for their growth and development. Calcium inhibits absorption of copper through active transport but women milk is the richest source of biocompatible copper. Physiologically, women are more vulnerable to various diseases. Anemia is the major setback for women health. Copper is essential for the iron transport and formation of red blood cells. Copper supplementation improves the health of anemic women. Copper metabolism is controlled by estrogen, so after menopause women bone health goes down severely and leads to bone disorders. They could be controlled by copper supplementation. Skin care and cosmetics products are big part of women health products. Various role of copper such as antimicrobial, angiogenesis promoter, healing power, free radical scavenging could make it important part of women health in dermatological section.

1.4. Postmenopausal osteoporosis

Osteoporosis is a common disease characterized by a systemic impairment of bone mass and micro-architecture that results in enhanced bone fragility and consequent increase in fracture risk. With an ageing population, the medical and socioeconomic effect of osteoporosis, particularly postmenopausal osteoporosis, will increase further. Severity of osteoporosis is measured in terms of bone mineral density (BMD) (Nakchbandi and van der Merwe, 2009). The World Health Organization diagnostic criterion for osteoporosis is a BMD measurement equal to or more than 2.5 standard deviations (SD) below the young female (age 20–29 years) reference mean (T-score ≤ −2.5 SD) and bone microarchitecture disruption resulting in brittleness of bones (Vasikaran et al., 2011).

Osteoporosis causes major disability in the older population, particularly in women. In women with more than 10 years after the menopause, nutrition seems to play a key role in rates of bone loss (Lowe et al., 2002).

1.4.1. Burden of Osteoporosis

Osteoporosis, a silent epidemic disease, has become a major health hazard in recent years, increases bone fragility and thereby the risk of fractures is associated with high mortality,
morbidity and high medical expenses throughout the world (Shirwaikar and Khan, 2010). More than 200 million people are presumably affected worldwide, with approximately 2 million hip fractures annually. This incidence will increase almost fourfold during the next 50 years. Primary osteoporosis typically occurs in postmenopausal women or younger women with estrogen deficiency after oophorectomia. Secondary osteoporosis occurs in men and women older than 75 years (Aaseth et al., 2012). Bone loss in women occurs most rapidly immediately after menopause at an annual rate of 3 % to 5 % over 5 years, mostly due to a decrease in circulating estrogens. Osteoporosis is estimated to affect approximately 1 in every 3 postmenopausal women worldwide (VanderWalde and Hurria, 2011). It has been projected (Table 1.3) that over half of the hip fractures in the world by the year 2050 will occur in Asia (Cooper et al., 1992, Shatrugna et al., 2005).

<table>
<thead>
<tr>
<th>Country</th>
<th>Disease percentage in 1990</th>
<th>Projected in 2050</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>0.2 %</td>
<td>0.6 %</td>
</tr>
<tr>
<td>Russia</td>
<td>8.8 %</td>
<td>4.4 %</td>
</tr>
<tr>
<td>USA</td>
<td>28 %</td>
<td>24.4 %</td>
</tr>
<tr>
<td>Europe</td>
<td>28.6 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Asia</td>
<td>31.2 %</td>
<td>51.1 %</td>
</tr>
</tbody>
</table>

In India, osteoporosis is a major cause of morbidity and mortality in the elderly. Based on a 2001 census, approximately 163 million Indian were above the age of 50 and 230 million Indians are expected to cross the age of 50 by 2015, 20 %., ~46 million, are postmenopausal women with osteoporosis. The prevalence of osteoporosis in India according to the orthopedic surgeons was expected to 38.4 % of all adult population and with female preponderance (Jhaveri et al., 2015, Malhotra and Mithal, 2008). Even a conservative estimate suggests that 20 % of women and about 10-15 % of men would be osteoporotic. If the lower bone density is shown to confer a greater risk of fracture, as is expected, the figure can increase up to 150 million (Mithal et al., 2014).

1.4.2. Bone growth and bone metabolism

Bones are very important for mechanical and protective functions. They are home for marrow and involve in homeostasis of calcium. Adult human has 206 bones, which have 99 % of total body calcium. Bone development start at the stage of embryo by the process of endochondral ossification where cartilage converts into bone. This process is very fast in infants and young age but slower in aged population. Structure of the bone can be classified into two types: (1) Cortical
or Compact bone and (2) Trabecular or Cancellous bone. Bone structure is composed of inorganic mineral nano-crystals, extracellular organic matrix, cells lipid and water. Hydroxyapatite, a mineral form of calcium along with few other mineral forms the inorganic nano-crystal. Extracellular organic matrix is composed of type 1 collagen mainly. Bones are composed of mainly three different cells, which control bone metabolism or bone modeling and remodelling; osteoblasts, osteoclasts and osteocytes. Osteoblasts are important for bone formation and osteoclast are responsible for bone resorption while osteocytes are helpful in bone remodelling. Bones have potential to convert their shape and size for changing their microarchitecture in response to life styles and mechanical loads by the process of bone modeling. The process of bone remodelling is a surface phenomenon which is performed by osteoblasts and osteoclasts. The process of bone remodelling renews the bones constantly throughout the life. In this process, bone resorption is done by osteoclasts cells and bone formation is done by osteoblasts cells. It has been found that at any given time 1/10\textsuperscript{th} of the total body bone would be on remodelling stage.

1.4.3. Bone remodelling

Bone remodelling or bone metabolism is a lifelong process and provides equilibrium between bone formation and bone resorption. The process of formation of new bone is called as bone formation or ossification. The process in which matured bone tissue is removed from the skeleton is called as bone resorption. The cycle of bone resorption and ossification also controls the reshaping and replacing of bone in case of injuries such as fractures and also during mechanical loading. Any disturbance in bone remodelling leads to weakening of bone. Bone remodelling occurs due to two types of cells namely osteoblasts and osteoclasts. Osteoblasts are bone forming cells with a single nucleus. Osteoblasts are terminally differentiated cells of mesenchymal stem cells. Osteoblasts synthesize collagen and proteins such as osteocalcin and osteopontin. Osteoblasts are also engaged in export of calcium and phosphate. In contrast, osteoclasts are bone resorption cells, which degrade the bone. Osteoclasts are giant, multinucleated cells derived from the hematopoietic stem cells in the bone marrow. The mineralized bone is broken into fragments, osteoclasts engulf these fragments and digests within cytoplasmic vacuoles. Calcium and phosphorous liberated by breakdown of mineralized bone are released into the bloodstream. Unmineralized bone termed as osteoid is protected against osteoclastic resorption (Summerlee, 2002).
1.4.4. Bone health and aging

Bone health is very critical issue for aging population. Bone remodelling is performed by various cells. Cell proliferation and cell differentiation get slower with aging which affect bone remodelling. Expression of extracellular bone matrix protein is age dependent and decreases with aging. Mineral content in bone increases with aging. This process makes the bones brittle. Aging leads to loss of bone mass and strength. Oxidative stress contributes faster in aging of the bone cells.

1.4.5. Pathophysiology of osteoporosis

Osteoporosis is a bone disease that increases the risk of fracture. It is caused by loss of bone density. Osteoporosis affects femoral neck or vertebral bodies and probability of fractures increase with decrease in bone mineral density. Osteoporosis leads to abnormally porous bone that is compressible like a sponge. The symptoms of osteoporosis are invisible unless there is a bone fracture. Osteoporotic bone fractures are associated with pain, decreased quality of life and disability. The causes of age-related changes in bone mass are multifactorial and include genetic predisposition, nutritional factors, endocrine changes, habitual exercise levels and body weight. Bone loss is accelerated to 2-5 % per year immediately before and for up to 10 years post-menopause. The pathophysiology of osteoporosis in aging women and men is largely described by the effects of sex hormone deficiency on the bone. In women, estrogen deficiency is the main cause of early rapid postmenopausal bone loss, whereas hyperparathyroidism and vitamin D deficiency are thought to explain age-related bone loss later in life. Other reasons for osteoporosis are defective bone formation by aging osteoblasts, impairment of the growth hormone, reduced peak bone mass, age-associated sarcopenia, leptin secreted by adipocytes and serotonin secreted by the intestine. In postmenopausal women has rapid development of osteoporosis by faster bone loss due decreased ovarian secretion of estrogens. Serum estradiol level goes down to 85-90% than premenopausal state. In this state, rate of bone resorption increased by 90% while rate of bone formation is increased by 45 % than in premenopausal women. Decrease in estrogen secretion lead to increase in the production of receptor activator of nuclear factor kappa B ligand (RANKL) and cytokines. This favors bone resorption by increased activation and decreased apoptosis of osteoclasts (Summerlee, 2002).
1.4.6. Detection techniques

Bone health is assessed by biochemical analysis of blood and urine, physical parameter of bone and bio-imaging of bone. Bone turnover markers (BTM) are biochemical products measured usually in blood and/or urine that predict fracture risk, and reflect the metabolic activity of bone. They are traditionally categorized as markers of bone formation (direct or indirect products of active osteoblasts) or bone resorption (produced by active osteoclasts) (Luhmann et al., 2012, Aaseth et al., 2012). Most studied biochemical BTM are calcium, phosphorus, copper, alkaline phosphatase, tartrate resistant alkaline phosphatase. BTM for bone formation are alkaline phosphatase, procollagen type I N propeptide (PINP), procollagen type I C propeptide (PICP) and osteocalcin (OC). BTM for bone resorption are tartrate resistant alkaline phosphatase, pyridinium cross-links (pyridinoline (PYD) and deoxypyridinoline (DPD)) and C-terminal and N-terminal cross-linking telopeptides (CTX, NTX) of the type I collagen molecule (Vasikaran et al., 2011). Physical parameter, which are used for detection of osteoporosis are bone mineral density (BMD) and bone mineral content (BMC). BMD and BMC are measured by dual energy X-ray absorptiometry (DEXA or DXA). BMD is also detected by ultrasonic bone densitometer. Bio-imaging for detection of osteoporosis is done by bone scan using computer tomography (CT), quantitative CT (qCT), micro CT, high resolution CT and high-resolution magnetic resonance imaging (MRI) to understand 2D and 3D geometry, microarchitecture, modeling and remodelling of different bones (Adams, 2013). Tracer techniques using radionuclides is an another technique to quantify bone turnover at specific site. Positron emission tomography and gamma camera are very useful tracer techniques to study bone turnover (Blake et al., 2014).

1.4.7. Present treatment regimen for osteoporosis

Currently, therapeutic approaches for osteoporosis treatment include estrogen replacement therapy, selective estrogen receptor modulator (SERM) such as raloxifene, phytoestrogen (plant steroid), tibolone (synthetic steroid) bisphosphonates (such as alendronate, risedronate), calcitriol, calcitonin, teriparatide (recombinant parathyroid (PTH) hormone), calcium, denosumab (human monoclonal antibodies) and vitamin D etc. Since ovarian hormone deficiency is thought to be the major causative factor for postmenopausal osteoporosis, hormone replacement therapy (HRT) is perhaps the most effective treatment, but is not preferred because it increases the risk of breast cancer, endometrial damage and of cardiovascular diseases. The other therapeutic agents said afore are also associated with moderate to serious adverse effects. Cochrane systematic review showed
that aerobics, weight bearing and resistance exercises are also very effective in increasing the BMD of osteopenic individuals. However cannot be recommended to the affected individuals due to the risk of fractures (Ip et al., 2013). Table 1.4 summarizes the effect of most commonly used drugs, which are antiresorptive agents and act by inhibiting osteoclasts activity (Waalen, 2010).

### Table 1.4: Current and emerging therapies for the treatment of osteoporosis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Category</th>
<th>Mechanism of action</th>
<th>Route of administration</th>
<th>Effect</th>
<th>↓ risk of fracture</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisphosphonates</td>
<td>Antiresorptive</td>
<td>Inhibit osteoclasts</td>
<td>Oral</td>
<td>3-8%</td>
<td>40-70%</td>
<td>Osteonecrosis, Bioavailability</td>
</tr>
<tr>
<td>e.g. Risedronate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen</td>
<td>Antiresorptive</td>
<td>Inhibit osteoclasts</td>
<td>Oral</td>
<td>5-6%</td>
<td>34%</td>
<td>Heart disease</td>
</tr>
<tr>
<td>SERM* e.g. Raloxifene</td>
<td>Antiresorptive</td>
<td>Inhibit osteoclasts</td>
<td>Oral</td>
<td>2-3%</td>
<td>30-50%</td>
<td>Thromboembolism</td>
</tr>
<tr>
<td>Calcitonin e.g.</td>
<td>Antiresorptive</td>
<td>Inhibit osteoclasts</td>
<td>Intranasal</td>
<td>1-2%</td>
<td>36%</td>
<td>Induce cancer on long term</td>
</tr>
<tr>
<td>Salmon</td>
<td></td>
<td>activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTH* e.g. Teriparatide</td>
<td>Anabolic</td>
<td>Stimulate osteoblasts</td>
<td>Subcutaneous</td>
<td>8-10%</td>
<td>35-65%</td>
<td>Osteoporosis on long term, Bone cancer</td>
</tr>
<tr>
<td>Strontium ranelate</td>
<td>Anabolic and</td>
<td>Stimulates osteoblasts and</td>
<td>Oral</td>
<td>Improved</td>
<td>13-43%</td>
<td>Osteomalacia</td>
</tr>
<tr>
<td>antiresorptive</td>
<td></td>
<td>inhibits osteoclasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denosumab</td>
<td>Antirepportive</td>
<td>Anti-RANKL monoclonal Antibody</td>
<td>Subcutaneous</td>
<td>Improved</td>
<td>43%</td>
<td>Skin infection</td>
</tr>
<tr>
<td>Odanacatib</td>
<td>Antiresorptive</td>
<td>Cathepsin K inhibitor</td>
<td>Oral</td>
<td>3.3-5.5%</td>
<td>Reduced</td>
<td>Under Clinical Trials</td>
</tr>
</tbody>
</table>

SERM- Synthetic Estrogen Receptor Modulator, PTH- Para Thyroid Hormone, RANKL- Receptor-Activator of Nuclear Factor Kappa Beta Ligand

### 1.4.8. Limitations of current therapy

Estrogen and bisphosphonates are most preferred therapy but they have certain severe side effects. Estrogen and its analogues have the limitation for increasing risk for breast cancer, endometrial cancer and thromboembolism and cardiovascular disorders. Osteonecrosis is severe side effect associated with Bisphosphonates. PTH is the only approved anabolic drug but recommended to use only up to 2 years. High cost and daily subcutaneous injection have limited its applications. Cost of denosumab has limited its application (Bhutani and Gupta, 2013, Kumar et al., 2012, Waalen, 2010). Most of the drugs used for the osteoporosis treatment act by antiresorptive mechanism where they inhibit the activity of osteoclasts. Long term application of antiresorptive agents would lead to aging of bone by disturbing normal cycle of bone remodelling. This would
result in aged bone having good mineral density but with less strength and higher brittleness. Therefore, antiresorptive agents are recommended for short term therapy. Most of these therapy acts by inhibiting bone resorption but lacks promoting bone formation.

1.4.9. **Newer drug for osteoporosis treatment**

Limitations of current therapies has led to development of new drug for the treatment of osteoporosis with lesser side effects and other limitations. Newer drugs are in various stages of drug development. Newer antiresorptive drug therapy includes, new SERM analogous (bazedoxifene, lasofoxifene), osteoprotegerin (a decoy receptor for RANKL), C-src kinase inhibitors (inhibit maturation of osteoclasts), αVβ3 integrin antagonists (antagonize binding of osteoclasts with bone), cathepsin K inhibitors (inhibit breakdown of collagen type I), chloride channel inhibitors (inhibit development of acidic pH for bone resorption) and nitrates (by NO formation). Newer anabolic drugs are PTH related peptides therapies (transdermal patch, nasal spray, related proteins), calcium-sensing receptor antagonism (stimulate release of PTH), sclerostin neutralizing antibodies (stimulate bone remodelling), dickkopf-1 (Dkk-1) inhibition (stimulate bone formation), statins (stimulate bone formation), matrix extracellular phosphoglycoprotein (MEPE) fragments (stimulate bone formation), activin inhibitors (improve bone remodelling ), and cannabinoid agonists (enhance osteoblast differentiation) (Bhutani and Gupta, 2013, Kumar et al., 2012, Waalen, 2010). Development of both antiresorptive and anabolic drugs would help in improving the quality of life of osteoporosis patients.

1.4.10. **Role of trace elements in osteoporosis**

Nutritional factor is one of the potential contributor in manifestation of osteoporosis. Apart from calcium and vitamin D, several other elements like copper, zinc, magnesium, manganese are essential for bone formation. Elements like aluminium, cadmium and lead have toxic effects on the bone formation. In case of divalent metal, calcium and magnesium have been studied well for their role to reduce osteoporosis process. Copper, zinc and manganese are other essential elements with significant role in bone formation where copper has dual action as cofactor for lysyl oxidase and superoxide dismutase (Aaseth et al., 2012).

1.5. **Copper and postmenopausal osteoporosis**

1.5.1. **Copper in postmenopausal osteoporosis**

Osteoporosis causes major disability in the older population, particularly women. In women with more than 10 years past the menopause, nutrition seems to play a key role in rates of bone loss.
Older adults often have reduced dietary intake and compromised nutrient bioavailability resulting from the use of multiple medications and increased excretion of copper (Johnson et al., 1992). Ongoing research efforts are seeking to establish the role of copper in relation to the health of this population and investigate its potential therapeutic value. The most important nutrient identified to date is calcium, which is often given as a supplement administered with vitamin D. However, copper is known to play an important role in the health of the skeleton, and there are evidences linking marginal copper deficiency with osteoporosis. Changes in architecture of osteoporotic patient bone, are the most universal sign of copper deficiency (Strain, 1998). Copper in western diet has been decreasing at least since the 1930 and osteoporosis is one of the most likely human illnesses from low copper intakes (Klevay, 2011). Observational studies show that serum copper levels in elderly people with bone fractures are significantly lower than those of age-matched control (Conlan et al., 1990, Noor et al., 2012). Postmenopausal women whose diets had a high copper intake had significantly greater bone mineral densities than those with a low dietary intake even when both groups calcium intake was similar (Howard et al., 1990). Moreover, a low dietary intake of copper over a period of six weeks significantly increased the rate of bone resorption — an early indicator of increased bone turnover in healthy adult males aged between 20 and 59 years (A Baker, 1999). Several studies support the idea that increased copper intakes may reduce the rate of postmenopausal osteoporosis. For example, one study showed that copper supplementation with an additional 3 mg per day for two years in women aged between 45 and 56 years reduced loss of bone mineral density at the lumbar spine (Eaton-Evans et al., 1996). The usual dietary intake of these women was about 1 mg a day. Another study revealed that a mixture of trace minerals including copper, manganese and zinc given alongside calcium for two years increased bone mineral density by 1.48 % compared to a loss of 1.25 % in a group given calcium alone and a loss of 3.53 % in women given placebo (Lowe et al., 2002). In a study to evaluate calcitonin therapy in postmenopausal women, copper level was found to be significant low as compare to control and improved by calcitonin therapy (Gür et al., 2002). In a study on monitoring 5 days food dairies of healthy postmenopausal women for two years shows that daily copper in intake < 0.9 mg is associated with poor bone health (Nielsen et al., 2011). In a meta-analysis, it was reported that low serum level of copper is important risk factor of osteoporosis well-designed studies with adequate control for confounding factors are required in future
investigations (Zheng et al., 2014). Such findings raise important questions about the possible therapeutic role of copper for osteoporosis.

1.5.2. **Role and mechanism of copper for improving bone remodelling**

Continuous bone remodelling is essential to maintain good health of bone. Disturbance in the process of bone remodelling lead to bone disorder like osteoporosis. Copper plays important role in bone remodelling. Copper finds various mechanisms to alter bone remodelling process in osteoporosis patients for improving BMD by bone formation and inhibiting bone resorption (Figure 1.2).

![Bone remodelling diagram](image)

**Bone remodelling**

- **Bone specific alkaline phosphatase**
  - Osteocalcin
  - Calcium phosphate crystals
  - Crosslinked collagen fibrils
  - Bone formation

- **Lysyl oxidase**
  - **Bone**
  - **RANKL**
  - **Osteoblasts**
  - **Osteoclasts**

- **Tropocollagen**
  - **ROS**
  - **Tartrate-resistant acid phosphatase**
  - **Bone resorption**

---

*Figure 1.2. Mechanism of action of copper in the prevention of osteoporosis*

- Copper is a cofactor for lysyl oxidase which responsible for cross-linking the collagen fibrils to form triple helix of collagen fibrils. This triple helix of collagen fibrils help to form organic matrix of bone which act as platform for mineralization of calcified crystals. This mechanism of bone formation helps in development of bone mineral density and bone strength.

- Copper is a cofactor for superoxide dismutase which helps in scavenging free radicals and help in reducing activation of osteoclasts. This prevents the bone from oxidative damage and maintains BMD.
Copper is a known angiogenesis promoter and play important role in growth and development. This help in improving the rate of bone formation.

- Copper induce secretion of vascular endothelial growth factor (VEGF). VEGF enhances production of NO which inhibits osteoclasts and augments osteoblasts.
- Copper helps in gene expression and protein synthesis. It is essential to increase protein levels in osteoporosis patients to improve bone mineral density.
- Copper helps in improving organic matrixes which provide groves for mineralization and improve BMD.

1.6. References


Chapter 1  Copper and Postmenopausal Osteoporosis


