ISSN PRINT 2319-1775 Online 2320-7876, www.ijfans.org
Vol.11, Iss.12, Dec- 2022

STUDY OF ALUMINIUM

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Abstract

This article gives a brief introduction to the Aluminium (Al), Al and its effects, several of organs. The physical and chemical properties of Al, where it naturally by occurs, its production, uses as well as the kinetic & metabolism of Al are also discussed in detail. Present study reported that Al chloride capable of caused marked alterations in some biochemical parameters. Significant decrease was found in the levels of glutathione peroxidase, catalase, superoxide dismutase, and increased level was observed in TBARS in brain. Al alterations on major biochemical constituents such as proteins, nucleic acids and glycogen along with phosphodiester linkages, tryptophan bands, tyrosine doublet, disulfide bridge conformations, aliphatic hydrophobic residue, and salt bridges lipids and nucleic acids due to the overproduction of ROS in brain, liver, kidney, spleen, bone tissues of mice. The administration of deferiprone and deferoxamine and deferiprone significantly improved the level of protein, and recovered the progression of Al induced Alzheimer's disease. Al toxicity is a wide spread crisis in all living organisms, including both flora and fauna, furthermore causes wide spread degradation of the environment and health. DFO and DFP are efficient chelators for Al poisoning and they reduce the Al concentration. Ca, Mg, and P contents of the Al exposed bone were less than those of the control group, and there was an increase in the mineral contents of the bone after DFO and DFP treatments.

Introduction

Al is a universal toxicant and ingestion of excessive amount of Al leads to accumulation in target organs, which has been associated with damage of testicular tissue of both humans and animals (1). Al is about 8% of the earth's crust content and is rich in air, soil and water. Consequently, it is unavoidable for us to intake the metal via various kinds of routes. Although the physiological action of Al is not clear, it is well known that Al overload can induce serious brain injury and neurodegeneration. Al is the most widely distributed metal in the environment and is extensively used in modern daily life. Until the early 1970s, Al toxicity was first implicated in the pathogenesis of clinical disorders in patients with chronic renal failure or dialysis encephalopathy. From then on, the neurobiology of the metals is now receiving growing interest (2). There are now considerable evidences to demonstrate that the accumulation of Al in body tissues is associated with damages of the target organs. Al accumulation is most pronounced in human via high-Al dialysate, but it can also occur in individuals due to dietary, occupational, cosmetic, medications and adjuvant exposure (3). Al is ubiquitous, exposure to this element is in fact unavoidable. It means that pregnant women may be potentially exposed to Al in food, drinking water, soil ingestion, and some medications, whereas they may be also concurrently exposed to various types of stress, either at home or in the workplace. Because Al and maternal stress during pregnancy have been shown to produce adverse developmental effects in mammals, the purpose of this study was to assess the developmental toxicity in mice of a combined exposure to both Al and maternal restraint (4). Al is a chemical element in the boron group with symbol Al and atomic number 13. It is silvery white in color and insoluble in water under normal circumstances. Al is the third most prevalent element and the most abundant metal in the earth's crust, representing approximately 8% of total mineral components (5). It is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere (6, 7). Al existing primarily as polymorphous aluminosilicates (Al_2O_5Si) in rocks and soils (8, 9). Due to its reactivity, Al in nature is found only in combination with other elements. The ongoing acidification of our environment has increased both the solubilization and conversion of these inert forms of Al in biologically active species (10, 11, 12). Al is remarkable for the metal's low density and for its ability to resist corrosion due to the phenomenon of passivation. Structural components made from Al and its alloys are vital to the aerospace industry and are important in other areas of transportation and structural materials. The most useful compounds of Al, at least on a weight basis are the oxides and sulfates. Despite its prevalence in the environment, Al salts are not known to be used by any form of life. In keeping with its pervasiveness, Al is well tolerated by plants and animals. Owing to their prevalence, potential beneficial (or otherwise) biological roles of Al compounds are of continuing interest. The impact of Al on biological systems has

been the subject of much controversy in the past few decades (13, 14, 15, 16). Upto this time, no biological function has been attributed to this metal and more importantly, Al accumulation in tissues and organs results in their dysfunction and toxicity (17).

Dietary (food and water) exposure

Food is the primary source of Al under physiological conditions, but its bioavailability from food has not been adequately determined (18). While Al is ubiquitous in the environment, the major route of Al exposure by the general public is considered to be dietary exposure, particularly through foods containing such Al compounds as food additives (19). Al compounds are widely used as food additives in various food products; for example, Al ammonium sulfate is added as a firming agent or stabilizer in egg products, processed fish and vegetables, candied fruit, etc. (20) and acidic and basic sodium Al phosphate (SALP), sodium and calcium Al silicate, Al sodium sulfate, and Al lakes of various food dyes and colors (21). A small amount of Al is ingested by human infants through breast milk (22). Total dietary exposure to Al has been calculated to range from 14 to 280 mg Al/week in the adult population (21). Many types of foods contain Al because they are grown in soil that contains Al. When the soil pH is lower than 4.5 – 5.0, Al is solubilized in the soil water and absorbed by plant roots (23). Fluoride addition to potable water supply is a factor that should be considered when Al availability is evaluated. Increased concentration dramatically affects the speciation and the solubility of Al (24). Recently, it has been shown that the method of beverage preparation significantly affects the Al content in coffee infusions. In particular, the difference between coffee beverage prepared in Al and stainless steel moka pots was significant due to Al leaching to the coffee infusion (25). Another source of Al for commercial formulations is the container. Since, all liquid formulations are stored in glass containers and glass contains Al in its constitution, the additives which present an affinity for Al may cause it to migrate from the container into the solution (26).

Iatrogenic exposure

Al compounds have many medical implications as antacids, phosphate binders, buffered aspirins, vaccines, antiperspirants and allergen injection (27).The commonly used vaccines, diphtheria, tetanus, hepatitis, rabies and anthrax, all are based on Al adjuvant. The two most prevalent sources of Al are water used to prepare dialysate and Al – containing phosphate binders (28). Al – containing adjuvants are widely used in a variety of vaccine products such as recombinant proteins, virus – like particles, conjugated polysaccharides and recently, DNA vaccines (29). The patients affected by Down's syndrome, a genetic disease caused by trisomy of chromosome 21, are at risk of Al intoxication due to a markedly increased gastrointestinal absorption of this metal (29). The absorption of Al may be markedly increased through unusual routes: it may be absorbed through the underarm skin when contained in antiperspirant or may be inhaled with dust in polluted environment (30); Even a single dose of Al containing antacid can increase the serum Al and the level of increase varies with the type of liquid taken with it (30). Flaten (2001) reported that regular consumers of antacids ingest gram amounts of Al daily (31).

Occupational exposure

The exposure to Al is unavoidable due to the increased use of Al in day–to–day life and industrializations. The potential of Al exposure is expected to be highest among certain occupational groups such as workers of Al refining and metal industries, people employed in printing and publishing and in automotive dealerships and service stations, and individuals involved in fabricated metal products. During the past two decades, a considerable number of studies reported cognitive changes and possible impairment and other occupational hazards in relation to exposure to Al dusts and fumes (32). Recently, it has been implicated that the risk of hypertension increases in the Al exposure in manufacturing employees (33). Schlesinger et al., (2000) supported the concept of gradual accumulation and long – term retention of Al within the respiratory tract of individuals repeatedly exposed in occupational settings (33). Both objective neurophysiological, neuropsycological measures and subjective symptomatology indicated mild but unequivocal dose – dependent increase in Al burden in the body in Al welders and current mild steel welders (34, 35). A study of Zatta et al., (2003) also suggested the occupational and environmental exposure to the three metals (Al, manganese and zinc) as a possible cause of neurodegenerative disorders (36).

Entry, intestinal absorption and distribution of Al in the body

Al absorption seems to be very low, but many factors can enhance it in animals and humans. One of the significant routes of Al absorption is through the gut (37). Cochran et al., (1993) suggested the presence of two Al– specific proteins, which bind Al with the intestinal mucosa and thereby regulate Al absorption (38). Dermal applications of Al compounds in cosmetic, antiperspirant and health care products generally do

not induce harmful effects on skin or other organs (39). Underarm application of ^{26}Al chlorohydrate was associated with upto 0.012% absorption of the ^{26}Al through the skin (40). Thus, the skin may be a minor route of Al entry into the body. *In vivo* animal studies suggested that the Al absorption was generally very low (1%) and the percentage absorbed was sensitive to amount of Al intake (41). To gain entry into the body, Al has to cross a layer of epithelial barrier. The interaction of gastrointestinal, olfactory, pulmonary and dermal epithelia with Al was not well understood. However, intravenous, intramuscular and parenteral administrations of Al compounds bypass such barriers.

The intimate mechanism by which Al ions are transported across the brush border of the intestinal cells is not yet well known. In normal rats, 0.05 – 0.1% of ingested Al is absorbed in the intestine (42). A few experimental studies have suggested that the Al ions are absorbed in the gut through the paracellular route (43). The influence of food on Al absorption was demonstrated by the observation that Al absorption was higher in the post – prandial state (42). The amount taken up into cells or organs was very difficult to measure accurately (36). Al is absorbed from the alimentary tract in the small intestine and, because of favorable pH, in the stomach and duodenum, too. Al intestinal absorption is determined by a number of factors, for instance solubility of Al compound, the presence in the alimentary tract of organic acids (citric, ascorbic), diet deficient in Fe and Ca, renal insufficiency, immaturity of the alimentary tract, gastric hyperacidity, severe systemic diseases (44).

Distribution of the metal in different target organs varies with route, dose, and duration of exposure (45). Al accumulates in all tissues of mammals, preferentially in kidneys, liver, heart, bones and brain (46). Unequal distribution of Al to various tissues has been reported in normal, Al – exposed humans and in Al – treated experimental animals. Studies of tissue distribution after subcutaneous administration of Al in rabbits have also shown differential distribution of Al in different organs (6-9). The results of Wu et al., (2012) indicated that glutamate and citrate enhanced Al absorption in the intestine *in vitro* and glutamate increased Al deposition in red blood cell, brain and kidney *in vivo (11-17)*.

In comparison with many other polyvalent cations including gallium, lanthanides and actinides, Al binds rather weakly to plasma components, so maximizing its facility to be transferred to binding sites within tissues (44). It has been estimated that 45 – 75% of intravenously injected ^{26}Al was excreted in the first 24 hrs after injection (45). Since, only 0.5% of injected Al remains in human plasma one day after injection, it follows that Al transfer to different tissues was very rapid. The molecular mechanisms, through which tissues uptake Al, probably differ from tissue to tissue and depend on the type of compound. Uptake of Al ions *via* transferrin receptor – mediated exocytosis might have toxic consequences, as demonstrated in cultured hepatocytes (44) as well as in bone marrow cells (46).

Al and oxidative stress

Al enhances oxidative stress (OS) through enhanced iron – mediated Fenton reactions by increasing the redox active iron concentration. Al activates superoxide dismutase, while it inhibits catalase. The increased H_2O_2 pool enhances the presence of redox active iron either from loosely bound Fe or by modulating the electron transport chain (7-15). This favours the enhancement of Fe – mediated oxidative stress. All these events lead to the generation of hydroxyl free radicals and results in neuronal cell death by way of damage to DNA, proteins and lipids. Al promotes the iron induced ROS in the cells (7-15). Fig. 1 and 2 shows the ROS interferes with numerous cellular constituents including lipids, protein, and nucleic acids, and the Mechanism of Al induced oxidative stress.

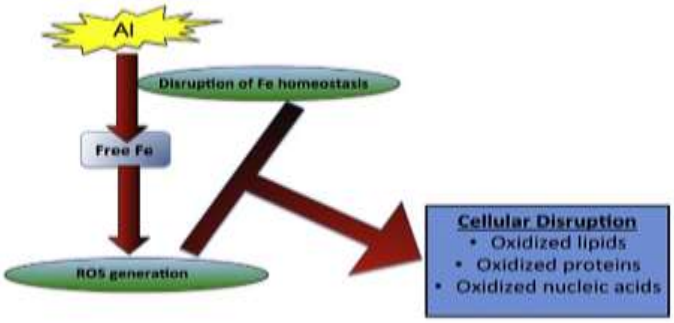

Fig.1. Al toxicity leads to defective Fe homeostasis and oxidative stress.

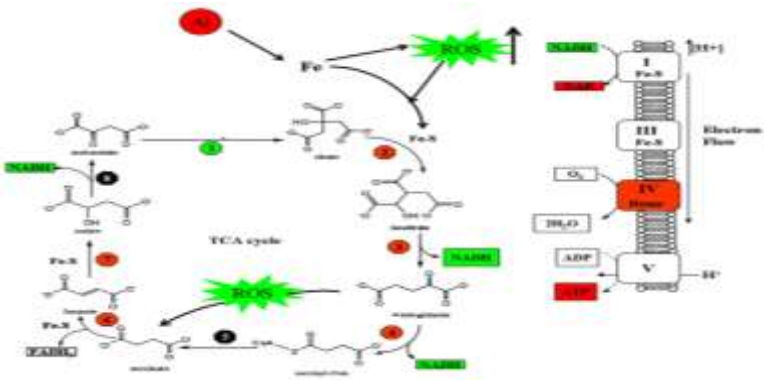

Fig.2. Mechanism of Al induced oxidative stress.

Antioxidant defense and al toxicity

Excessive generation of ROS leads to damage of cellular lipid membrane, proteins and DNA. Several mechanisms exist in the body to cope over production of free radicals. The basic and the most predominant defense mechanism of the human body are antioxidants that are involved in prevention, repairing and physical defense against oxidants. Antioxidants are generally categorized to non – enzymatic and enzymatic. Non – enzymatic antioxidants include dietary compounds (vitamins C and vitamins E), minerals (selenium and zinc), glutathione, uric acid and ubiquinol. Superoxide Dismutase (SOD), Catalase (CAT) and Glutathiode Peroxide (GPx) are the main enzymatic antioxidants (12-17). Al is considered a pro–oxidation and in exposure results in the production of free radicals (5-9), being responsible for neurotoxicity. Al induces oxidative stress by changing in the levels of SOD, CAT and biomarkers of cellular peroxidation (9-14).

Nicotinamide adenine dinucleotide phosphate (NADPH) is central to any anti–oxidative defense strategy and it fuels most, if not all the enzymes involved in combating oxidative stress. In fact, these cellular systems would not be able to function without NADPH. Intricate NADPH – generating machinery is activated under the influence of Al toxicity, a situation that favors the biosynthesis of fatty acids and the subsequent accumulation of lipids (5-17).

Effects of al on autoimmunity

The immune system is one of the main systems affected by Al accumulation, which plays a premier presage role in reflecting Al toxicity (6-9). Studies have shown that there is an association between Al and immune functions, but it is ambiguous whether Al induces adverse effects on the immune system (5-17). Al has the potential of immunotoxicity to human and animals. Now, effects of Al on the immune function are controversy. Some researchers showed that Al suppressed the immune function (7-9), but other studies observed the opposite results. The association of Al with the immune system has consequences of allergy or autoimmunity. Autoimmunity, oral tolerance, expression of the immune cells, hypersensitivity and erythrocyte immune function are important components of the immune function. As the immune system appears to be sensitive to Al exposure, exploration of these components above should help to better understand the toxic effects of Al on the immune function.

The autoimmune conditions were observed after a long time immunization with Al adjuvant because Al was a toxic metal. In the past very limited data reported the autoimmune cases induced by Al. High Al dose or long time Al exposure will make human and animals exert toxic effect.

Selection of organs

Brain

Brain is highly vulnerable to oxidative stress due to its high metabolic rate, the reduced capacity for cellular regeneration, and numerous cellular oxidative stress targets like lipids, nucleic acids, and proteins. The general function of the brain is to receive and analyze sensory inputs, and to integrate different sensory inputs in specific order to initiate the appropriate motor outputs. The central nervous system has many sensory and motor primary nerve pathways and subsequently transported towards the brain by axonal transport, thus, circumventing the apparently tight blood–brain barriers (BBB) and its causes various perturbations of brain as shown in Fig.3. The neurotoxic actions of Al have been a primary focus of attention in the last few years.

ISSN PRINT 2319-1775 Online 2320-7876, www.ijfans.org
Vol.11, Iss.12, Dec- 2022

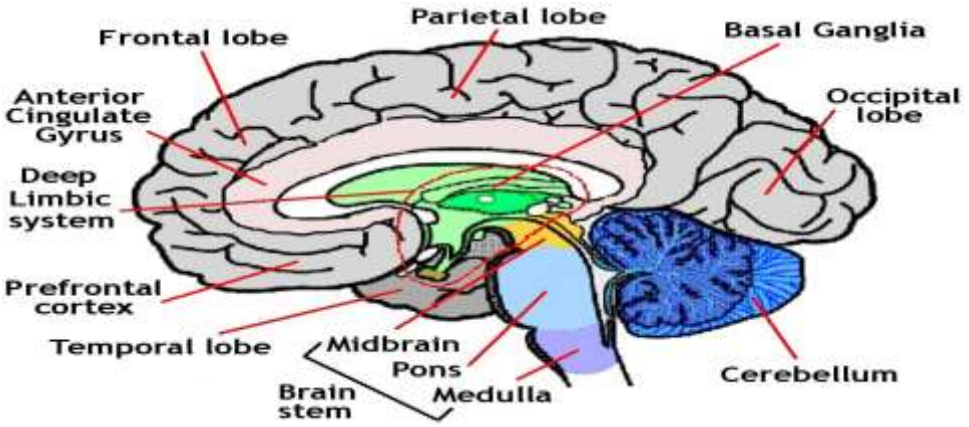

Fig.3. Labelled diagram of brain tissue.

Impacts of Al on the neural system

Al is highly neurotoxic and at high levels inhibits prenatal and postnatal development of the brain in humans and experimental animals (8) and this may be involved in the progression of neurodegenerative processes (8). Infants subjected to prolonged feeding with Al containing intravenous solutions exhibited impaired neurological development. Al is a possible contributing factor in Alzheimer's disease (8). Epidemiological studies have indicated a link between Al in drinking water and AD and a variety of human and animal studies have implicated learning and memory deficits after Al exposure (8).

$AlCl_3$ has a potential to get accumulated in specific brain regions (8) which was correlated with degenerative changes. The possible role of Al in Alzheimer's disease gives rise to the important question whether Al can enter the brain, and if it does, what the mechanisms of entry are as shown in Fig. 4. Three routes have been proposed by which Al could enter the brain from systemic circulation: blood – brain barrier (BBB), nasal – olfactory pathway and cerebrospinal fluid (CSF) (8). More rapid exchange is possible through the BBB, as many carriers of Al have been identified at the BBB. Transferrin (Tf) – mediated transport of Al has been suggested to be one of the mechanisms (8). Another important carrier for brain Al influx may be monocarboxylate transporter (MCT), a proton co – transporter which is located at both the luminal and abluminal surfaces of the BBB. Epidemiological, experimental as well as clinical studies have indicated a strong association between Al accumulation and several neurological disorders such as Alzheimer's disease, Down syndrome.

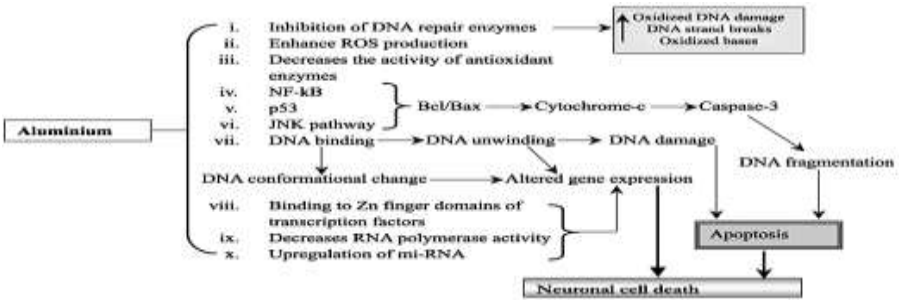

Fig. 4. Al (Al) causes neurotoxicity in multifaceted way.

Liver

Liver is the main organ for the detoxification of toxic substances and likely to be the target of heavy metals as shown in Fig.5. Heavy metals mainly accumulate in metabolically active tissues. The liver is highly active in the uptake and storage of heavy metals. Mice responded to heavy metal exposure by producing large amount of metallothionein in liver tissues. Liver is also a good indicator of chronic exposure to heavy metals, because it is vulnerable to Al toxicity. As the main organ of various key metabolic pathways, the effects of chemical usually appear primarily in the liver. This is turn provides toxicologists with a definitive site for the investigation.

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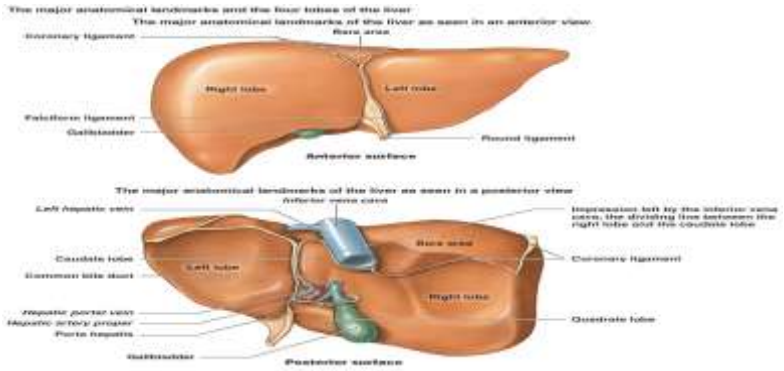

Fig.5. Labelled diagram of liver tissue.

Effects of Al exposure on liver tissue

The liver is a critical organ which contains most of the accumulated metals and where toxic effects can be expected (6). Al accumulated higher in the liver than in the brain, muscle, heart or lung (6). Al – induced hepatic dysfunctions, DNA cross – linking in rat ascites hepatoma cells (6, 10). No single mechanism emerges to explain all the systemic effects of Al. One of the mechanisms involves free radical –induced oxidative cell injury in Al toxicity (10). As a matter of fact, interactions between oxidative stress and hepatic damage may accelerate the progression of chronic hepatodegenerative disorders, including enzymes increase induced by Al (10).

Kidney

Kidney is the usual pathway for metal excretion as shown in Fig.6. In the present study, we chose kidney because it is most responsible for inactivation and excretion of drugs and other chemicals. During the excretion process the excess amount of Al ions are rapidly eliminated from the body through the kidney mainly detoxification mechanism. Hence kidney is clearly a major target organ for both acute and chronic Al exposures (5). Kidney plays a major role in preventing accumulation of Al by excreting it out through urine (5,11). Kidney may be highly vulnerable to nephrotoxic effects of Al during normal process of its excretion (11).

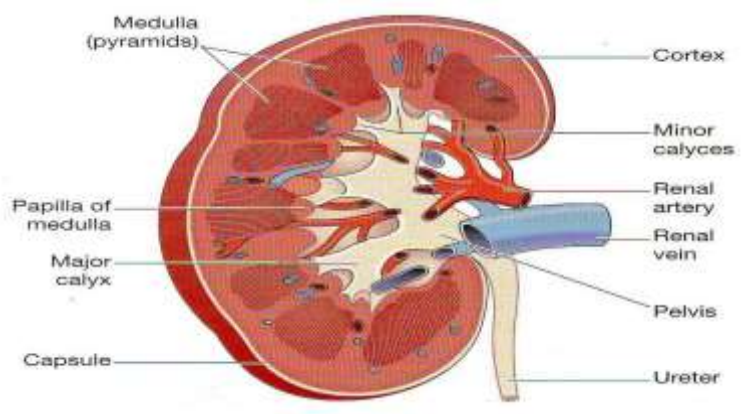

Fig.6. Labelled diagram of kidney tissue.

Effects of Al exposure on kidney tissue

The kidney appears to be the major route for the elimination of systemic Al (5). Al accumulation in renal tissue affects cellular metabolism, promotes oxidative stress, alterations in renal tubular p–aminohippuric acid transport and renal tubular phosphate reabsorption, together with impairment in sodium and water balance, without important changes in global renal functions (11). Under ordinary conditions in healthy people, it is thought to be able to excrete all of the absorbed Al. However, in patients fed by total parenteral nutrition receiving Al – contaminated solutions, its capacity was exceeded even in those with normal renal function, and part of the infused metal is retained (5). To which extent, the normal kidney can excrete the Al absorbed by patients treated with large oral loads is difficult to quantify. Al renal excretion can be inefficient (11) for a number of reasons. One of these is protein – binding, which limits Al ultrafilterability, even though the fraction of metal that is protein bound decreases with increasing plasma Al concentration, and at very high plasma Al

levels, the ultrafilterability of the metal is very low (~1%) as a consequence of formation of colloid (11). In addition to proteins, small legends capable of significantly binding Al in plasma are also expected to interact with the renal excretion of the metal. Further, when Al was given as the citrate the urinary excretion was significantly higher than when given as the chloride or sulphate (5,11). Such findings suggested the potential of the kidney for Al storage and thus toxicity.

Spleen

The spleen is an organ found in virtually all vertebrate animals. Similar in structure to a large lymph node, it acts primarily as a blood filter. The spleen plays important roles in regard to red blood cells (also referred to as erythrocytes) and the immune system (9). It removes old red blood cells and holds a reserve of blood, which can be valuable in case of hemorrhagic shock, and also recycles iron. As a part of the mononuclear phagocyte system, it metabolizes hemoglobin removed from senescent erythrocytes as shown in Fig.7. A study published in 2009 using mice found that the spleen contains, in its reserve, half of the body's monocytes within the red pulp (9). These monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cells and macrophages while promoting tissue healing.

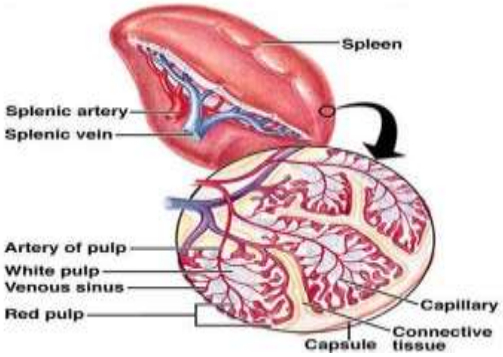

Fig.7 Labelled diagram of spleen tissue.

Effects of Al exposure on spleen tissue

Spleen is a peripheral organ of the immune system. The accumulated Al may alter the immune function. $AlCl_3$ can suppress the growth of spleen and suppresses the immune function, disorder the balance of trace elements and inhibit the immune regulation of cytokines in the spleen. Body weight of rats and growth index of spleen can reflect the physical conditions of organism with intoxication, and they are important in toxicology experiment (9). The growth index of spleen is the ratio of spleen weight and BW, and it indicates the change of the immune function of spleen (9). $AlCl_3$ decreased growth indexes of spleen in chickens (9).

Bone

Bones are rigid organs that constitute part of the endoskeleton of vertebrates. They support and protect the various organs of the body, produce red and white blood cells and store minerals. Bone tissue is a type of dense connective tissue. Bones come in a variety of shapes and have a complex internal and external structure, are lightweight yet strong and hard, and serve multiple functions. The largest bone in the human body is the femur and the smallest bones among the 206 are the auditory ossicles. Bones have eleven main functions. These are bones can serve to protect internal organs, such as the skull protecting the brain or the ribs protecting the heart and lungs. Bones provide a frame to keep the body supported and leverage system for skeletal.

Fig.8. Labelled diagram of bone tissue.

Muscles, tendons, ligaments and joints function together to generate and transfer forces so that individual body parts or the whole body can be manipulated in three–dimensional space. The interaction between bone and muscle is studied in biomechanics. Bones are important in the mechanical aspect of overshadowed hearing. The marrow, located within the medullary cavity of long bones and interstices of cancellous bone, produces blood cells in a process called hematopoiesis. Bones act as reserves of minerals important for the body, most notably calcium and phosphorus as shown in Fg.1.8. Mineralized bone matrix stores important growth factors such as insulin–like growth factors, transforming growth factor, bone morphogenetic proteinsand others. The yellow bone marrow acts as a storage reserve of fatty acids. Bone buffers the blood against excessive pH changes by absorbing or releasing alkaline salts. Bone tissues can also store heavy metals and other foreign elements, removing them from the blood and reducing their effects on other tissues. These can later be gradually released for excretion. Bone cells release a hormone called osteocalcin, which contributes to the regulation of blood sugar (glucose) and fat deposition (8).

Effects of Al exposure on bone tissue

Bone is a complex material with a hierarchical multi–scale organization from the molecule to the organ scale (8). The cycle of Al in bones starts with the transfer of the metal ions from transferrin and citrate in the blood stream to bone surfaces, where Al adheres. Within the skeleton, Al ions are first deposited on bone surfaces, including internal endosteal and external periosteal surfaces, trabecular surfaces and the surface of the vascular channels that permeate compact bones. Subsequently, Al ions may be transferred to osteoclasts, large multinucleated bone resorbing cells, or incorporated in the bone matrix. Osteoclasts may release Al to macrophages in the bone marrow. Macrophages could act as a temporary store of Al ions, before releasing the metal to citrate and/or transferrin, allowing Al re–entering the blood stream (8). Al deposits are present at the mineralised bone front on both growing and resting bones. In recent years, the association between increased Al bone stores in dialyzed patients and the development of osteomalacia, previously known as "renal osteodistrophy" has been well established (8).

Genotoxicity of Al

Al, being a non–physiological metal accumulates in the body is dispersed in different regions of the cell. The major sites of localization are mitochondria, lysosomes and nucleus in the cell (Dobson et al., 1998). Since Al is a Lewis base, it might bind to oxygen donors generated in the cell. It binds to biomolecules like nucleic acids, phosphate group of ATP and phosphorytated proteins and carboxylic groups of the molecules (8). Walton (2006) showed that the Al was centrally localized in the nuclear region compared to other intracellular organelles. Al acts as a pro–oxidant in the cells. Al induces DNA damage in the human peripheral blood lymphocytes at a concentration of 10 µg/ml. An increase in oxidized bases was observed in DNA at this concentration of Al as validated by digestion with formamido–pyrimidine DNA glycosylase. This indicates that the mechanism of DNA damage is oxidatively linked (5-15).

Chelating Therapy

Chelating agents are drugs with complex metallic ions, forming ring structures within their molecule (Greek: Chele = crab; the compounds hold the metal like carbs claw). The chelating agents possess the common ability to form complexes with heavy metals and thereby prevent or reverse the binding of metalic captions to body ligands. Chelating therapy is recommended for heavy metal poisoning. Heavy metals exert their toxic effects by combining with one or more reactive groups (ligands) essential for normal physiological functions. Chelating agents are designed specifically to compete with these groups for the metals and thereby prevent or reverse toxic effects and enhance the excretion of metals.

Desfferroxamine (DFO)

It is the principal product of the various sideramines obtained from streptomyces Pilosus (7-17). DFO consist of one molecule of acetic acid, two molecules of succinic acid, and three molecules of 1–amino–5–hydroxylamino–pentane Fig.9. The organic units of DFO are interlinked to form a chain, in which three hydroxamine groups are inside and one free amino group at the end. The free amino group is capable of reacting with organic and inorganic acids to form DFO salts. A good Al chelator should be capable to mobilize Al and to reduce its body burden, so reversing both encephalopathy and osteomalacia. The first Al chelator introduced in clinical practice for Al related osteomalacia was desferrioxamine (7-17). Long – lasting deferoxamine therapy might reduce not only bone Al deposits, but also the Al burden in the brain in humans (7). Desferrioxamine treatment has been even used

successfully on patients with acute encephalopathy due to severe Al intoxication caused by Al bladder irrigation.

Fig.9. Structure of Desfferroxamine (DFO)

Deferiprone (DFP)

Seventeen years after the first clinical trials DFP, which is orally active, has emerged as a suitable ameliorate for patients for whom DFO is, for one reason or another, inadequate as shown in Fig.10. Many patients are successfully treated usually a dose of DFP 75mg/ kg/ day. Some patients may need higher doses (upto 100mg/Kg), or combination therapy of DFP every day. The side effects of DFP are agranuclocytosis, neutropenia, gastrointestinal symptoms, arthropathy, transient changes in liver enzymes, and zinc deficiency (8-12).

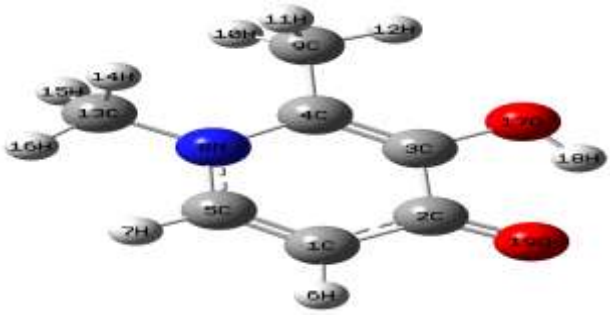

Fig.10. Structure of Deferiprone (DFP)

Spectroscopic analysis

Spectroscopy is the use of the absorption, emission, or scattering of electromagnetic radiation by matter to qualitatively or quantitatively study the matter or to study physical processes. The matter can be atoms, molecules, atomic or molecular ions, or solids. The interaction of radiation with matter can cause redirection of the radiation and transitions between the energy levels of the atoms or molecules. Biology relies on a vast set of molecular and macromolecular machines, each performing a well defined task. Our understanding of living systems is therefore intimately connected to the characterization of structure and function in molecular and macromolecular systems. Spectroscopy can offer the necessary tools to investigate the relevant structure and function, but the size and complexity of biological systems is beyond that usually encountered in the physical and chemical sciences and therefore poses a particular challenge. This challenge is met by extraordinary efforts to extend the sensitivity, specificity, information content and in some cases spatial resolution of spectroscopic methods.

UV–visible absorption spectroscopy

UV spectroscopy deals with the recording of absorption spectrum of light in the visible and ultraviolet region. The region for UV and Visible cover from 200 –800 nm (2000 – 8000 Å) where UV region is further subdivided into near ultraviolet region (200 – 400 nm) and far (or) vacuum region (15 – 200 nm). Ultraviolet and visible (UV–Vis) absorption spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels, and UV–Vis spectroscopy is usually applied to molecules or inorganic complexes in solution. The UV–Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The

concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer–Lambert Law.

Instrumentation

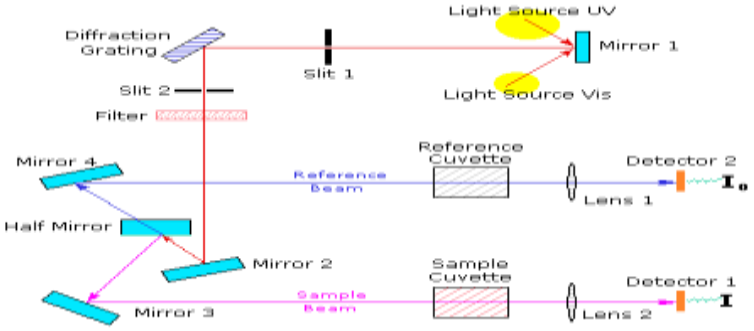

Fig.11. Schematic diagram of a dual–beam UV–visible spectrophotometer.

The light source is usually a deuterium discharge lamp for UV measurements and a tungsten – halogen lamp for visible and NIR measurements. Schematic of a dual – beam UV – visible spectrophotometer as shown in Fig.11. The instruments automatically swap lamps when scanning between the UV and visible regions. The wavelengths of these continuous light sources are typically dispersed by a holographic grating in a single or double monochromator or spectrograph. The spectral band pass is then determined by the monochromator slit width or by the array–element width in array – detector spectrometers. Spectrometer designs and optical components are optimized to reject stray light, which is one of the limiting factors in quantitative absorbance measurements. The detector in single–detector instruments is a photodiode, phototube or photomultiplier tube (PMT). UV–Vis–NIR spectrometers utilize a combination of a PMT and a Peltier – cooled PbS IR detector. The light beam is redirected automatically to the appropriate detector when scanning between the visible and NIR regions. The diffraction grating and instrument parameters such as slit width can also change. In single – beam UV –visible absorption spectroscopy, obtaining a spectrum requires manually measuring the transmittance of the sample and solvent at each wavelength. The double – beam design greatly simplifies this process by measuring the transmittance of the sample and solvent simultaneously. The detection electronics can then manipulate the measurements to give the absorbance. The dual – beam design greatly simplifies this process by simultaneously measuring P and Po (P is the power of light after it passes through the sample and Po is the initial light power) of the sample and reference cells, respectively. Most spectrometers use a mirrored rotating chopper wheel to alternately direct the light beam through the sample and reference cells. The detection electronics or software program can then manipulate the P and Po values as the wavelength scans to produce the spectrum of absorbance or transmittance as a function of wavelength.

Fourier Transform–Infrared (FT–IR) Spectroscopy

The vibrational spectroscopic techniques are potential tools for noninvasive optical tissue diagnosis. In recent years, applications of spectroscopic techniques in biological studies have increased a great deal, and particularly clinical investigations related to malignancy and cancer detection by spectroscopic means have attracted attention both by the clinical and non – clinical researchers. The total internal energy of a molecule in a first approximation can be resolved into the sum of rotational, vibrational and electronic energy levels. Infrared spectroscopy is the study of interactions between matter and electromagnetic fields in the IR region. In this spectral region, the EM waves mainly couple with the molecular vibrations. In other words, a molecule can be excited to a higher vibrational state by absorbing IR radiation. The probability of a particular IR frequency being absorbed depends on the actual interaction between this frequency and the molecule. In general, a frequency will be strongly absorbed if its photon energy coincides with the vibrational energy levels of the molecule. IR spectroscopy is therefore a very powerful technique which provides fingerprint information on the chemical composition of the sample. The mid – IR (400 – 4000 cm^{-1}) is the most commonly used region for analysis as all molecules possess characteristic absorbance frequencies and primary molecular vibrations in this range (8-11).

ISSN PRINT 2319-1775 Online 2320-7876, www.ijfans.org
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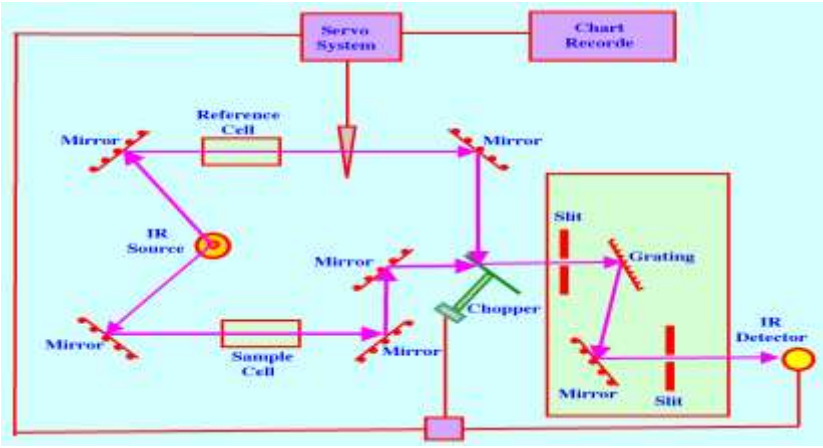

Fig. 12. Schematic diagram of FT–IR Spectrometer.

IR spectroscopy is based on the absorption of electromagnetic radiation. The sample is radiated by IR light in IR spectroscopy and the vibrations induced by electrical dipole moment are detected as shown in Fig. 12. Thus, the vibrational technique may provide complete information, by the shapes and positions of spectral bands on the energy scale are closely located for the specific functional groups of the molecule. Many vibrations are observed in the spectra to serve as a valuable confirmatory evidence of samples. Therefore, the using IR spectroscopy can offer a more potential approach for analyzing intact sample and provides more detailed chemical information. A common FT–IR spectrometer consists of a source, interferometer, sample compartment, detector, amplifier, A/D convertor and a computer. The source generates radiation which passes the sample through the interferometer and reaches the detector. Then the signal is amplified and converted to digital signal by the amplifier and analog–to–digital converter, respectively. Eventually, the signal is transferred to a computer in which Fourier transform is carried out.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP–OES)

Inductively coupled plasma optical emission spectrometry (ICP–OES), also referred to as inductively coupled plasma atomic emission spectroscopy (ICP–AES), is an analytical technique used for the detection of trace elementals. Its high specificity, multi–element capability and good detection limits result in the use of the technique in a large variety of applications. All kinds of dissolved samples can be analyzed, varying from solutions containing high salt concentrations to diluted acids. Fig.13 Shows the Steps involved in the analysis of aqueous samples by ICP–AES. A plasma source is used to dissociate the sample into its constituent atoms or ions, exciting them to a higher energy level. They return to their ground state by emitting photons of a characteristic wavelength depending on the element present. This light is recorded by an optical spectrometer. When calibrated against standards the technique provides a quantitative analysis of the original sample.

In inductively coupled plasma–atomic emission spectrometry as shown in Fig 14, the sample is usually transported into the instrument as a stream of liquid sample. Inside the instrument, the liquid is converted into an aerosol through a process known as nebulisation. The sample aerosol is then transported to the plasma where it is desolvated, vaporized, atomized, and excited and/or ionized by the plasma. The excited atoms and ions emit their characteristic radiation which is collected by a device that sorts the radiation by wavelength. The radiation is detected and turned into electronic signals that are converted into concentration information for the analyst.

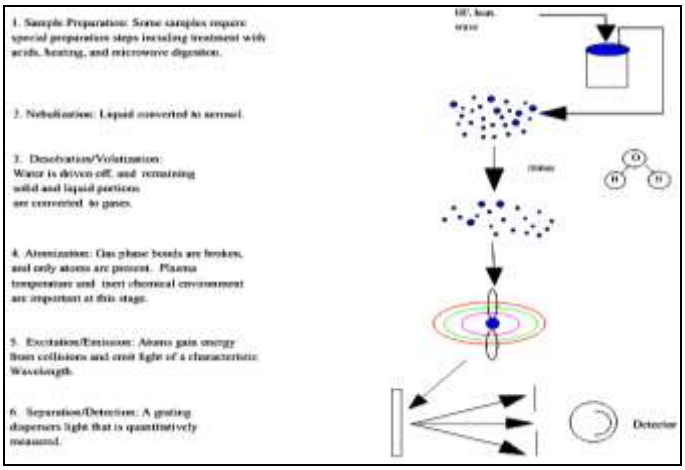

Fig.13. Steps involved in the analysis of aqueous samples by ICP–OES.

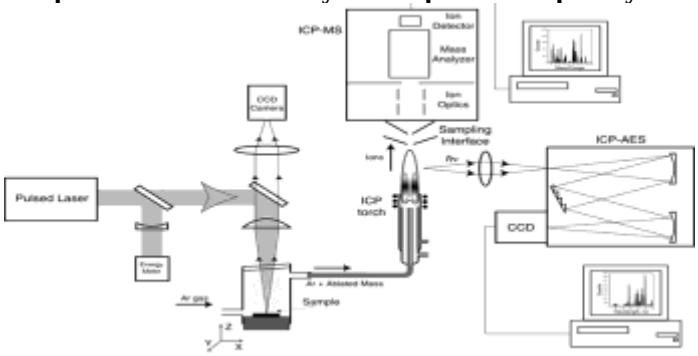

Fig. 14. Schematic diagram OF ICP–OES spectrometry.

Fourier Transform Raman (FT–Raman) Spectroscopy

Recently, spectroscopy has emerged as one of the major tools for biomedical applications and has made significant progress in the field of clinical evaluation. Research has been carried out on a number of natural tissues using spectroscopic techniques, including Raman spectroscopy. Raman spectroscopy is a vibrational spectroscopic technique that can be used to optically probe the molecular changes associated with diseased tissues (7). These vibrational spectroscopic techniques are relatively simple, reproducible, nondestructive to the tissue, and only small amounts of material (micrograms to nanograms) with a minimum sample preparation are required. In addition, this technique provides molecular–level information, allowing investigation of functional groups, bonding types, and molecular conformations. Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. These bands are relatively narrow, Raman spectroscopy has been widely used in various fields of science. It has been successfully utilized to qualitatively and quantitatively determine the molecular compositions of solid, liquid, and gaseous samples.

A typical laboratory Raman spectroscopy system showed in Fig.15. Raman spectroscopy typically uses a non-ionizing laser as the excitation source. The incident photons can be absorbed, scattered, and/or pass through the material without interaction. If the energy of the incident photon matches the energy gap between the ground state and an excited state of a molecule, the photon will most likely be absorbed and the molecule is promoted to the excited state. Fluorescence occurs when the excited molecule subsequently relaxes to the ground state by emission. Scattering takes place, on the other hand, as the incident photon distorts the electron clouds. Two types of scattering typically exist in the visible–light and near–infrared spectral range: Rayleigh and Raman scattering. The more intense form, Rayleigh scattering, happens when only the electron clouds are distorted. This is considered an elastic process, as no appreciable energy exchange occurs. However, if the vibrational state of the molecule is altered, energy transfer occurs, either from the photon to the molecule or vice versa. The process becomes inelastic and is named Raman scattering. This is in general a weak process involving approximately one in every 10^6 –10^8 scattered photons (7).

ISSN PRINT 2319-1775 Online 2320-7876, www.ijfans.org
Vol.11, Iss.12, Dec- 2022

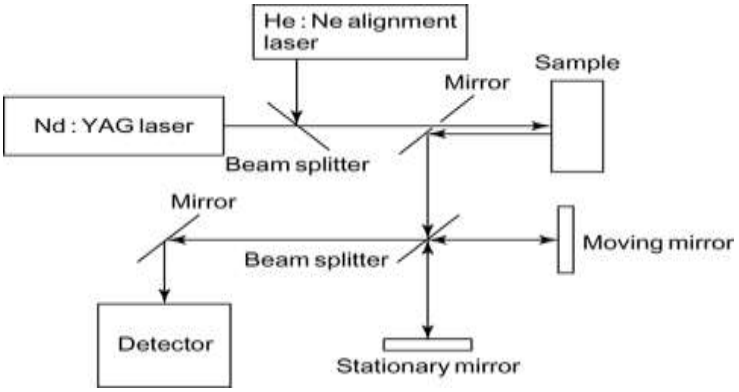

Fig. 15. A typical laboratory Raman spectroscopy system.

Depending on the direction of energy transfer between the molecule and the photon, Raman scattering can be further categorized into two subtypes: Stokes and anti–Stokes. Stokes scattering takes place when the molecule absorbs energy from the incident photon and elevates itself from a lower energy state to an excited vibrational state. On the other hand, because of thermal perturbation or prior external excitation, the molecule may already be in an excited vibrational state before interacting with the incident photon. In this case anti–Stokes scattering may result, such that the molecule releases energy upon interacting with the incident photon and subsequently returns to a lower energy state. The scattered photons in an anti–Stokes process higher energy than the incident photons. Because at room temperature most molecules are in the ground state, Stokes scattering typically dominates. Stokes scattering is therefore commonly recorded for Raman spectroscopy unless special experimental conditions are arranged. Raman band shifts (i.e., the energy difference between the incident and scattered photons) are typically described in wavenumber.

Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) has many and varied applications in life sciences, earth sciences, and in engineering. The scanning electron microscope (SEM) uses a focused beam of high–energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron–sample interactions reveal information about the sample including external morphology, chemical composition, and crystalline structure and orientation of materials making up the sample. In most applications, data are collected over a selected area of the surface of the sample, and a 2–dimensional image is generated that displays spatial variations in these properties. Areas ranging from approximately 1 cm to 5 microns in width can be imaged in a scanning mode using conventional SEM techniques (magnification ranging from 20x to approximately 30,000x, spatial resolution of 50 to 100 nm).

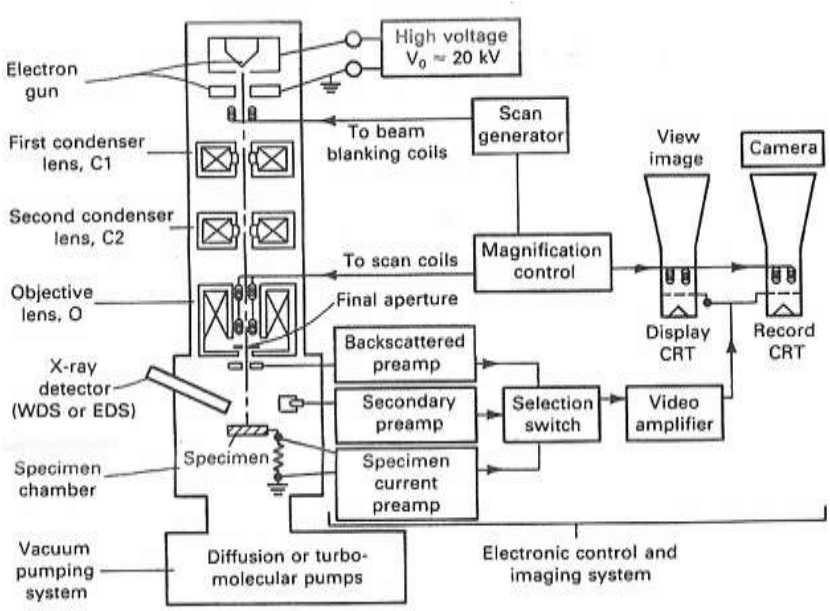

Fig. 16. Schematic diagram of a Scanning Electron Microscope.

Schematic diagram of a Scanning Electron Microscope showed in Fig.16. Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron–sample interactions when the incident electrons are decelerated in the solid sample. These signals include secondary electrons (that produce SEM images), backscattered electrons (BSE), diffracted backscattered electrons (EBSD that are used to determine crystal structures and orientations of minerals), photons (characteristic X–rays that are used for elemental analysis and continuum X–rays), visible light (cathodoluminescence – CL), and heat. Secondary electrons and backscattered electrons are commonly used for imaging samples: secondary electrons are most valuable for showing morphology and topography on samples and backscattered electrons are most valuable for illustrating contrasts in composition in multiphase samples (i.e. for rapid phase discrimination). X–ray generation is produced by inelastic collisions of the incident electrons with electrons in discrete shells of atoms in the sample. As the excited electrons return to lower energy states, they yield X–rays that are of a fixed wavelength (that is related to the difference in energy levels of electrons in different shells for a given element). Thus, characteristic X–rays are produced for each element in a mineral that is "excited" by the electron beam. SEM analysis is considered to be "non–destructive" i.e. x–rays generated by electron interactions do not lead to volume loss of the sample, so it is possible to analyze the same materials repeatedly.

X–ray Diffraction (XRD)

The X–ray diffraction is a powerful tool for investigations in solid state. Therefore much effort and ingenuity have been directed towards the developing technique employing X–ray diffraction. These techniques differ both in the apparatus they use and in the information they are designed to provide.

The diffraction of X–ray by crystals will provide information regarding the crystal structure. The interpretations of the results are done by making use of Bragg's law ($n\lambda = 2d \sin \theta$).

The basis of information revealed by X–ray diffraction

Diffraction is a phenomenon that is a property of wave motion. The useful information is provided by these peak's characteristics. They are: **Position**: The position of the peak, measured as the angle θ, yields: size (i.e., lattice parameter and d value), shape (cubic, tetragonal, etc.,) and orientation (hence it can be used for crystal orientation) and preferred orientation determination of the unit cell. **Intensity**: The relative intensity of the peaks, measured either as the peak height or more correctly as the area under its profile, provides data for determining the position of the atoms in the unit cell. **Shape**: The shape of the peak, of which the breadth is useful guide, provides information regarding crystallite size and lattice imperfections, including strain.

The fundamental measurement in X–ray diffraction

Most X–ray diffraction techniques are concerned with properties of the diffraction peaks, hence the fundamental aim in X–ray diffraction experiments is to delineate the peak sufficiently well for the characteristic of interest to be obtained with adequate precision. The basic operation is the measurement of the intensity of the diffracted ray as function of angle. The experimental record of this measurement can be obtained: Photographically or electronically using diffractometers which have electronic counter connected with chart recorders, digital print–out or controlling computers, where the record can be held in the computer store for further processing or can be printed out (7).

The radiation

The wavelength of X–rays used in X–ray diffraction is of the same order as the distance between the atoms in a crystal (about 0.1 nm). X–ray emitted by the X–ray tube is of two types. Firstly, the characteristic radiation which is due to the element of which the anode is composed. This radiation consists of sharp lines of which one may be selected, and hence monochromatic radiation is obtained. The most commonly used radiation of Cu Kα is a doublet with $\lambda\alpha1$= 0.154051nm and $\lambda\alpha2$= 0.154433 nm. Secondly, the continuous spectrum or "white" radiation, which is a function of the voltage applied to the x–ray tube.

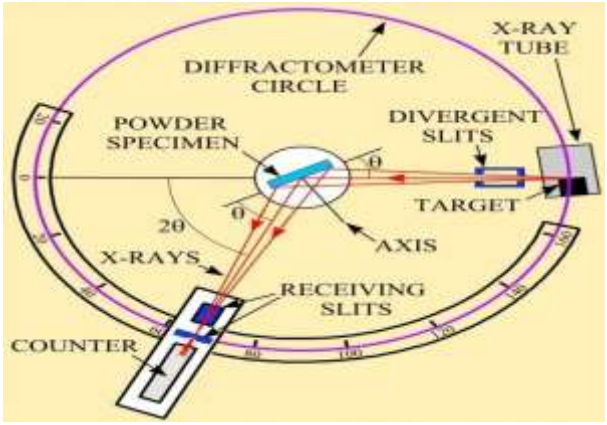

Fig.17. Layout of a typical powder Diffractometer

The large number of sets of parallel planes is possible in each crystal. Each set of planes has its own value of θ for which the incident X–ray beam will be diffracted and θ is related to the inter planar spacing 'd' Reinforcement will only take place when the X–ray diffracted by parallel planes of atoms are in phase with one another, that is when their path difference is an integral number of wavelength. This condition is represented by Bragg's law [2d sin θ=nλ] where λ is the wavelength, d–inter planar spacing, θ angle of incidence (and diffraction) of the X–rays and 'n' is the order. The 'd' values of each pattern were compared with the standard data published by Joint Committee on Powder diffraction Standards.

Histopathological Analysis

Histopathology refers to the study of cells, tissues and organ systems. It embraces a study of function as well of structure. Study of histology provides not only the information regarding the gross anatomy of the organ but also the structural basis for the physiology of the organ system. Knowledge of the normal is a necessary prelude to the study of the abnormal (pathology), which deals with the alterations in structure and function of the body and its organs, tissues and cells exposed to xenobiotics. Hence, the present work has been designed to study the effects of Al histopathogical alterations on the various organs of the mice mus musculus using Light Microscope.

Conclusion

Aluminium (Al) is the third most prevalent element and the most abundant metal in the earth's crust i.e., approximately 8% of total mineral components which is widely distributed in the environment and extensively used in daily life resulting easy exposure to human beings. Additionally, aluminium and its salts are commonly used in daily life as it is believed that it is a non-toxic and is quickly excreted in the urine. However, this element can have negative impact on human health. Aluminium exposure results in the production of free radicals and reactive oxygen species (ROS), which can react with lipids, proteins and nucleic acids, and leads to cell death *via* apoptosis or necrosis.

Chelating agents are drugs with complex metallic ions, forming ring structures within their molecule. Chelating therapy is recommended for heavy metal poisoning. For the last several decades, the most widely used chelators in the treatment of aluminium intoxication were Desferrioxamine (DFO) and Deferiprone (DFP). Aluminium causes neurotoxicity, changes in metabolism of liver, suppress autoimmunity, kidney dysfunction, spleen dysfunction, changes in metabolism of bone and morphological changes in bone. Some studies have been explained the entry, intestinal absorption and distribution of aluminum in the body. DFO and DFP inhibited lipid peroxidation may be due to scavenging of free radicals and is expected to its radical scavenging property. Furthermore, free radical scavenging enzymes such as superoxide dismutase, catalase, and glutathione peroxidase decreased in aluminum intoxicated mice but enhanced but enhanced by treatment with chelating agents DFO and DFP.

In the brain, liver tissue, bone, tissues the FT-IR, ICP-OES, FT-Raman and Biochemical estimations revealed the decreased quantity of protein due to the decreased level of free radical scavengers, Aβ deposition, increases of lipid peroxidation, oxidants, antioxidants, increases of lipid peroxidation, interaction of aluminium with adenosine triphosphate (ATP) metabolism and calcium homeostasis, decreased level of glycogen in aluminium intoxicated mice, and qualitative alterations in amino acids and quantitative changes in glutathione content due to aluminium exposure in aluminium intoxicated mice and progression of aluminium induced Alzheimer's disease. Also, provides the detailed explanation of phosphodiester linkages, tryptophan bands, tyrosine doublet, disulfide bridge conformations, aliphatic hydrophobic residue, and salt bridges. Further, revealed the aluminium induced alterations on the major

biochemical constituents such as lipids, proteins, phosphate, carbonate and proline due to the aluminum poisoning. ICP–OES study found the degree of trace elements contents and it suggested that there is a close relationship between the Ca:P ratio in bone tissue.

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