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COCOS NUCIFERA WATER AS A THERAPEUTIC AGENT IN LEAD-INDUCED ALTERATIONS OF CARDIO-PULMONARY TISSUES

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Abstract
The coconut (Cocos nucifera L.) is an important fruit tree in the tropical regions and the fruit can be made into a variety of foods and beverages. Lead is a naturally occurring toxic metal found in the earth's crust. Young children and adults are particularly vulnerable to the toxic effect of lead and can suffer profound and permanent adverse impact with the heart and lungs being the major organs affected. The aim of this study was to evaluate the histological effect of coconut water on lead induced toxicity in the heart and lungs of adult male wistar rats. Twenty- Eight (28) Adult Male Wistar rats were purchased from the Pharmacological Department of Niger Delta University. The rats were weighed and grouped into 5. The rats were given growers marsh and water for 12 hours cycle and were allowed to acclimatize for 14 days. The rats in the treatment groups were administered with 2mg/kg of Lead acetate intraperitoneally and there treated with a varying concentration of coconut water. At the end of 4 weeks of administration, the heart and lungs of animals were harvested, processed and stained with Haematoxylin and Eosin. Result showed the lungs of the lead only group showed enlargement of the alveoli, thickening of the septa and infiltration of inflammatory cells while rats in the lead group treated with coconut water also showed thickening of the interalveolar septa. However, animals in the coconut water only group showed normal appearance of lungs while the heart result revealed no alteration to it's morphology upon lead administration and also there were maintenance of the normal cardiac structure. Our conclusion reveals coconut water dosage administered in this study did not ameliorate lead toxicity in the heart and lungs of adult male wistar rats. Future research should focus on molecular pathways involved in these changes and explore alternative protective agents for lead-induced toxicity.

Keywords: *Coconut water, lead, Cardio-pulmonary, interalveolar septa, Toxicity, Inflammatory cells.*

INTRODUCTION

The coconut (Cocos nucifera L.) is an important fruit tree in the tropical regions and the fruit can be made into a variety of foods and beverages. The edible part of the coconut fruit (coconut meat and coconut water) is the endosperm tissue. Endosperm tissues undergo one of three main modes of development, which are the nuclear, cellular and helobial model and the development of coconut endosperm belongs to the nuclear mode. Initially, the endosperm is a liquid containing free nuclei generated by a process, in which the primary endosperm nucleus undergoes several cycles of division without cytokinesis (the process in which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells). Cytokinesis then occurs, progressing from the periphery towards the center, thus forming the cellular endosperm layer (Lopez *et al*., 1993). At first, the cellular endosperm is translucent and jelly-like, but it later hardens at maturity to become white flesh (coconut meat). Unlike the endosperms of other plants (e.g., wheat and corn), the cellularization process in a coconut fruit does not fill up the entire embryo sac cavity, but instead leaves the cavity solution-filled. This solution is commonly known as coconut water and it is of cytoplasmic origin (Janick *et al.*,2008]. Nutrients from coconut water are obtained from the seed apoplasm (surrounding cell wall) and are transported symplasmically (through plasmodemata, which is the connection between cytoplasms of adjacent cells) into the endosperm (Patrick *et al.,* 2001).

The coconut tree (Cocos nucifera) is a member of the palm tree family (Arecaceae) and the only living species of the genus Cocos.The term "coconut" (or the archaic "cocoanut") (Pearsall *et al.,* 1999) can refer to the whole coconut palm, the seed, or the fruit, which botanically is a drupe, not a nut. They are ubiquitous in coastal tropical regions and are a cultural icon of the tropics.The coconut tree provides food, fuel, cosmetics, folk medicine and building materials, among many other uses. The inner flesh of the mature seed, as well as the coconut milk extracted from it, forms a regular part of the diets of many people in the tropics and subtropics. Coconuts are distinct from other fruits because their endosperm contains a large quantity of an almost clear liquid, called "coconut water" or "coconut juice". Mature, ripe coconuts can be used as edible seeds, or processed for oil and plant milk from the flesh, charcoal from the hard shell, and coir from the fibrous husk. Dried coconut flesh is called copra, and the oil and milk derived from it are commonly used in cooking – frying in particular – as well as in soaps and cosmetics.

Sweet coconut sap can be made into drinks or fermented into palm wine or coconut vinegar. The hard shells, fibrous husks and long pinnate leaves can be used as material to make a variety of products for furnishing and decoration. The coconut has cultural and religious significance in certain societies, particularly in the Austronesian cultures of the Western Pacific where it is featured in their mythologies, songs, and oral traditions. The fall of its mature fruit has led to a preoccupation with death by coconut. (Nayer, 2017) It also had ceremonial importance in precolonial animistic religions. (Nayer, 2017) It has also acquired religious significance in South Asian cultures, where it is used in rituals of Hinduism. It forms the basis of wedding and worship rituals in Hinduism. It also plays a central role in the Coconut Religion founded in 1963 in Vietnam (Sandhya *et al.,* 2008).

Coconut water should not be confused with coconut milk, although some studies have used the two terms interchangeably (Sandhya *et al*., 2008). The aqueous part of the coconut endosperm is termed coconut water, whereas coconut milk, also known as "santan" in Malaysia and Indonesia, and "gata" in the Philippines refers to the liquid products obtained by grating the solid endosperm, with or without addition of water. Coconut water is served directly as a beverage to quench thirst, while coconut milk is usually used as a food ingredient in various traditional cooking recipes. The main components of coconut milk are water (ca. 50%), fat and protein, Seow & Gwee, 1997, whereas coconut water contains mainly water (ca. 94%). Unlike coconut water, coconut milk, which is the source of coconut oil, is generally not used in plant tissue culture medium formulations (George *et al.*, 1984).

Compared to coconut water, there are only limited studies on the aqueous extract of coconut solid endosperm (coconut meat or copra). Used coconut meat extract in orchid tissue culture to study its effects on orchid seed germination (Mariat *et al*., 1951). Subsequently, Mauney et al. purified a growth factor from the aqueous extract of coconut meat which was found to be very potent in promoting growth of tissue cultured plants (Mauney *et al.*, 1952). Another group, Shaw and Srivastava demonstrated the presence of purine-like substances in coconut meat extract. The purine-like substances were able to delay senescence (the process of ageing in plants) in detached cereal leaves, which exhibited similar known physiological effects of cytokinin. Zakaria et al. showed that the aqueous extract of coconut meat exhibited anti-inflammatory and wound healing properties when on mice (Zakaria *et al.*, 2006).

The heart is a muscular organ found in humans and other animals. This organ pumps blood through the blood vessetestedls, Moran & Micheal, 2013. Heart and blood vessels together make the circulatory system. The pumped blood carries oxygen and nutrients to the tissue, while carrying metabolic waste such as carbon dioxide to the lungs, Guyton & Hall, 2011. In humans, the heart is approximately the size of a closed fist and is located between the lungs, in the middle compartment of the chest, called the mediastinum (Moore *et al.*, 2009). In humans, the heart is divided into four chambers: upper left and right atria and lower left and right ventricles (Reed *et al.,* 2008). Commonly, the right atrium and ventricle are referred together as the right heart and their left counterparts as the left heart.(Gray's Anatomy, 2008). In a healthy heart, blood flows one way through the heart due to heart valves, which prevent backflow. The heart is enclosed in a protective sac, the pericardium, which also contains a small amount of fluid. The wall of the heart is made up of three layers: epicardium, myocardium, and endocardium (Betts & Gordon 2013).

Justification of the study:

Medical researchers have carried out researches / practical on the oral uses, therapeutic dose and mode of actions of coconut Water, but little or no consideration has not been given to the antiinflammatory and analgesic activity of coconut Water in cardio-toxicity and pulmonary-toxicity of Lead induced damage in the heart and lungs of albino rat.

Aim Of Study:

To determine the histological effect of cocos nucifera water on lead induced damage of heart and lungs using albino wistar rats.

Specific Objectives:

To evaluate the histological effect of coconut water in Lead induced toxicity in the Heart and Lungs of albino rats.

To find out the dosage of coconut water that would show a mitigating effect.

Research Questions:

What are the Histological effects of Lead on the Heart and Lungs?

What are the Histological effect of Coconut water on the Heart and Lungs?

Histological effect of Lead on Coconut water ameliorated Heart and Lungs?

Research Hypothesis: Null Hypothesis(H0)

1. Coconut Nucifera (coconut) water has no significant histopathological effect on lead induced heart and lung damage in albino wistar rats
2. There is no histopathological effect of lead on the heart and lungs

3. There is no histopathological effect of coconut water on the heart and lungs

Alternate Hypothesis(H1)

1. Coconut Nucifera (coconut) water has a significant histopathological effect on lead induced heart and lung damage in albino wistar rats
2. There is histopathological effect of lead on the heart and lungs
3. There is histopathological effect of coconut water on the heart and lungs

Chemical Components of Coconut Water:

Some of the most significant and useful components in coconut water are cytokinins, which are a class of phytohormones. The first cytokinin, N6-furfuryladenine (kinetin) was isolated from an autoclaved sample of herring sperm DNA in 1955 (Miller *et al.*,1955). In 1963, Letham isolated trans-zeatin, the first naturally-occurring cytokinin, from a plant source (unripe corn seeds). In addition to various plant-related functions, it was also found that some cytokinins (e.g., kinetin and trans-zeatin) showed significant anti-ageing, anti-carcinogenic, and anti-thrombotic effects (Vermuelen *et al.*, 2002).

Furthermore, micronutrients (nutrients needed in small quantities) such as inorganic ions and vitamins in coconut water play a vital role in aiding the human body antioxidant system (Evans & Halliwell, 2001) Hypermetabolism gives rise to an increased production of reactive oxygen species (or free radicals), as a result of increased oxidative metabolism. Such increase in free radicals will cause oxidative damage to the various components of the human cell, especially the polyunsaturated fatty acids in the cell membrane, or to the nucleic acids in the nucleus. Fortunately, living organisms have well developed antioxidant systems to neutralize the most detrimental effects of these oxidizing species. Micronutrients have important functions in this aspect. For example, they act directly to quench free radicals by donating electrons, or indirectly as a part of metallo enzymes (a diverse class of enzymes that require a catalytic metal ion for their biological activity) such as glutathione peroxidase (selenium) or superoxide dismutase (zinc, copper) to catalyse the removal of oxidizing species (Shenkin, 2005).

Other components found in coconut water include sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids and enzymes and they play different functional roles in plant and human systems due to their distinct chemical properties. The myriad of compounds, both known and unknown, provide coconut water with the special biological properties that is known to the typical layman (Ge *et al.*,2006)

Phytohormones:

Phytohormones are a group of naturally occurring organic compounds that play crucial roles in regulating plant growth in a wide range of developmental processes. Initially, the term phytohormone was synonymous with auxin. Later on, the other plant growth regulators such as gibberellins (GAs), ethylene, cytokinins, and abscisic acid (ABA) were categorized together with auxins as the “classical five” hormones. Coconut water contains auxin, various cytokinins, GAs and ABA (Ma *et al.*, 2008).

Auxin:

Coconut water contains indole-3-acetic acid (IAA), the primary auxin in plants (Wu & Hu, 2009). IAA is a weak acid (pKa = 4.75) that is synthesized in the meristematic regions located at the shoot apex and subsequently transported to the root tip in plants. For many years, tryptophan was assumed to be the precursor of IAA and this was later confirmed using experiments involving seedlings of Phaseolus vulgaris subjected to stable isotope labeling studies. IAA occurs not only in the free form, but is also conjugated to various amino acids, peptides, or carbohydrates. These IAA conjugates are biologically inactive and appear to be the IAA storage forms in seeds and are probably involved in hormonal homeostasis (Jakubowska *et al*., 2005).

Auxin is implicated in many regulatory processes in plants especially those relating to plant growth and development (Dharmasiri *et al*., 2005). Auxin functions in the relay of environmental signals such as light and gravity, the regulation of branching processes in shoots and roots, and as discovered more recently, the patterned differentiation of cells in meristems and immature organs. Undoubtedly, it is a versatile spatial-temporal signal. Auxin transport generates auxin concentration maxima and gradients within tissues that are instrumental in the diverse regulation of various plant developmental processes, including embryogenesis, organogenesis, vascular tissue formation and tropisms. The unique signal-molecule transport mechanism of auxin to a large extent underlies the remarkable developmental plasticity of plants that allows their growth and architecture to fit the environment changing (Robert & Friml, 2009).

Cytokinins:

Cytokinins, being able to induce plant cell division, were discovered in the 1950s (Werner *et al.,* 2001). Natural cytokinins are N6-substituted adenine derivatives with various substituted groups, and the physicochemical behaviour of cytokinins is a function of side chain(s), sugar, phosphate and degree of purine ring and/or side chain modification. The auxin-cytokinin hypothesis predicted that cytokinins, together with auxins, play an essential role in plant morphogenesis by controlling the formation of roots and shoots and moderating their relative growth. Cytokinins are a class of phytohormones that exert various roles in the different aspects of plant growth and development, e.g., cell division, formation and activity of shoot meristems, induction of photosynthesis gene expression, leaf senescence, nutrient mobilization, seed germination, root growth and stress response (Tantikanjana *et al*., 2001). Evidently, cytokinin-deficient plants generally develop stunted shoots with smaller apical meristems. The plastochrone of these cytokinin-deficient plants is prolonged, and leaf cell production is only 3-4% of wild type plants (with normal cytokinin metabolism), indicating an absolute role of cytokinins in leaf growth. Cytokinins are required during leaf formation, both to drive the cell division cycle at normal rates and to obtain the required number of divisions in order to produce a normal leaf size. In addition, cytokinins are also involved in promoting the transition from undifferentiated stem cells to differentiated tissues. Unlike the growth-promoting role of cytokinins in the shoot apical meristem, cytokinins have a negative regulatory function in root growth whereby it suppresses cell division in plant roots (Werner *et al*., 2001). Furthermore, cytokinins play an important role in retarding or even reversing leaf senescence Haberer & Kieber, 2002) Gan and Amasino reported on the three approaches used to investigate the inhibitory role of the cytokinins in plant senescence: the external application of cytokinins, the measurement of endogenous cytokinin levels before and during senescence, and the manipulation of endogenous cytokinin production in transgenic plants. However, externally applied cytokinins were not always effective in blocking the senescence of excised leaves. The effect of cytokinins on senescence can also vary under different experimental conditions. These studies revealed an inverse correlation between cytokinin levels and the progression of senescence in a variety of tissues and plant species. Cytokinins can interfere with senescence in detached tissues of dicotyledons and monocotyledons but are often less effective in attached tissues. In addition, cytokinin levels, as well as the capacity to synthesize cytokinin, decline with the progression of leaf senescence (Gan & Amasino, 1996).

Coconut water is an important additive in the tissue culture media of several plants, including orchids and traditional Chinese medicinal herbs. The cytokinins found in coconut water support cell division, and thus promote rapid growth. They are mostly used to propagate protocorm-like bodies of orchids in plant industries (Huan *et al.,* 2004). However, it should be noted that cytokinins cannot completely substitute coconut water's effects. This is due to the presence of other phytohormones (such as auxin and gibberellins or even undefined chemical components which may exert synergistic effects with cytokinins. One advantage of coconut water is that it results in considerable plant cell proliferation without increasing the number of undesirable mutations (Arditti, 2008). Coconut water contains various cytokinins.

Gibberellins (GAs):

GAs are a class of phytohormones which exert certain effects on plant growth and development, in aspects such as seed germination, epidermal cell elongation, leaf expansion and flower development. The main biological action of GAs is their ability to stimulate the elongation of plant shoots and induce the growth of stems in rosette and dwarfish forms. Together with auxins, GAs stimulate cambial activity and in effect, causing the formation of large xylem and phloem cells in woody plants (Chen *et al., 2*009). Apart from the vital roles played in plants, recent study also showed that gibberellin derivatives have anti-tumor bioactivities. Chemically, all known GAs are gibberellic acids (a family of diterpenoids acids), and there are currently 136 members of GAs identified based on their chemical structures. GAs are numbered neither by their structural information nor by their functions, but rather in the order of their identification. GA1 and GA3 were successfully detected and quantified in coconut water (Ge *et al.,* 2008).

Inorganic ions:

Inorganic ions are required for normal cellular function, and are critical for enzyme activation, bone formation, hemoglobin function, gene expression, and the metabolism of amino acids, lipids and carbohydrates (Wall, 2006). Coconut water contains a variety of inorganic ions and these ions contribute to the therapeutic value inherent in coconut water. As the basic ion composition of coconut water can replenish the electrolytes of the human body excreted through sweat, such as sodium, potassium, magnesium and calcium, it can serve as an effective rehydration drink (Saat *et al.*, 2002). The concentration of these electrolytes in coconut water generates an osmotic pressure similar to that observed in blood, and it also does not affect hemostasis (plasma coagulation) . As a result, coconut water can be used as a short term intravenous hydration fluid under certain emergency situations. Interestingly, Anurag and Rajamohan showed that coconut water has cardioprotective effects in experimental myocardial infarction induced in rats and this was probably attributed to the rich content of mineral ions in coconut water, especially potassium (Anurag *et al*., 2003).

Vitamins:

Vitamins, which are essential for the normal functioning of the human body, are also found in coconut water. Greater consumption of fruits and vegetables is associated with the reduced risk of cardiovascular disease, stroke, and cancers of the mouth, pharynx, esophagus, lungs, stomach, and colon (Riboli &Norat, 2003), because they contain vitamins and minerals vital for normal physiological functions. Coconut water contains vitamins B1, B2, B3, B5, B6, B7 and B9. The B vitamins are water-soluble and are required as coenzymes for enzymatic reactions essential for cellular function (Depient *et al.*, 2006)

Vitamin B6 (which includes pyridoxal, pyridoxine and pyridoxamine) serves as a coenzyme in various enzymatic reactions, such as the transamination and decarboxylation reactions. For example, it is the coenzyme of γ-cystathionase, which catalyses the cleavage of cystathionine, releasing α-ketobutyrate and cystein (Caroll *et al.*,1949). The α-ketobutyrate molecule is subsequently converted into succinyl-CoA and fed to the tricarboxylic acid (TCA) cycle while cystein is involved in protein and gluthathione biosynthesis. Vitamin B6 deficiency can affect various processes of the body, such as inflammation and renal function (Depient *et al.,* 2006). Coconut water contains folate, also known as vitamin B9. It was identified in the late 1930's as the nutrient required to reduce anemia during pregnancy (Goh & Koren,2008) It can prevent mitochondrial toxicity induced by methanol metabolites. In addition, the active form of folate, 5methyltetrahydro-folate is believed to be one of the central methyl donors required for mitochondrial protein and nucleic acid synthesis. Lower blood levels of vitamin B6 and folate can increase the risk for atherosclerosis and other vascular diseases (Robinson *et al.,* 1998). Another study found that high plasma levels of vitamin B6 and folate may reduce the risk for breast cancer. In addition to vitamin B, coconut water also contains vitamin C (total ascorbic acid), which is an important dietary antioxidant (Shenkin, 2006).

Lead (Pb):

Lead is a chemical element; it has symbol Pb (from Latin plumbum) and atomic number 82. It is a heavy metal that is denser than most common materials. Lead is soft and malleable, and also has a relatively low melting point. When freshly cut, lead is a shiny gray with a hint of blue. It tarnishes to a dull gray color when exposed to air. Lead has the highest atomic number of any stable element and three of its isotopes are endpoints of major nuclear decay chains of heavier elements (Prohaska *et al.,* 2022).

Lead is a relatively unreactive post-transition metal. Its weak metallic character is illustrated by its amphoteric nature; lead and lead oxides react with acids and bases, and it tends to form covalent bonds. Compounds of lead are usually found in the +2 oxidation state rather than the +4 state common with lighter members of the carbon group(Ling *et al.,*2005). Exceptions are mostly limited to organolead compounds.

Like the lighter members of the group, lead tends to bond with itself; it can form chains and polyhedral structures.

Lead (Pb) is a naturally occurring metal and generally form lead compounds by combining with two or more elements. Lead reacts with air and water to form lead sulfate, lead carbonates or lead oxide. These compounds act as a protective barrier to prevent corrosion. Lead can also interact with both acid as well as base. It has a low melting point and located above hydrogen in the electromotive series. Although the existence of lead is indicated in nature but human activities has been found as the main reason for increasing lead content in the environment (Shahid *et al.,* 2015). Lead is released in air from mining of lead, factories utilizing lead compounds, alloys, vehicle exhaust and burning of fossil fuels (Violante *et al.,* 2010). The lead is removed from atmosphere by rain and transferred to soil or comes in contact with surface water. Moreover, lead is used as pesticide during vegetable and fruit cultivation (Gall *et al.,* 2015). Disposal of lead containing waste products, removal of lead based paints from bridges, buildings and damaged battery from industries further results into the accumulation of lead in municipal landfills. Lead combines very strongly with the soil particles and present in the top layer of soil (Gupta *et al.,* 2008). Lead enters water bodies or lakes when these soil particles are washed away by rain water. Thus, lead is transferred to animals and plants from air, water, soil and this cycle continues (Abadin *et al.,* 2007).

Lead is not a foreign material to the human body as it is distributed to the brain, liver, kidney, and bones and is stored in bones and teeth, However, this is only 10 µg/dL in adults and 1.4 µg/dL in children (Jusko *et al.,* 2008). The guideline value of lead indicated by world health organization is 0.01 mg/L. The Nervous system is mainly affected by lead. The normal functioning of nervous system is influenced if an individual is exposed to lead for a long time. Moreover, longer exposure also causes severe effects on kidney as well as brain (Abadin *et al.,* 2007). Lead is easily absorbed by the body. Children absorb higher amounts of lead than adults which is highly dangerous as they are developing. In children lead is not absorbed by the bones like in the case of adults therefore they are at a higher risk of poisoning as the other soft tissues absorb the excess lead. Lead acts as a calcium analog, thus it is easily absorbed in people with calcium, zinc, and iron deficiencies. Lead affects the reproductive systems of both males and females (Flora *et al.,* 2006). In the case of males, there is a reduction in sperm count and volume, the motility and the morphology of the sperm are also affected (Wu *et al.,* 2012). In females who have high exposure to this metal, miscarriage, premature birth, low birth weight, and developmental problems are seen. When toxicity of lead has reached higher amounts, spontaneous abortion of the fetus occurs (D. C, 2005). Lead can damage cell structure, cell membrane and most importantly it interferes with DNA transcription. At developmental stages, lead passes through the placenta into the body of the fetus. At developmental stages, lead passes through the placenta into the body of the fetus (Mason *et al*., 2014).

History of lead:

Metallic lead beads dating back to 7000–6500 BC have been found in Asia Minor and may represent the first example of metal smelting (Rich, 1994) At that time, lead had few (if any) applications due to its softness and dull appearance. The major reason for the spread of lead production was its association with silver, which may be obtained by burning galena (a common lead mineral). The Ancient Egyptians were the first to use lead minerals in cosmetics, an application that spread to Ancient Greece and beyond; the Egyptians had used lead for sinkers in fishing nets, glazes, glasses, enamels, ornaments. Various civilizations of the Fertile Crescent used lead as a writing material, as coins, Chapuruka, 2017 and as a construction material. Lead was used by the ancient Chinese as a stimulant, as currency, as contraceptive, and in chopsticks. The Indus Valley civilization and the Mesoamericans used it for making amulets; and the eastern and southern Africans used lead in wire drawing. (Bisson & Vogel, 2000).

Classical era:

Because silver was extensively used as a decorative material and an exchange medium, lead deposits came to be worked in Asia Minor from 3000 BC; later, lead deposits were developed in the Aegean and Laurion. (Wood *et al.*, 2021) These three regions collectively dominated production of mined lead until c. 1200 BC.[135] Beginning c. 2000 BC, the Phoenicians worked deposits in the Iberian peninsula; by 1600 BC, lead mining existed in Cyprus, Greece, and Sardinia (Bisson & Vogel, 2000).

Rome's territorial expansion in Europe and across the Mediterranean, and its development of mining, led to it becoming the greatest producer of lead during the classical era, with an estimated annual output peaking at 80,000 tonnes. Like their predecessors, the Romans obtained lead mostly as a by-product of silver smelting. Lead mining occurred in central Europe, Britain, Balkans, Greece, Anatolia, Hispania, the latter accounting for 40% of world production (Hong *et al.,*1994).

Lead tablets were commonly used as a material for letters, Ceccarelli 2013, Lead coffins, cast in flat sand forms and with interchangeable motifs to suit the faith of the deceased, were used in ancient Judea. Lead was used to make sling bullets from the 5th century BC. In Roman times, lead sling bullets were amply used, and were effective at a distance of between 100 and 150 meters. The Balearic slingers, used as mercenaries in Carthaginian and Roman armies, were famous for their shooting distance and accuracy (Calvo, 2019)

Lead was used for making water pipes in the Roman Empire; the Latin word for the metal, plumbum, is the origin of the English word "plumbing". Its ease of working, its low melting point enabling the easy fabrication of completely waterproof welded joints, and its resistance to corrosion ensured its widespread use in other applications, including pharmaceuticals, roofing, currency, warfare (Retired & Cilliers, 2006). Writers of the time, such as Cato the Elder, Columella, and Pliny the Elder, recommended lead (and lead-coated) vessels for the preparation of sweeteners and preservatives added to wine and food. The lead conferred an agreeable taste due to the formation of "sugar of lead" (lead (II) acetate), whereas copper vessels imparted a bitter flavor through verdigris formation (Grout, 2017).

The Roman author Vitruvius reported the health dangers of lead (Hodge, 1981) and modern writers have suggested that lead poisoning played a major role in the decline of the Roman Empire. Other researchers have criticized such claims, pointing out, for instance, that not all abdominal pain is caused by lead poisoning. According to archaeological research, Roman lead pipes increased lead levels in tap water but such an effect was "unlikely to have been truly harmful".When lead poisoning did occur, victims were called

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"saturnine", dark and cynical, after the ghoulish father of the gods, Saturn. By association, lead was considered the father of all metals. Its status in Roman society was low as it was readily available and cheap (Thornton *et al.*, 2001).

Middle Ages:

Lead mining in Western Europe declined after the fall of the Western Roman Empire, with Arabian Iberia being the only region having a significant output. The largest production of lead occurred in South Asia and East Asia, especially China and India, where lead mining grew rapidly (Rich 1994).

In Europe, lead production began to increase in the 11th and 12th centuries, when it was again used for roofing and piping. Starting in the 13th century, lead was used to create stained glass. In the European and Arabian traditions of alchemy, lead (symbol ♄ in the European tradition), Ede & Cormack, 2016 was considered an impure base metal which, by the separation, purification and balancing of its constituent essences, could be transformed to pure and incorruptible gold. During the period, lead was used increasingly for adulterating wine. The use of such wine was forbidden for use in Christian rites by a papal bull in 1498, but it continued to be imbibed and resulted in mass poisonings up to the late 18th century. Lead was a key material in parts of the printing press, and lead dust was commonly inhaled by print workers, causing lead poisoning (Sinha *et al*., 1993).

Lead also became the chief material for making bullets for firearms: it was cheap, less damaging to iron gun barrels, had a higher density (which allowed for better retention of velocity), and its lower melting point made the production of bullets easier as they could be made using a wood fire. Lead, in the form of Venetian ceruse, was extensively used in cosmetics by Western European aristocracy as whitened faces were regarded as a sign of modesty. This practice later expanded to white wigs and eyeliners, and only faded out with the French Revolution in the late 18th century. A similar fashion appeared in Japan in the 18th century with the emergence of the geishas, a practice that continued long into the 20th century. The white faces of women "came to represent their feminine virtue as Japanese women", with lead commonly used in the whitener (Nakashima *et al.,* 1998).

Modern era:

Further evidence of the threat that lead posed to humans was discovered in the late 19th and early 20th centuries. Mechanisms of harm were better understood, lead blindness was documented, and the element was phased out of public use in the United States and Europe. The United Kingdom introduced mandatory factory inspections in 1878 and appointed the first Medical Inspector of Factories in 1898; as a result, a 25-fold decrease in lead poisoning incidents from 1900 to 1944 was reported. Most European countries banned lead paint commonly used because of its opacity and water resistance for interiors by 1930 (Markowitz & Rosner, 2000).

The last major human exposure to lead was the addition of tetraethyllead to gasoline as an antiknock agent, a practice that originated in the United States in 1921. It was phased out in the United States and the European Union by 2000 (Riva *et al*., 2012).

In the 1970s, the United States and Western European countries introduced legislation to reduce lead air pollution (More *et al*., 2017). The impact was significant: while a study conducted by the Centers for Disease Control and Prevention in the United States in 1976–1980 showed that 77.8% of the population had elevated blood lead levels, in 1991–1994, a study by the same institute showed the share of people with such high levels dropped to 2.2%. The main product made of lead by the end of the 20th century was the lead–acid battery (Rich 1994).

From 1960 to 1990, lead output in the Western Bloc grew by about 31%. (Rich 1994) The share of the world's lead production by the Eastern Bloc increased from 10% to 30%, from 1950 to 1990, with the Soviet Union being the world's largest producer during the mid-1970s and the 1980s, and China starting major lead production in the late 20th century. Unlike the European communist countries, China was largely unindustrialized by the mid-20th century; in 2004, China surpassed Australia as the largest producer of lead. As was the case during European industrialization, lead has had a negative effect on health in China (Zhang *et al*., 2012).

Lead(pb) intake by humans:

Pb is an environmental pollutant. Despite the low amounts absorbed, prolonged exposure to Pb can accumulate in the human body system, resulting in lead poisoning or toxicity. Lead has a half-life of around 30 days in the blood, after which it diffuses into soft tissues such as the kidneys, brain, and liver and then distributed to bones, teeth and hair as lead phosphate (Engwa *et al.*, 2019). ROS (Reactive oxygen species) such as hydroperoxide, hydrogen peroxide, and singlet oxygen are produced as a result of lead poisoning. Pb generates these free radicals which leads to oxidative stress causing cellular damage to the body cells. The body suffers oxidative stress when there is an imbalance of ROS and antioxidant defences. Oxidative stress causes cell and tissue destruction, which increases the likelihood of adverse health outcomes like cardiovascular disease and cancer (Flora, 2011). Increased oxidative stress causes lipid peroxidation, which damages cell membranes resulting in cell damage. Lead inhibits the activity of 5-aminolevulinic acid dehydratase, resulting in hemoglobin oxidation and lipid peroxidation, which can cause red cell hemolysis. When there is increase in concentration of Pb, the equilibrium between the ROS and antioxidants is altered. The rise in ROS production causes depletion of antioxidant defense causing oxidative stress which eventually leads to cell damage. Lead also interferes with the activity of other antioxidant enzymes including superoxide dismutase and catalase. Glutathione in the body helps to maintain ROS in balance. Ninety percent of glutathione in the cell is reduced, while ten percent is oxidized, and it serves as an antioxidant defense mechanism. Glutathione stabilizes ROS and is reduced back to GSH by glutathione reductase after being oxidized to glutathione disulfide. By attaching to the sulfhydryl group of glutathione, Pb inactivates it, making GSH replenishment ineffective and increasing oxidative stress (Batool *et al.,* 2017). The deposition of a small amount of Pb in the human body causes cellular malfunction and has a negative impact on an individual's health

Physiological and Biochemical Effects of Lead Accumulation in Human Beings:

Lead (Pb) is one of the ancient heavy metals used by human beings. From time immemorial, Pb has huge applications in the manufacturing of instruments and tools due to its splendid physical and chemical properties. Lead is used in the manufacture of boats, bearings, buildings, paints, lead batteries, automobiles, gasoline, pipes, ceramics, plastics, and in smelting, mining processes, and the arms industry. Studies have revealed that both adults and children are affected by lead toxicity. In the case of children, both internal and external tissues are soft, hence making them more susceptible (Ara and Usmani, 2015). Its malleability, ductility, corrosion resistance, low melting point, and abundant availability are the reasons lead is used till today, even though its accumulation is hazardous (Wani & Usmani, 2015). Being non-biodegradable in nature, the removal of lead from the environment is inevitable. Lead toxicity and lead accumulation in humans is one of the major health concerns. While occupational causes like dermal contact and inhalation contribute to the indirect intake of lead in humans, consumption of Pb contaminated food and water are direct sources of accumulation. Acute Pb toxicity leads to dysfunction of the kidney, reproductive system, and brain while chronic damages are caused to the CNS and PNS. Lead also inhibits the synthesis of hemoglobin. Pregnant women with low calcium, iron or zinc levels are prone to the effects of lead accumulation (Kwong *et al.,* 2004). Lead is a poisonous metal and disturbs the functions of almost every organ in the human body. Common symptoms observed are behavioral changes, lowered IQ, slow learning in children, diarrhea, anemia, skin allergies, kidney malfunctioning and many more. Also, the Pb interacts with mechanisms and functions of the male reproductive system and affects the sperm count (B. Quintanilla-Vega *et al.*, 2000). Even low levels of Pb in the kidney cause chronic renal malfunctioning. It is found that Pb interferes with the activities of several enzymes, delta-aminolevulinicacid dehydratase (ALAD), ferrochetase catalase, superoxide dismutase (SOD) and many more. Lead-induced oxidative stress increases radical production damaging the cell membranes, cell functions and DNA (Hsu and Guo, 2002; Ahamed and Siddiqui, 2007).

MATERIALS AND METHODS:

Location of Study/Study Area:

The study was carried out in Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island Amasomma, Bayelsa state.

Procurement of Coco Nucifera Extract and Lead Acetate:

The seeds of the plant Coco Nucifera were obtained from Amasomma, Southern Ijaw LGA, Bayelsa State.

Animal Housing:

Twenty-eight albino rats weighing between 130.58g+ 179.6g- were used for this study. These rats were obtained from the Animal house of the Pharmacology Department of Niger Delta University, Bayelsa State, Nigeria. They were housed under standard condition of temperature (27- 20C) with twelve hours light and dark periodicity. These animals were housed in clean gated in groups and fed on standard feed pellets (Guinea feed Nigeria Plc) and clean water ad libitum throughout the duration of the study. ACC limitation was for two weeks. Animals were handled in the study according to institutions guidelines for experiments involving the use of animals.

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Experimental Design:

The rats were weighed and divided into five groups. The duration of this study was four weeks, and the animals were allowed to acclimatize for two weeks. After the acclimatization period 28 rats were randomly divided into 5 groups.

Lead Acetate:

Lead acetate amehydrate, Molychem (India) was purchased from K-Prime Medicals, Idumota, Lagos Island LGA, Lagos State.

Table Showing experimental designs in groups of the administration of extract and acetic acid.

GROUPS	Group 1 5	Group 2 6	Group 3 6	Group 4 6	Group 5 5
TREATMENT REGIME	Control (No administration)	Lead acetate only 2ml/kg	Lead acetate 2ml/kg and coconut water (1.3ml)	Lead acetate 2ml/kg and coconut water (0.9ml)	Coconut water only (1.1ml)

Protocol:

Title: Heamatoxylin and Eosin Staining Technique

Aim: To demonstrate the general tissue structure

Principle: Heamatoxylin is a basic dye which stains the acidic components of the cell (nucleus) while Eosin, the counterstain is an acidic dye that stains the basic components of the cell (cytoplasm)

Reagent composition:

A) Heamatoxylin

Saturated Aqueos Aluminium sulphate	750ml
Heamatoxylin	1.0g
1% iodine in 7% alcohol	50ml
Distilled water	250ml

B) 1% Eosin

Eosin	1g

Distilled water	100ml
Thymol as preservative	1 crystal

C) Scotch's tap water

Sodium bicarbonate	3.5g
Magnesium sulphate	20g
Tap water	100ml
Thymol as preservative	1 crystal

Procedure:

1. Tissue sections were brought down to water by dewaxing in xylene untill all the wax were removed properly then they were passed through descending grades of alcohol and then rinsed in water.
2. Sections were stained in Heamatoxylin for 10 minutes
3. Sections were rinsed in water
4. Sections were briefly differentiated in 1% acid alcohol and rinsed in water
5. sections were blued in scotch's tap water for 2 minutes
6. Sections were blued in scotch's tap water for 2 minutes
7. Sections were counterstained using 1% Eosin for 2 minutes
8. Sections were rinsed in water
9. Sections were later dehydrated in ascending grades of alcohol, cleared in xylene and mounted using dpx
10. examined under the microscope using x10 to focus and x40 to view

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RESULTS:

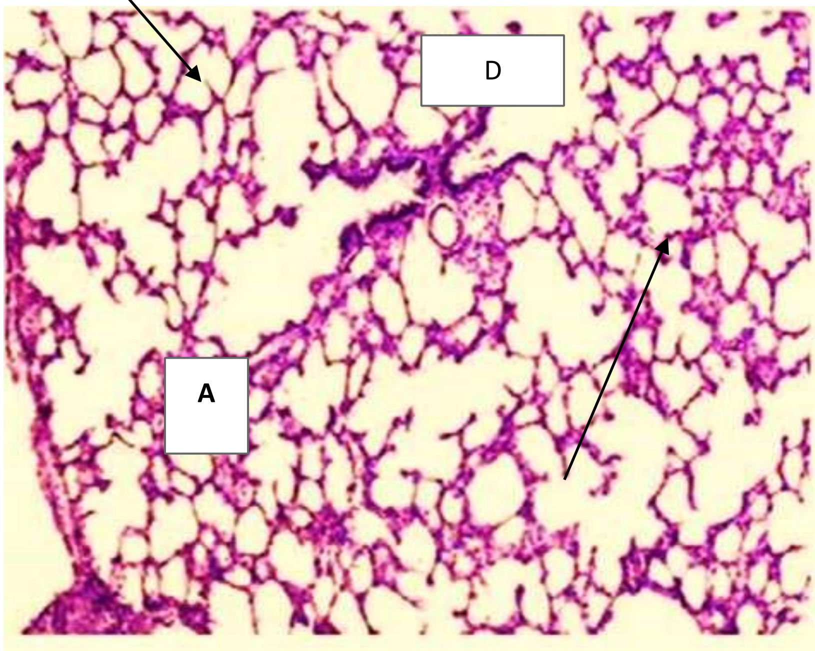

PLATE 4.1: Shows the Morphology of the lung (Control). Slide shows normal morphology of the lung, the alveolar sac (A), alveolar duct (D) the alveoli (A)and interalveolar septa(arrow). (X40)

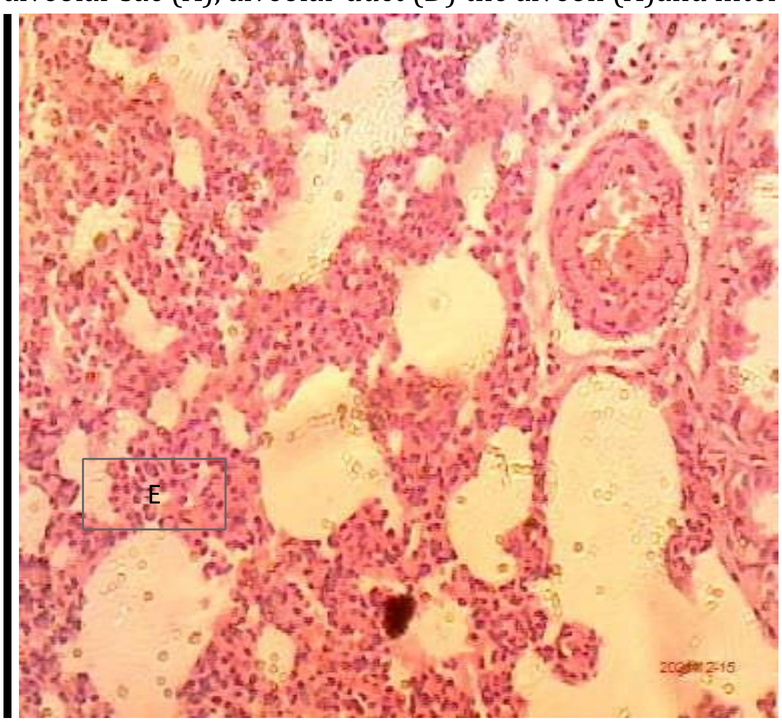

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PLATE 4.2: Shows the Morphology of the lung after the administration of Lead Acetate. Slide shows enlargement of the alveoli, thickening of interalveolar septa(E) with presence of inflammatory cells(I).(X10) H&E

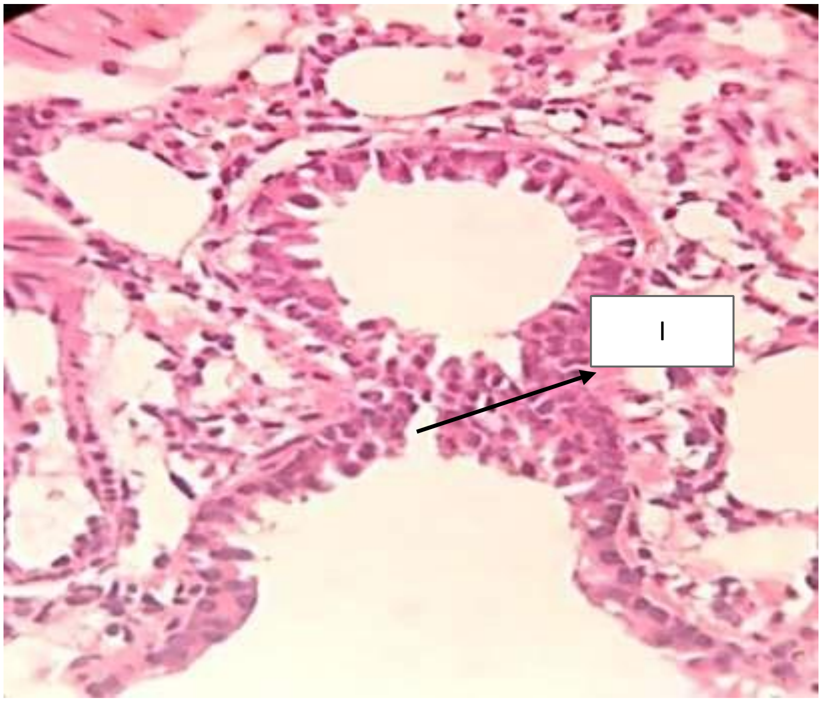

PLATE 4.3: Shows the Morphology of the lung after the administration of Lead Acetate and coconut water (high dose). Slide shows thickening of interalveolar septa with presence of inflammatory cells(I) (X40) H&E.

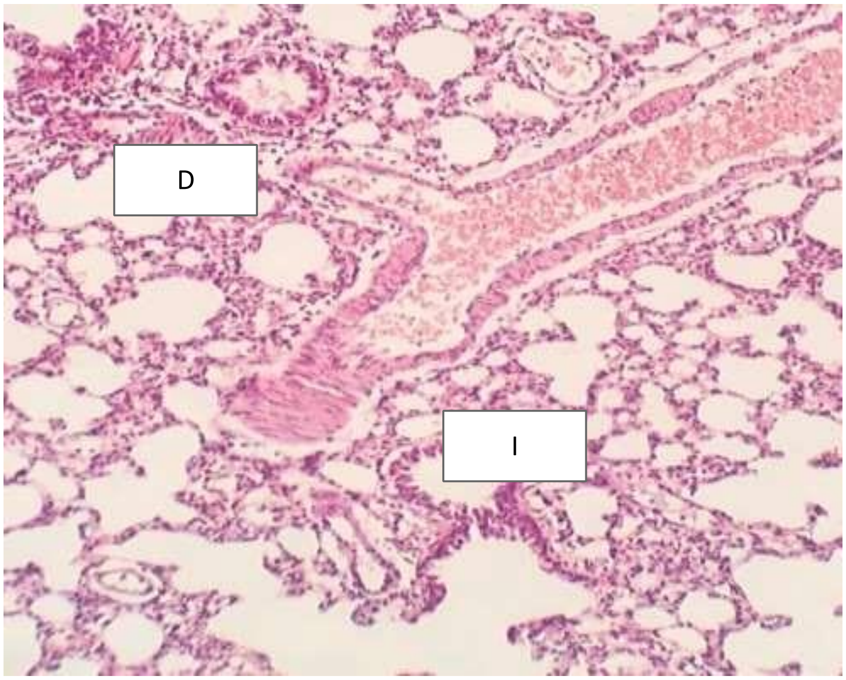

PLATE 4.4: Shows the Morphology of the lung after the administration of Lead Acetate and coconut water (low dose). Slide shows areas of hemorrhage (H) in the blood vessel, with presence of inflammatory cells(I), .(X10) H&E.

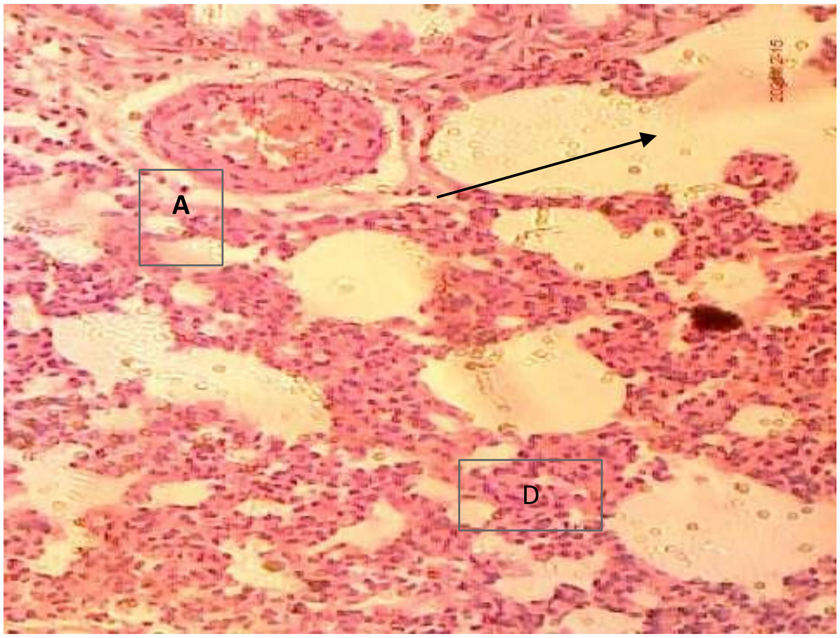

PLATE 4.5: Shows the Morphology of the lung after the administration of Coconut water. Slide shows normal morphology of the lung, the alveolar sac (A), alveolar duct (D) the alveoli (A)and interalveolar septa(arrow) (X40)

DISCUSSION AND CONCLUSION:

PLATE 4.1: Shows the Morphology of the lung (Control). Slide shows normal morphology of the lung,the alveolar sac (A), alveolar duct (D) the alveoli (A)and interalveolar septa(arrow).(X40).

PLATE 4.2 Shows the morphology of the lungs that lead acetate exposure elicits a distinct pathological reaction in lung tissue. The enlargement of the alveoli coupled with the thickening of the septa and the infiltration of inflammatory cells points to a disruption in normal lung architecture and function. These changes could compromise the efficiency of gas exchange, reflecting the potential for impaired respiratory function following exposure to toxic substances like lead acetate (X10) H&E.

PLATE 4.3: The most notable change observed on this slide is the thickening of the interalveolar septa. In a normally functioning lung, the interalveolar septa are thin walls that separate the tiny air sacs (alveoli) where gas exchange occurs. Their delicate structure is essential for rapid diffusion of oxygen into the blood and removal of carbon dioxide. However, in this treated sample, the septa appear thicker. Such thickening is often a hallmark of an inflammatory response or edema accumulation of fluid in the tissue triggered by an injurious stimulus. In this context, lead acetate is known for its toxic properties, which can damage tissue and provoke inflammation. The increased septal thickness may be due to a combination of inflammatory cell infiltration, deposition of extracellular matrix components, or even the early formation of fibrotic tissue.

One prominent feature noted is the presence of areas of hemorrhage, marked as "H," seen in the blood vessel regions. Hemorrhage here indicates that there has been bleeding or leakage of blood due to damage from the toxic agent. This may be a result of injury to the vascular walls, leading to a disruption of the normal barrier function. The hemorrhage suggests that the blood vessels are not intact and that their permeability has been compromised, which is a common finding in toxic lung injury. Another key observation is the infiltration of inflammatory cells, indicated by "I." The presence of these cells shows that the lung tissue is responding to the toxic insult by mobilizing the immune system to the site of damage. These inflammatory cells, which may include macrophages, neutrophils, and lymphocytes, aim to remove damaged cells and any harmful particles, but their accumulation can also contribute to further tissue injury if the inflammatory response becomes excessive.

PLATE 4.4: This displays lung tissue following exposure to lead acetate, revealing a clear pathological response. The slide demonstrates that the alveoli become enlarged, the interalveolar septa thicken, and inflammatory cells infiltrate the tissue. Together, these alterations indicate a disruption of the lung's normal structure and function, potentially hindering efficient gas exchange and leading to impaired respiratory performance as a result of toxic exposure (X10) H&E.

PLATE 4.5: Shows the Morphology of the lung after the administration of Coconut water. Slide shows normal morphology of the lung, the alveolar sac (A), alveolar duct (D) the alveoli (A)and interalveolar septa(arrow). (X40).

PLATE 4.6: This slide depicts the normal morphology of the heart, highlighting the intricate branching of cardiac myocytes. The cardiac muscle tissue is visualized in both transverse (labeled T) and oblique (labeled Q) sections, with the central nuclei clearly indicated by an arrow. The image is captured at 10× magnification.

Summary of Findings

Lung Morphology Observations:

Lead Acetate exposure resulted in alveolar enlargement, thickened interalveolar septa, and inflammatory cell infiltration, indicating lung tissue damage. Coconut Water (high dose) showed partial improvement, but inflammatory cells persisted, suggesting ongoing tissue repair.

Coconut Water (low dose) resulted in vascular hemorrhage, indicating incomplete protection against Lead Acetate toxicity while Coconut Water alone maintained normal lung morphology, suggesting a protective or neutral effect on lung tissue.

Heart Morphology Observations:

Unlike the lung, Lead Acetate exposure did not significantly alter heart morphology, suggesting cardiac resilience against lead toxicity. Coconut Water (high and low doses) did not induce major changes, indicating no adverse effects on heart tissue. Coconut Water alone maintained normal cardiac structure, reinforcing its safety in heart tissue. (Ajibogun & Oboma.,2013) A similar study by Otobele Akpesiri from the University of Benin, (Otobele *et al.,*2019) supports these findings. Akpesiri's research demonstrated that oral exposure to lead significantly increased lead accumulation in the heart, gut, kidney, and liver of rats, while treatment with coconut water lowered lead concentration. Additionally, coconut water helped remediate weight loss effects associated with lead exposure. The therapeutic effect was attributed to coconut water's antioxidant and scavenging properties, confirming its role in reducing bioaccumulation of lead and mitigating toxicity.

Similarly, the administration of Coconut Water at a high dose appears to partially mitigate lung damage, while lower doses exhibit vascular hemorrhage, suggesting that dosage plays a crucial role in its protective effects.

Another related study, by Enagbonma Ben Jesuorsemwen, further corroborates the beneficial effects of coconut water (Enagbonma *et al*.,2023). The research observed that lead exposure reduced RBC, MCV, MCH, MCHC, and RDW levels, potentially due to erythrocyte fragility and the inhibitory effects of lead on erythrocyte enzymes. When lead-exposed groups were treated with coconut water, their RBC and related values improved, likely due to the iron and folate content in coconut water, which supports red blood cell production and reduces oxidative damage.

Additionally, lead-fed rats exhibited increased WBC and lymphocyte levels, possibly due to inflammation caused by lead toxicity. However, these values declined in coconut water-treated groups, highlighting its anti-inflammatory, anti-thrombotic, and antioxidant properties.

The collective findings indicate that Lead Acetate toxicity significantly impacts lung and hematological parameters, while coconut water demonstrates protective and therapeutic effects against lead-induced damage. Its ability to reduce bioaccumulation, improve hematological markers, and mediate inflammation, reinforces its potential as a natural remedy, for lead poisoning. However, proper dosage is crucial, as lower doses may not provide full protective benefits.

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The collective findings indicate that Lead Acetate toxicity significantly impacts lung and hematological parameters, while coconut water demonstrates protective and therapeutic effects against lead-induced damage. Its ability to reduce bioaccumulation, improve hematological markers, and mediate inflammation, reinforces its potential as a natural remedy, for lead poisoning. However, proper dosage is crucial, as lower doses may not provide full protective benefits.

CONCLUSION

The findings suggest that Lead Acetate induces significant lung damage, characterized by alveolar enlargement, septal thickening, and inflammatory infiltration. However, heart tissue appears more resistant to Lead Acetate toxicity, maintaining normal morphology across all experimental groups.

Similarly, the administration of Coconut Water at a high dose appears to partially mitigate lung damage, while lower doses exhibit vascular hemorrhage, suggesting that dosage plays a crucial role in its protective effects.

These results highlight the potential therapeutic role of Coconut Water in reducing lung inflammation and structural damage caused by Lead Acetate. Further studies are needed to:

1. Investigate the biochemical mechanisms behind Coconut Water's protective effects.
2. Determine optimal dosage for mitigating heart and lung toxicity.
3. Explore long-term effects of Coconut Water on both lung and heart tissues.

RECOMMENDATION

This study provides valuable insights into the histopathological effects of Lead Acetate and the potential protective role of Coconut Water. While the lung tissue shows significant alterations, the heart remains structurally intact, suggesting organ-specific responses to toxicity. Future research should focus on molecular pathways involved in these changes and explore alternative protective agents for lead-induced toxicity.

CONFLICT OF INTEREST: There is no conflict of interest whatsoever in this research work.
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