

Research Article

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Effects of Concurrent Administration of Lead and Dioxin on The Liver of Adult Female Wistar Rat

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Abstract

Lead (Pb) and dioxin have the potential to cause damage to the liver by exposure. The current study was therefore designed to investigate the histopathological effects of lead (Pb) and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) (singly and in combination) on the liver of adult female rats. Thirty-two (32) female wistar rats were randomly divided into four groups (A – D) of eight (8) rats each. Group A rats served as control and were given distilled water, group B fed 7.0 mg/kg body weight of lead (Pb) only, group C fed 30 µg/kg body weight of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) only, group D fed 7.0 mg/kg body weight of lead (Pb) and 30 µg/kg body weight of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) for twentyeight (28) consecutive days. Experimental Animals were sacrificed on the 29th day by cervical dislocation. The liver was collected after sacrifice and fixed in 10 % formol calcium and subsequently processed for histological observation using Hematoxylin and Eosin staining principle. In co-exposed rats, hepatic tissue damages were significantly increased when compared to the individual toxic effects of lead (Pb) and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin). These toxicants exhibited synergism which was supported by histopathological studies. Result revealed that when the animals were co-exposed to lead (Pb) and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin), the hepatic tissues became more vulnerable. Thus, the chances of hepatotoxicity increase when the subjects are being exposed to both lead (Pb) and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) than either one of these toxicants alone. The chances of hepatotoxicity increase when the subjects are being exposed to both lead (Pb) and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) than either one of these toxicants alone. The distortions of the liver morphology may adversely affect liver functions and subsequently predispose to liver failure.

Keywords: lead; liver; wistar rat

Introduction

Lead is the most toxic heavy metal in the environment (Henretig, 2016). Lead exposure is widespread and has an impact on public health (Dart et al., 2014). It is used to produce many products, including lead crystal tools, printing pigments, hair coloring pigments, and cosmetics (Patrick, 2016). Exposure to lead occurs through lead-related professions and in industrial processes such as lead smelting, combustion, pottery making, paint, battery recycling dyes, book printing, house dust, and polluted soil (Levin et al., 2018). Occupational exposure to lead occurs in smelters, mines, and battery factories, as well as welding of lead-coated metal. The magnitude of the response to lead toxicity depends on the dose, age of the person exposed, duration of exposure, nutritional status and occupational exposure (Kosnett, 2015). Lead spreads to all organs and systems in the body, but the prime target of lead toxicity is the central nervous system (Ekong et al., 2016). It can affect the

neurological and biological functions of the animal body. It causes several problems in various organs, such as mental retardation, behavioral problems, violence, and growth delay, damage to the central nervous system and learning abilities, damage to sensory nerves, and impairment in cognitive function, hearing and vision (Patrick, 2016). Lead can cause adverse health effects in three ways inhalation, ingestion and skin contact (Mycyk et al., 2015). It also causes toxic effects on genes, especially in the brain, bone marrow, lungs, and liver (Mahaffey, 2016). Dioxins are permanent environmental pollutants that pose a potential risk to human health (Guo et al., 2019). Among all dioxin compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin is a highly toxic environmental contaminant (Knutsen et al., 2018). In the experimental animals exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin, various biochemical changes and diseases like dermal toxicity, immunotoxicity, hepatotoxicity, carcinogenesis,

teratogenicity, behavioral and endocrine changes are identified (Li et al., 2021). In addition to such diseases, exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin is determined to cause oxidative stress (Ishiniwa et al., 2013). Oxidative stress is defined as defects in the oxidant-antioxidant balance (Devito 2012). In an early study, oxidative stress is identified in various tissues exposed acutely to high doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (Mikolajczyk et al., 2021). It is reported that free oxygen species, deoxyribonucleic acid damage and lipid peroxidation increase in experimental animals exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (Guo et al., 2019). Most of the toxicological studies on water contaminating of permanent environmental pollutants deal with the single metal exposure. Little is known about the interaction among these metals due to their co-exposure through drinking water and their combined toxic effects on animals and humans. Therefore, the present study was to address interaction of metals by assessing the effects of exposure to combination of lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) on liver in rats.

The Liver

The liver is a reddish-brown gland located immediately inferior to the diaphragm, filling most of the right hypochondriac and epigastric regions. It is the body's largest gland. The liver has numerous functions. From their variety and importance, it is not hard to understand why such liver diseases as cirrhosis, hepatitis, and liver cancer are so serious and often fatal. Only one of these functions contributes to digestion; the secretion of bile (Saladin, 2018). Bile is a yellow-green fluid containing minerals, cholesterol, neutral fats, phospholipids, bile pigments, and bile acids. The principal pigment is bilirubin, derived from the decomposition of hemoglobin. Bacteria of the large intestine metabolize bilirubin to urobilinogen, which is responsible for the brown color of feces. In the absence of bile secretion, the feces are grayish white and marked with streaks of undigested fat (acholic feces). Bile acids (bile salts) are steroids synthesized from cholesterol. Bile acids and lecithin, a phospholipid, emulsify fat; breaking globules of dietary fat into smaller droplets with more surface area exposed to enzyme action. Emulsification greatly enhances the efficiency of fat digestion (Lan and Muralitharan, 2015).

Anatomy Of the Liver

The liver weighs about 1.36 kilograms (kg) and is located in the right upper quadrant of the abdomen, tucked against the inferior surface of the diaphragm (Elaine, 2016). The posterior surface of the liver is in contact with the right ribs 5–12. It is divided into two major lobes, the right lobe and the left lobe, which are separated by a connective tissue septum, the falciform ligament. Two smaller lobes, the caudate lobe and the quadrate lobe, can be seen from an inferior view. Also seen from the inferior view is the porta, which is the “gate” through which blood vessels, ducts, and nerves enter or exit the liver (Lan and Muralitharan, 2015).

Functions Of the Liver

The liver performs important digestive and excretory functions, stores and processes nutrients, detoxifies harmful chemicals, and synthesizes new molecules (Saladin, 2018). The liver produces and secretes about 600–1000 mL of bile each day. Bile (bīl) contains no digestive enzymes, but it plays an important role in digestion by diluting and neutralizing stomach acid and by dramatically increasing the efficiency of fat digestion and absorption. Digestive enzymes cannot act efficiently on large fat globules. Bile salts emulsify fats, breaking the fat globules into smaller droplets, much like the action of detergents in dishwater. The small droplets are more easily digested by digestive enzymes. Bile also contains excretory products, such as cholesterol, fats, and bile pigments, including bilirubin; a bile pigment that results from the breakdown of hemoglobin. Gallstones may form if the amount of cholesterol secreted by the liver becomes excessive and is not able to be dissolved by the bile salts (Saladin, 2018). Neural and hormonal stimuli regulate the secretion and release of bile. Bile secretion by the liver is stimulated by parasympathetic stimulation through the vagus nerve. Secretin, which is released from the duodenum, also stimulates bile secretion and release. Cholecystokinin stimulates the gallbladder to contract and release bile into the duodenum. In addition, most (90% of) bile salts are reabsorbed in the ileum. The blood carries the bile salts back to the liver, where they stimulate additional bile salt secretion and are once again secreted into the bile. The loss of bile salts in the feces is reduced by this recycling process (Lan and Muralitharan, 2015). The liver can remove sugar from the blood and store it in the form of glycogen. It can also store fat, vitamins, copper, and iron. This storage function is usually short-term. Foods are not always ingested in the proportion needed by the tissues. If this is the case, the liver can convert some nutrients into others.

For example, if a person eats a meal that is very high in protein, a large number of amino acids and only a small amount of lipids and carbohydrates are delivered to the liver (Saladin, 2018).

The liver can break down the amino acids and cycle many of them through metabolic pathways to produce adenosine triphosphate and to synthesize lipids and glucose. The liver also transforms some nutrients into more readily usable substances. Ingested fats, for example, can be combined with choline and phosphorus in the liver to produce phospholipids, which are essential components of cell membranes (Saladin, 2018). Many ingested substances are harmful to body cells. In addition, the body itself produces many by-products of metabolism that, if accumulated, are toxic. The liver is an important line of defense against many of those harmful substances. It detoxifies them by altering their structure, which makes their excretion easier. For example, the liver removes ammonia, a toxic by-product of amino acid metabolism, from the circulation and converts it to urea, which is then secreted into the circulation and eliminated by the kidneys in the urine. The liver also removes other substances from the circulation and excretes them into the bile (Lan and Muralitharan, 2015).

Literature Review

Dioxins

Dioxins, polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), are the most toxic group of environmental pollutants that have been described to date (Gotz et al., 2017). Chemically, dioxins consist of two aromatic rings linked via either one or two atoms of oxygen, and give rise, respectively, to polychlorinated dibenzofurans or polychlorinated dibenzodioxins. This extremely stable structure contains one to eight positions that can be chlorinated, which confers both high structural stability and extreme hydrophobicity. Depending on the number and position of chlorination ($P = 1-8$), the dioxin group includes 75 polychlorinated dibenzodioxins and 135 polychlorinated dibenzofurans congeners that vary significantly in terms of their overall toxicity (Ishiniwa et al., 2013). The number and position of chlorines in dioxin molecules affect their toxicity by modifying their shape, determining thus their binding ability to the AhR receptor (Pajurek et al., 2023). Typically, congeners with chlorine atoms substituted in the lateral 2, 3, 7 and 8 positions of the aromatic rings are

considered as the most toxic. Of these, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), with a toxic equivalency factor (TEF) of 1.0, is the most toxic congener of all the dioxins (Pius et al., 2020). Dioxins are formed as a by-product from incomplete thermic reactions, including industrial processes, combustion processes such as burning of household-, municipal- and medical waste, or burning fuels. Dioxins often occur as a mixture of chemical congeners in the environment and can act both synergistically, inducing a stronger toxic effect, or antagonize the effect of another (Fromme et al., 2016). Dioxins are formed as a by-product from incomplete thermic reactions, including industrial processes, combustion processes such as burning of household-, municipal- and medical waste, or burning fuels (Sindik et al., 2015). Dioxins released in the environment because of improper disposal are extremely persistent to degradation and can remain in both water and soils for years. Dietary intake of dioxins is the main exposure route for humans and animals, because of their lipophilic properties and the high accumulation potential. Dioxins bioaccumulate in marine foodwebs and seafood is the main source of exposure for humans (Pajurek et al., 2022). Dioxins are emitted into the environment from two main sources. The first one, which causes emission of only a small proportion of these chemical contaminants, is due to natural combustion-related process such as forest fires and volcanoes (Goto et al., 2017). Over the last decade, the incidence of forest fires has increased resulting in higher dioxin releases from this source (Wikoff et al., 2012). Secondly, dioxins are released into the environment from industrial sources, such as processes involving melting of metals, production of polyvinyl chloride plastics, treatment of white paper pulp with chlorine, the combustion of coal, natural gas and petroleum products and wastes, and the manufacture of chlorinated pesticides and herbicides for agriculture. The incineration of municipal and medical wastes as wastes from fossil fuels and fly ash storage are also important sources of dioxins (Zhang et al., 2016). Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans are also released from plastic bottles and containers exposed to microwave radiation or freezing, and from the vast and ever-increasing amounts of electronic wastes that are estimated to reach 52.2 million tonnes by 2021 (Sindik et al., 2015). Ironically, recycling methods in processing of so-called e-waste, such as manual disassembly, roasting, acid leaching and open burning

also result in the formation of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (Sindik et al., 2015). Finally, road transport emissions due to the combustion of carbon-based fuels are an important source of several halogenated organic pollutant, like polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans, polychlorinated biphenyls and polybrominated diphenyl ethers (PBDEs) (Goto et al., 2017).

Health Effects of Dioxins

Chlorinated dioxins are considered as one of the most toxic man-made substances, because of the low dose required to cause lethality (even though not leading to an instant death) (Pajurek et al., 2023). The 2,3,7,8-tetrachlorodibenzo-p-dioxin congener is used as a model compound since it is the most toxic congener (Devito, 2012). Exposure to dioxins can cause several serious health outcomes such as severe wasting, thymic atrophy, teratogenesis, reproductive effects, chloracne, immunotoxicity, enzyme induction, decreases in T4 and vitamin A, and increased hepatic porphyrins (Fromme et al., 2016). One effect of severe dioxin exposure is chloracne, a condition that is extremely persistent and easily identified. There are examples of chloracne which lasted for more than 30 years (Wikoff et al., 2012). According to Mikolajczyk et al. (2021), the biochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin can be grouped into three classes, Altered metabolism resulting from changes in enzyme levels. Altered homeostasis resulting from changes in hormones and their receptors. Altered growth and differentiation resulting from changes in growth factors and their receptors (Mikolajczyk et al., 2021).

The various symptoms of the diseases caused by exposure to chlorinated dioxins are mediated through the Aryl hydrocarbon (Ah) receptor pathway (Kannan et al., 2012). The Ah receptor is a ubiquitous, high affinity protein that responds to planar aromatic ligands (e.g. 2,3,7,8-tetrachlorodibenzo-p-dioxin) by forming an active complex. The activated complex binds to a specific position on deoxyribonucleic acid, and probably functions as transcriptional enhancers (Bi et al., 2020). Several parts of the normal growth, development and differentiation, as well as hypoxia, aging and circadian rhythms are regulated by the Ah receptor (Mikolajczyk et al., 2021). Dioxin is absorbed in the gastrointestinal tract and transported into the systematic circulation. It then binds into lipid rich tissue, accumulates and/or is eliminated. The elimination of dioxin is affected by the amount of

body fat (the less body fat the faster elimination) and metabolism. Degradation rate varies among species and even within species (Kulkarni, 2019). Chlorinated dioxins and brominated dioxins have many similar characteristics, and this also applies to their affinity to the Ah receptor (Fromme et al., 2016). Although the studies and knowledge of the toxic properties of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans are limited, the toxic properties shown e.g. lethality, wasting, thymic atrophy, teratogenesis, reproductive effects, chloracne, immunotoxicity, enzyme induction, decreases in T4 and vitamin A, and increased hepatic porphyrins – are the same as for chlorinated dioxins (Vanden-Berg et al., 2013). There are major challenges in assessing risks of chemicals, and usually there are a number of uncertainties involved. It is also complex to estimate the exposure of a substance, as it may involve different pathways, such as dietary, dermal, inhalation and the rate of this exposure (Devito, 2012). To be able to conduct risk evaluations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, the method of toxic equivalence factors was developed (Wikoff et al., 2012). The model is based on the relationship between congeners and their ability to mediate biological and toxic effects in various in vitro and in vivo test systems (Kulkarni, 2019). In order to grade the toxicity of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, relative potency factors were determined for individual polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans congeners. The relative potency factors estimate a relative potency compared with 2,3,7,8-tetrachlorodibenzo-p-dioxin, the prototypical congener for this class (Rovira et al., 2010). According to Kannan et al. (2012), the toxic equivalence factor values are estimated according to the following guidelines

Toxic equivalence factor values have been calculated by comparing the ratio of the molar dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin to produce a 50% effect for a given toxic or biochemical end point to the molar dose of the chemical required to generate a 50 % effect. A panel of experts assesses the relative potency factor values and gives the substance a specific toxic equivalence factor value, which is used to more easily compare the toxicity of different dioxins. The toxic equivalence factor values and method have been evaluated and corrected since the late 1980s (Wikoff et al., 2012). In a mixture of different congeners, it is

relevant to provide an estimate of the total potential toxicity, and because of that the term toxic equivalent quantity (TEQ) was introduced. With 1,2,3,7,8-tetrachlorodibenzo-p-dioxin being the model congener, the total potency of a mixture of dioxins can be expressed as $TEQ = \sum (Compound_1 \times TEF_1 + Compound_2 \times TEF_2 + \dots + Compound_n \times TEF_n)$ (Kannan et al., 2012).

The toxic equivalence factor model has its limitations and challenges. The toxic equivalence factor methodology uses a single toxic equivalence factor value for all end points and for all mammals, including humans. Some argue that it is unlikely that a single factor can describe the relative potency of a chemical for all end points and all species. In addition, there is a discussion about the fact that the toxic equivalence factor methodology only provides an estimation of the potential health effects of dioxin or dioxin-like chemical. It does not take into account exposure to any other chemicals, and the health effects different combinations may cause (Devito, 2012). On the basis of current knowledge, for human risk assessment it is recommended to use similar toxic equivalent factors for both brominated and chlorinated congeners (Kulkarni, 2019).

Tetrachlorodibenzo-P-Dioxin (Tcdd)

2,3,7,8-tetrachlorodibenzo-p-dioxin is the most toxic and well-studied halogenated aromatic hydrocarbons and serves as the prototype for understanding how the halogenated aromatic hydrocarbons family of chemicals elicits toxicity (Barone et al., 2019). The basic chemical structure of all dioxins consists of two benzene rings connected by two oxygen atoms and substituted with four to eight chlorine atoms. The various positions of the chlorine atoms give rise to up to 75 dioxin congeners (Zhang et al., 2016). 2,3,7,8-Tetrachlorodibenzo-p-dioxin contains chlorine atoms at positions 2, 3, 7, and 8 on the benzene rings. 2,3,7,8-tetrachlorodibenzo-p-dioxin is often found in complex mixtures of halogenated aromatic hydrocarbons, and it can persist in both environmental and biological samples that are exposed to such mixtures. In the environment, 2,3,7,8-tetrachlorodibenzo-p-dioxin is primarily degraded by photolysis, which occurs when ultraviolet light (in the presence of a hydrogen donor) splits chlorine atoms off of 2,3,7,8-tetrachlorodibenzo-p-dioxin (Goto et al., 2017). However, the penetration of ultraviolet light into soil is typically shallow, which contributes to the long half-life of 2,3,7,8-tetrachlorodibenzo-p-dioxin in soil, which can be 10

to 100 years (Sindik et al., 2015). 2,3,7,8-tetrachlorodibenzo-p-dioxin also persists in the human body because there is no process for metabolizing it. Adipose tissue and liver are major storage sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin (Mikolajczyk et al., 2021). The half-life in the body is dependent on dose and body composition, and high amounts of body fat led to increased persistence. Recent studies report that the half-life in humans is approximately 1 to 2 years (Bi et al., 2020).

2,3,7,8-Tetrachlorodibenzo-P-Dioxin Toxicity

In animals, the toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin include endocrine alterations, developmental and reproductive toxicity, hepatotoxicity, immunosuppression, carcinogenesis and death (Zhang et al., 2016). Studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in humans have been primarily based on accidental exposures in which 2,3,7,8-tetrachlorodibenzo-p-dioxin was found as part of a complex mixture (Fromme et al., 2016). The most appropriate way to estimate the potential health risk of mixtures of dioxins is to express the toxicity as if a chemical mixture under study were pure 2,3,7,8-tetrachlorodibenzo-p-dioxin (Barone et al., 2019). The total dioxin toxic equivalency (TEQ) value has been adopted internationally to express the toxicity of mixtures of dioxins. The total dioxin toxic equivalency equals the toxic equivalency factor, which expresses the toxic potencies of halogenated aromatic hydrocarbons relative to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin, multiplied by the weight of the compound (Vanden-Berg et al., 2013). In the general population, it is estimated that the mean 2,3,7,8-tetrachlorodibenzo-p-dioxin total dioxin toxic equivalency level is 6 ng/kg in adipose tissue and 2 ng/kg in serum (WHO, 2010). Current mean serum lipid 2,3,7,8-tetrachlorodibenzo-p-dioxin levels are estimated to be between 0.5 and 1 ng/kg in the general population (Barone et al., 2019). These levels are within the margins of exposure limits for some toxicological outcomes of 2,3,7,8-tetrachlorodibenzo-p-dioxin, such as developmental reproductive toxicity, which is associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin body burden levels as low as 0.1 to 8 ng/kg (Devito, 2012). The observation of toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin at these low exposure levels underscores the importance of understanding mechanisms of 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity in order to adequately assess human health risks (Li et al., 2021).

Sources of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin

2,3,7,8-tetrachlorodibenzo-p-dioxin is typically produced as an unintentional byproduct of industrial manufacturing and combustion processes. A classic historical example of unintentional 2,3,7,8-tetrachlorodibenzo-p-dioxin production occurred during the manufacturing of the herbicide Agent Orange, which was used as a defoliant during the Vietnam War from the early 1960s to the early 1970s (Gonzalez and Domingo, 2021). Agent Orange was a 50/50 mixture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and dichlorophenoxyacetic acid (Kannan et al., 2012). 2,4,5-Trichlorophenol serves as the raw material to produce 2,4, 5-trichlorophenoxyacetic acid, and 2,3,7,8-tetrachlorodibenzo-p-dioxin was produced as a byproduct of this reaction.

2,4,5-trichlorophenoxyacetic acid was further used to produce other herbicides. Although the quantity of 2,3,7,8-tetrachlorodibenzo-p-dioxin impurity was small in these products, their widespread use sometimes resulted in the release of 2,3,7,8-tetrachlorodibenzo-p-dioxin into the environment at levels that required clean up (Fromme et al., 2016).

In addition to the use of 2,3,7,8-tetrachlorodibenzo-p-dioxin contaminated herbicides and other products, industrial accidents represent another source of human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. For example, in 1976, in the town of Meda, Italy, an explosion at a chemical manufacturing plant produced a chemical cloud that was blown by the wind to the south. This cloud, which contained 2,3,7,8-tetrachlorodibenzo-p-dioxin and a mix of other toxic chemicals, fell on the town of Seveso, where it contaminated soil, killed small animals, and caused dermal lesions in exposed humans. From the most contaminated areas, 730 people were eventually evacuated (Gonzalez and Domingo, 2021).

Blood levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin in random samples of inhabitants in the most contaminated zones were between 9.8 and 89.9 ppt (Bi et al., 2020). Another example of human exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin was in the early 1970s in the town of Times Beach, Missouri. A contractor used 2,3,7,8-tetrachlorodibenzo-p-dioxin contaminated oil from a chemical manufacturing plant to spray unpaved streets to control dust (Kannan et al., 2012). By 1982, the town of Times Beach was evacuated and eventually disincorporated. It was not until 2001 that the Times Beach site was determined to no longer pose a significant threat to public health or the environment (Guo et al., 2019). Since the mid-1980s, when it became apparent that

dioxins were a public health issue, lower amounts of dioxins have been entering the environment due to the abandonment of chlorinated phenol chemistry by large sectors of the chemical industry (Fromme et al., 2016).

Currently, the combustion of waste products particularly municipal waste and backyard burning of household waste is the prominent source of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the environment (Knutsen et al., 2018). These wastes often contain chlorine-based plastics, which form 2,3,7,8-tetrachlorodibenzo-p-dioxin during combustion. Once released into the atmosphere, 2,3,7,8-tetrachlorodibenzo-p-dioxin adsorbs onto dust particles and settles onto vegetation and in bodies of water, and accumulates in the soil. Livestock are exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin through consumption of contaminated vegetation and feedstock, whereas humans are primarily exposed to dioxin through the consumption of 2,3,7,8-tetrachlorodibenzo-p-dioxin laden animal products (Fromme et al., 2016). Because it is lipophilic and poorly metabolized, 2,3,7,8-tetrachlorodibenzo-p-dioxin partitions into the lipid compartments of organisms and bioaccumulates in the food chain. Therefore, the more animal fats consumed, the greater the risk of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Lead

Lead is the most important toxic heavy element in the environment. Due to its important physicochemical properties, its use can be retraced to historical times. Globally it is an abundantly distributed, important yet dangerous environmental chemical (Mahaffay, 2016). It's important properties like softness, malleability, ductility, poor conductivity and resistance to corrosion seem to make difficult to give up its use. Due to its non-biodegradable nature and continuous use, its concentration accumulates in the environment with increasing hazards.

Human exposure to lead and its compounds occurs mostly in lead related occupations with various sources like leaded gasoline, industrial processes such as smelting of lead and its combustion, pottery, boat building, lead based painting, lead containing pipes, battery recycling, grids, arm industry, pigments, printing of books, etc. Though its widespread use has discontinued in many countries of the world, it is still used in many industries like car repair, battery manufacturing and recycling, refining, smelting, etc. Lead is a highly poisonous metal affecting almost

every organ in the body. Of all the organs, the nervous system is the mostly affected target in lead toxicity, both in children and adults. The toxicity in children is however of a greater impact than in adults. This is because their tissues, internal as well as external, are softer than in adults. Long-term exposure of adults can result in decreased performance in some tests of cognitive performance that measure functions of the nervous system. Infants and young children are especially sensitive to even low levels of lead, which may contribute to behavioural problems, learning deficits and lowered intelligence quotient (Rubin and Strayer, 2018). Long-time exposure to lead has been reported to cause anaemia, along with an increase in blood pressure, and that mainly in old and middle-aged people. Severe damage to the brain and kidneys, both in adults and children, were found to be linked to exposure to heavy lead levels resulting in death. In pregnant women, high exposure to lead may cause miscarriage. Chronic lead exposure was found to reduce fertility in females (Sokol and Berman, 2015). Blood disorders and damage to the nervous system have a high occurrence in lead toxicity.

Signs And Symptoms of Lead Poisoning

Lead poisoning causes a variety of symptoms, including abnormal behaviour which varies from person to person, while time of exposure plays an important role (Kosnett, 2015). It usually takes weeks to months to build up the manifestations in the body by chronic lead exposure; however acute symptoms and signs also occur from short time intense exposures (Dart et al., 2014). Organic lead is perhaps more toxic than inorganic lead because of its lipid soluble nature, which results in rapid consequences (Timbrell, 2018). However, the lead levels at which signs and symptoms appear vary widely, depending on unknown characteristics of each individual (Bellinger, 2014). Blood lead levels from 25 and 60 µg/dL give rise to neuropsychiatric effects such as delayed reaction times, irritability, and difficulty in concentrating, as well as slowed down motor nerve conduction and headache (Merill et al., 2017). Anaemia may appear at blood lead levels higher than 50 µg/dL (Merill et al., 2017). In adults, abdominal colic, involving paroxysms of pain, may appear at blood lead levels higher than 80 µg/dL (Kosnett, 2015). High blood lead levels which exceed 100 µg/dL cause very severe manifestations, like signs of encephalopathy (condition characterized by brain swelling) accompanied by increased pressure within the skull, delirium, coma, seizures, and headache (Henritig,

2016). However, such manifestations appear in children at lead levels of 70 µg/dL and more. Central nervous system and neuromuscular manifestations usually result from intense exposure, while gastrointestinal features usually result from exposure over longer periods (Brunton et al., 2017). Signs and symptoms of chronic exposure include loss of short-term memory or concentration, depression, nausea, abdominal pain, loss of coordination, and numbness and tingling in the extremities (Patrick, 2016). Fatigue, problems with sleep, headaches, stupor, slurred speech, and anaemia are also found in chronic lead poisoning (Pearce, 2017). Children with chronic poisoning generally show aggressive behaviour and refuse to play.

Effects Of Lead Poisoning

All along human history, lead poisoning has been reported to have severe effects. Occasional lead poisoning was found to be caused by lead salts used in pottery glazes leached by acidic fruit juices. Beethoven's death has been treated in various reports. Many of them have concluded that he died because of the toxic doses of lead-based treatment administered by his doctor. Analysis of his hair was found to contain elevated levels of lead (Mai, 2016). It is also assumed that in the eighteenth and early nineteenth century lead was illegally added to wine both as a sweetener and to make it appear fresh (Mai, 2016). Lead poisoning is believed to be primarily responsible for the collapse of the Roman Empire, in which lead acetate was used as a sweetener of wine. Its prolonged use was considered to have caused dementia to many Roman emperors. Lead poisoning has also been found to be the cause of anaemia in a number of cases as lead inhibits uroporphobilinogen synthase and ferrochelatase, preventing both uroporphobilinogen formation and the incorporation of iron into protoporphyrin IX, which prevents heme synthesis (Cohen et al., 2015) or causes ineffective heme synthesis and subsequently microcytic anaemia. One of the mechanisms by which lead interferes with cognition is that it acts as calcium analogue which interferes with ion channels. It has been observed that Pb^{2+} is a potent reversible and selective blocker of voltage-dependent calcium channels at low concentrations (Busselberg et al., 2013). In a recent study, the authors showed that the toxic effect on blood cells of rats caused by lead nitrate was alleviated by sodium selenite. They also showed that effects of lead nitrate were more harmful in diabetic than in non-diabetic rats (Bas et al., 2015). Oxidative stress

was studied by low level lead exposure in first grade Uruguayan children, suggesting its potentially adverse effects on oxidative stress (Roy et al., 2015). Impaired respiratory function was observed in workers exposed to lead with elevated blood lead concentration and zinc protoporphyrin concentration (Jurdziak et al., 2015). In a recent study lead and cadmium contents were investigated in counterfeit cigarettes seized in the United States by various law enforcement agencies. Both lead and cadmium levels were found to be markedly higher than in their genuine equivalents. The results suggest the possibility of a higher risk of serious consequences to public health caused by counterfeit cigarettes (He et al., 2015).

Possible Pathway of Lead Poisoning

Poisoning due to lead occurs mainly by ingestion of food or water contaminated with lead. However accidental ingestion of contaminated soil, dust or lead based paint may also result in poisoning. Lead is thought to be quickly absorbed in the blood stream and is believed to have adverse effects on certain organ systems like the central nervous system, the cardiovascular system, kidneys, and the immune system (Bergeson, 2018). Most pharmaceutical companies have set a limit for maximum daily intake of lead as 1.0 µg/g; however prolonged intake of even this low level of lead is hazardous to human beings. Occupational exposure also results in elevated blood lead levels. Increased blood levels are associated with delayed puberty in girls (Schoeters et al., 2018). There is no threshold value for the level of lead present in blood below which its concentration can be considered safe. Extremely low yet permanent levels of lead exposure were found to reduce the cognitive capacity of children (Needlemann et al., 2016). Dangers from lead poisoning due to paint pigments mainly in children have sharply reduced the use of lead in paints. However old houses may still contain substantial amounts of lead paint (Levin et al. 2018). This sometimes causes an accidental contamination of lead in children. In industrialized countries white lead paint has been completely withdrawn from sale, however yellow lead chromate is still in use. Old paint should not be stripped by sanding, as this produces inhalable dust (Marino et al., 2016). Traditional medicines were also found to contain heavy metals including lead. A number of diseases have been reported due to consumption of traditional medicine (Karri et al., 2018). Ayurvedic medicines are considered to be heavily contaminated with heavy metals. In one recent study the blood lead levels were

evaluated in consumers of ayurvedic medicines. Of the 115 participants 40 % were found to have an elevated blood lead levels of 10 µg/dL or above and 9.6 % had blood lead levels above 50 µg/dL (Breeher et al., 2015). Recently a patient taking Chinese folk remedies was reported to suffer from dysplastic changes in erythroid precursors due to lead poisoning (Lv et al., 2015).

Effects on Children

Pregnant women who have elevated blood lead levels are at a risk of premature birth or of babies with a low birth weight. The foetus may be adversely affected at blood lead concentrations well below 25 µg per deciliter (Bellinger et al., 2017). Blood lead levels in the neonate were found to be higher than simultaneous maternal lead levels (Bellinger, 2015). Emaciated women with substantial exposure to lead prior to pregnancy are considered to be at increased risk. Children have been repeatedly reported to be at higher risk for lead poisoning because their bodies are in a state of growth and development (Chisolm and Harrison, 2016). Moreover, the absorption of lead occurs more quickly in children than in adults. Children, due to their childish behaviour, are more prone to ingest and inhale dust contaminated with lead (Landrigan, 2012).

Effects of Lead Poisoning in The Body System Renal System

A number of studies found low level environmental lead exposure to be associated with accelerated deterioration of chronic renal insufficiency (Yu et al., 2013). Even at levels far below the limits of normal ranges in the general population, both blood lead level and blood lead burden were found to be increased, predicting accelerated progression of chronic renal disease. Wedeen et al. (2015) studied the effects of occupational lead hazards on patients. They observed tubular dysfunctions in patients who underwent biopsy. Such studies suggest that lead nephropathy may be an important occupational hazard. Excretion of the waste product urate was reported in lead poisoning, suggesting that, in which urate builds up in the body (Ekong et al., 2016). Prolonged exposure to lead is associated with increase in blood pressure and some studies showed a connection between lead exposure and coronary heart disease, heart rate variability, and death from stroke, but this evidence is limited. Chronic lead nephropathy occurred due to years of lead exposure manifested in kidney biopsy by moderate focal

atrophy, loss of proximal tubules and interstitial fibrosis (Benjelloun et al., 2017).

Reproductive System

The reproductive system of both females and males is affected by lead. In females' sperm count is reduced and other changes occur in the volume of sperm when blood lead levels exceed 40 $\mu\text{g}/\text{dL}$. Activities like motility and the general morphology of sperm are also affected at this level (Navas-Acien et al., 2017). The problems with the reproductivity of females due to lead exposure are more severe. Toxic levels of lead can lead to miscarriages, prematurity, low birth weight, and problems with development during childhood (Park et al., 2018). Blood lead levels in mothers and infants are usually similar as the lead present in mother blood passes into the fetus through the placenta and also through breast milk (Dart et al., 2014). As bones store the highest content of lead, if metabolic changes mobilize the lead from bones into the blood due to pregnancy, the lead toxic risks increase. However increased calcium intake during pregnancy can help mitigate this phenomenon. Low doses of lead were found to significantly reduce the number of sperms within the epididymis of mice, while high doses reduced both the sperm count and percentage of motile sperms and led to an increased percentage of epididymal abnormal sperms. Lead directly targets testicular spermatogenesis and also the sperms in the epididymis inducing reproductive toxicity (Wadi and Ahmad, 2019). In lead treated groups suppressed rates of serum testosterone, intratesticular sperm counts, and sperm production rates were reported (Apostoli et al., 2015).

Nervous System

The brain is the most sensitive organ to lead exposure (Cleveland et al., 2018). In a child's developing brain, synapse formation is greatly affected in the cerebral cortex by lead. Lead also interferes with the development of neurochemicals, including neurotransmitters, and organization of ion channels (Dart et al., 2014). Lead poisoning also causes loss of neuron myelin sheath, reduction in the number of neurons, it interferes with neurotransmission and decreases neuronal growth (Pearson and Schonfeld, 2013). The brain of adults exposed to increased lead levels during their childhood also shows a decreased volume, especially in the prefrontal cortex on magnetic resonance imaging (Cleveland et al., 2018). Lead is able to pass through the endothelial cells at the blood brain barrier because it can substitute for

calcium ions and be taken up by calcium-ATPase pumps, thereby interfering with synapse formation. Children with blood lead concentration greater than 10 $\mu\text{g}/\text{dL}$ are at higher risk for developmental disabilities (Brunton et al., 2017). The effect of lead on children's cognitive abilities take place at very low levels. There is apparently no lower threshold to the dose-response relationship below which lead exposure is treated as safe (Meyer et al., 2013). Blood lead levels lower than 5 $\mu\text{g}/\text{dL}$ were found to be associated with reduced academic performance (Bellinger, 2018). Blood lead levels below 10 $\mu\text{g}/\text{dL}$ were reported to be associated with lower intelligence quotient and behaviour problems such as aggression, in proportion with the given blood lead level. Between the blood lead levels of 5 and 35 $\mu\text{g}/\text{dL}$, an intelligence quotient decreases of 2–4 points for each $\mu\text{g}/\text{dL}$ increase were reported in children (Brunton, 2017). Increased blood lead levels are also associated with a decrease in cognitive performance and with other psychiatric conditions like depression and anxiety (Kosnett, 2015). An increase in the blood lead levels from 50 to about 100 $\mu\text{g}/\text{dL}$ in adults was found to be associated with more severe conditions, like permanent impairment of central nervous system function (Bellinger, 2018). The hippocampus is a part of the brain involved in learning and memory (Nedlemann, 2014). The main reasons for lead interfering with learning particularly in children is that it damages the cells within the hippocampus. In rats exposed to lead, structural damage such as irregular nuclei and denaturation of myelin were reported (Mycyk et al., 2015).

Materials and methods

Experimental animals

Female *Rattus norvegicus* (albino rats) were chosen for this study. They were kept at the animal house section of Department of Biological Sciences, Federal Polytechnic Offa, Kwara State, Nigeria. Randomized block design was used in this study. Thirty-two (32) apparently healthy adult female albino rats were incorporated in the study. They were kept to acclimatize for fourteen (14) days before treatment and weigh about 179–184 g.

Housing Conditions

The animal house was free of pathogens and other extraneous factors with restricted access. The rats were kept in cages and marked respectively for identification. The animal room's temperature was

maintained at about 22 °C ($\pm$ 3°C) and humidity of at least 30 %. With respect to lightning, the pattern was 12 hours light and 12 hours dark. They were fed with a conventional laboratory diet and water ad libitum. There was adherence with the existing protocols for the use of lab animals strictly.

Drug Source

Lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) were purchased from Yomi-Esthyon Enterprise, Nigeria. All other chemicals were obtained from Sigma Chemical Company (Poole, Dorset, United Kingdom). Other chemicals and solvents used in this study were of analytical grade.

Animal Grouping and Treatment

Female albino wistar rats were randomly divided into four (four) groups (A-D) of eight (8) rats each and the treatment protocol was for twenty-eight (28) consecutive days in a single dose as follows;

Group A: The control groups were given ad libitum access to mineral water in 100 ml bottle/day for 28 consecutive days.

Group B: Given lead acetate in a dose of 7 mg/kg body weight by orogastric tube once daily for 28 days.

Group C: Given dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in a dose of 30 µg/kg body weight by orogastric tube once daily for 28 consecutive days.

Group D: lead acetate in a dose of 7 mg/kg body weight and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in a dose of 30 µg/kg body weight by orogastric tube once daily for 28 consecutive days.

Termination Of Treatment

Twenty-four hours after the last day of administration, the animals were weighed and were sacrificed using cervical dislocation. After dissection of the abdomen, the liver was removed and fixed in 10 % formol calcium for 24 hours.

Preparations Of Chemicals

Formol calcium: 100 ml of 40 % formaldehyde, 9 g Calcium chloride and 100 ml of distilled water.

Xylene

Graded alcohol

50 % alcohol: 50 ml of absolute alcohol + 50 ml of distilled water.

70 % alcohol: 70 ml of absolute alcohol + 30 ml of distilled water.

90 % alcohol: 90 ml of absolute alcohol + 10 ml of distilled water.

100 % alcohol: Absolute alcohol.

Glycerin

Fixation

The liver tissues were fixed in formol calcium. All tissues were fixed for 24 hours at room temperature. This prevents dehydration and decomposition of the liver tissues.

Dehydration

The recommended procedure of Pattanayak and Majumder (2009) was adopted. All tissues were dehydrated through ascending grades of alcohol by immersion.

Procedure

50% alcohol	1 hour
70% alcohol	1 hour
90% alcohol	1 hour
Absolute alcohol 1	1 hour
Absolute alcohol 2	1 hour

Clearing

The dehydrated tissues were cleared in xylene as follows;

Absolute alcohol and xylene	1 hour
Xylene I	1 hour
Xylene II	1 hour

Impregnation And Infiltration

Molten paraffin wax (melting point 56 °C) was used for infiltration. The wax was placed in an electrically heated, thermostatically regulated oven. The temperature of the oven was maintained at 60 °C. Infiltration was done twice for 1 hour each to remove the clearing agent prior to embedding.

Embedding

Thermostatically fixed (melting point 60°C) wax with 2 Lenkers (L-metal pieces) metal, each of appropriate size were used to form blocks of molten wax. The tissues were then immersed in the block and oriented to desired planes before the solidification in the wax on it.

Trimming

The blocks of tissues were trimmed to a suitable size and shape with a knife, leaving 1-2 mm of embedding medium on both sides. Thereafter the blocks were mounted on wooden blocks that can be held by the Olympus rotary microtome clamps during sectioning.

Sectioning

Each block was clamped on the microtome chuck and was brought a few microns to the knife edge by manual control. Ribbons of tissues were obtained using Olympus rotary microtome. The microtome was set at a thickness of 5 µm while the knife was placed

at an angle of 45 °C to the block of wax containing the tissue. Ice blocks were applied to the surface of the tissue blocks before and during sectioning to reduce the heat generated. After the trimming, a knife was used for the sectioning proper and ribbons were produced.

Floating Of Sections

The sections were allowed to flatten out in warm water bath to enhance the spreading out of sections.

Mounting Of Sections

To lift out the sections at an angle to the plane of the water, the glass slides were dipped vertically into the warm water.

Dewaxing

The slides were placed on a hot plate for a short period of time, this was to dry the slides. The slides were then stored in slide racks before staining.

Histochemical Techniques

Haematoxylin And Eosin

Technique: Cut sections were;

Dewaxed in two changes of xylene for 3 minutes each.

Hydrated in descending grades of alcohol.

Rinsed in distilled water.

Stained in Haematoxylin for 10 minutes.

Washed in running tap water for 3 minutes.

Differentiated in 1 % acid-alcohol for few seconds.

Blued in running tap water for 15 minutes.

Stained in 1 % alcoholic Eosin for 5 minutes.

Dehydrated rapidly in ascending grades of alcohol.

Cleared in xylene.

Mounted in Distrene Plasticizer Xylene (DPX) using clear cover slips.

Statistical Analysis

The statistical software package SPSS 10.0 for windows was used to analyze the data. Statistical analysis was undertaken by using student's t-test. $P < 0.05$ was considered statistically significant.

Results

Table 1: Table Showing the Body Weights of the Animals

Parameters	Group A	Group B	Group C	Group D
Initial weight	182.3 ± 0.52	178.4 ± 0.02	181.4 ± 0.05	176.1 ± 0.03
Final weight	224.5 ± 0.81	186.2 ± 0.08	192.2 ± 0.07	184.6 ± 0.07

Mean ± SEM; SEM = Standard Error of Mean; Key Group A = Control rats given distilled water throughout the study period. Group B = Experimental rats treated with lead acetate in a dose of 7.0 mg/kg body weight. Group C = Experimental rats treated with dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in a dose of 30 µg/kg body weight. Group D = Experimental rats treated with lead acetate in a dose of 7.0 mg/kg body weight and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in a dose of 30 µg/kg body weight

physical observation

During the period of administration, the physical activities of experimental animals (wistar rats) were observed. Rats in the control group were observed to exhibit normal physical activities, such as movement and playfulness, whereas animals in the treatment groups exhibited decreased activity. The hair of the rats of control group was healthy while in the treated groups, the hair was brittle, pale and scarce.

Body Weights Of the Animals

Table 4.1 revealed the weight changes in the control group and the treated groups. In this study, reduction of body weight was observed in treated rats in comparison with the control. There was significant difference in body weight of the treated rats compared to the control group. These results showed that the oral administration of dioxin and lead (Pb) for 4 weeks significantly reduced the weight of the rats.

Histopathological Findings

Histological section of liver of control rat treated with normal mineral water showed normal architecture of the liver tissue. The liver section was devoid of any cellular alterations. The central vein in liver was normal without any signs of inflammation, congestion, and vacuolization. The hepatocytes were normal with central nuclei (Figure 4.1A). The liver sections of group B which were treated with lead acetate only showed degenerative changes of the hepatocyte and dilation of the central vein as shown in Figure 4.1B. The liver sections of group C which were treated with dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) showed mild swelling of the hepatocytes in places with areas of necrosis, inflammatory leukocytic infiltration, and moderate congestion of central vein (Figure 4.1C). The liver sections of group D which were treated with lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) showed that the liver cells were degenerated with necrosis, severe congestion and hemorrhage hypertrophy of hepatocytes, hepatocyte swelling was observed (Figure 4.1D).

Microscopic Plates of The Liver

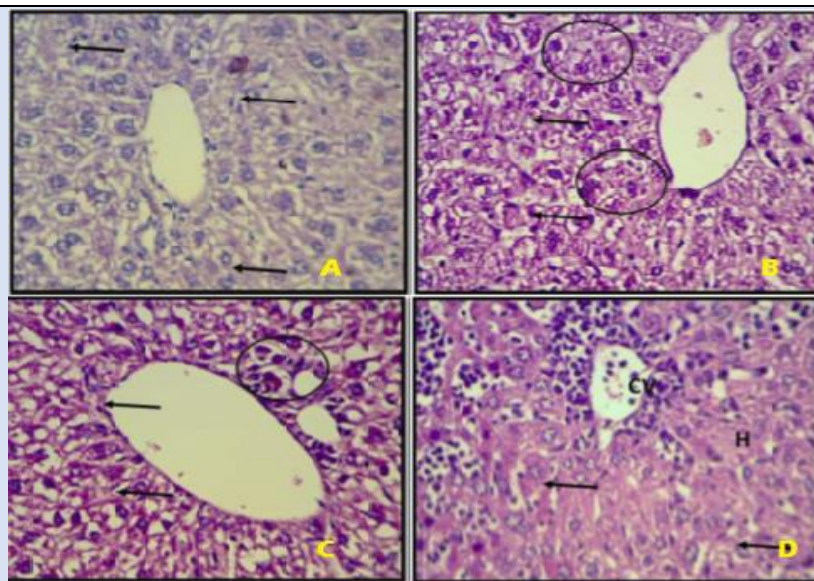


Figure 1: Photomicrography of the Experimental Rats.

(A) Photomicrograph of a Liver section of adult albino rats in control group (Group A), showing normal central vein (CV). The sinusoids are not occluded (S); the hepatocytes appear normal (H), and the central portal vein is devoid of any occlusion (CV). (B) Photomicrograph of a Liver section of rats treated with lead acetate in a dose of 7 mg/kg body weight (Group B), showing mild degenerative changes of the hepatocyte and dilation of the central vein. (C) Photomicrograph of a Liver section of rats treated with dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in a dose of 30 μ g/kg body weight (Group C), showing dilation of the central vein (arrows). (D) Photomicrograph of a Liver section rats treated with lead acetate in a dose of 7 mg/kg body weight and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in a dose of 30 μ g/kg body weight showing marked degeneration of hepatocytes (H) and occlusion of the central portal vein (CV) (H & E X400).

Discussion

Environmental pollution is the presence of a pollutant in an environment such as air, water, soil, and consequently in food which may be toxic and will cause harm to living things in the polluted environment. Lead and dioxin in the environment are considered the most dangerous toxins and pollutants that cause neurological disorders (Rubin and Strayer, 2018). In addition, dioxin and lead acetate caused liver toxicity in albino rat (Nault et al., 2021; Aldahmash and El-Nagar, 2016). The present study investigates toxicity of lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) in the histology of the liver of adult female albino rats. Body weight is important indicators of the toxic effect of any exogenous compound on the body. In the present study, lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) exposed rats registered less gain in body weight as compared with the control. This decrease in gain of body weight might be associated with lack of food consumption occurring due to loss of appetite or gastrointestinal disturbances (Bhattacharjee et al., 2016). Similar decrease in body weight has also been observed by Ciftci and Ozdemir

(2013) in rats exposed to dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin). Bhattacharjee et al. (2016) registered decrease in body weight in rats fed lead acetate. Dardouri et al. (2016) reported decrease in body weight of rats due to combined effects of Cadmium and Mercury in wistar rats.

Light microscopic examination of control group (group A) revealed normal hepatic architecture. Haematoxylin and Eosin (H & E) stained sections of liver of the control animals (group A) revealed normal characteristic of hepatic architecture; the hepatic lobules appeared to be made up of hepatocytes arranged in cords radiating from the central veins. The histological outline of the liver from the rats in the control group was well preserved. The liver sections of group B which were treated with lead acetate showed degenerative changes of the hepatocyte and dilation of the central vein as shown in Figure 4.1B. Mild swelling of the hepatocytes in places with areas of necrosis, inflammatory leukocytic infiltration, and moderate congestion of central vein were found in rats administered with dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) (group C). The liver cells were degenerated with necrosis, severe congestion and

hemorrhage hypertrophy of hepatocytes, hepatocyte swelling, was evident in rats fed with combination of lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) (group D). Histopathological alterations in the liver due to lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) administration causes liver damage as evidenced by the structural changes, i.e., disruption of the normal architecture, necrosis, cellular infiltration, and leading to pathological damage. Similar histopathological alterations in liver of rats after ingestion of lead acetate have been reported by Aldahmash and El-Nagar (2016). Nault et al. (2021) have also reported that dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) causes liver damage including changes in the normal histology, increased permeability and necrosis of hepatocytes. The liver is well-known target organ of the toxic impact regarding its function in biotransformation and excretion of xenobiotics. After entering uptake, liver is the first organ to be exposed by portal circulation. Hepatotoxicity is toxicity to the liver, bile duct, and gall bladder. The liver is particularly susceptible to xenobiotics due to a large blood supply and its role in metabolism. In this study, after oral administration of lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin), histopathological changes were observed in the liver of rats in all treatment groups compared with control group, most especially, in the rats co-exposed to lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) showed severe histopathological changes in the liver. The most pronounced effect of these metals was observed in the groups of rats co-exposed to lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin). The combined effects of different metals on the liver of experimental animals were studied by many investigators. These results are in accordance with the study of Bhattacharjee et al. (2016) who revealed that necrotic lesions, congestion areas, and occlusion of central vein were more prominent in the rat liver treated with combination of Lead, Cadmium and Arsenic than when exposed to single metal. Dardouri et al. (2016) reported that liver tissue was completely damaged and qualitative degenerative and necrotic changes were observed in almost all structures of the liver tissues after Cadmium and Mercury co-exposure in wistar rats. Ezedom et al. (2020) reported that wistar rat's liver co-exposed to Cadmium and Arsenic showed changes in hepatic trabecular structure and increased hepatocytes with cytoplasmic vacuoles. Results showed that lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-

dioxin) co-exposure induced drastic histopathological effects on the liver of rats. Lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) co-exposure causes liver damage which includes dilation of sinusoidal spaces with irregular nuclear shape, degenerative changes include binucleated cells, hypertrophy of hepatocytes and lymphocytic infiltration in the central vein.

Conclusion

The present study demonstrates the effects of lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) on the histology of the rat liver. It may be concluded from the study that exposure to combination of metals produced more deleterious effects than when exposed to single metal. The study revealed that lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) co-exposure caused deleterious effects on rat's liver which was evidenced in histological changes.

Recommendations

This study shows that lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) exposure is associated with deleterious effects in the liver. Thus, people should avoid exposure to lead acetate and dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) because it may have adverse effect on the histology of the liver.

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