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Hepatoprotective Potential of *Allium cepa* Bulb Extract in Mitigating Lead Toxicity in *Rattus norvegicus*

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Abstract
Rapid industrialization and agricultural expansions had redistributed the metals at regional as well as global levels with consequent environmental pollution producing deleterious effects on diverse living systems. Heavy metals are the most hazardous pollutants as they are non-degradable and undergo bioaccumulation. Lead is one of the major environmental contaminants, occurring in soil, sediments, air, and water. Lead toxicity is a significant public health issue, known to cause severe hepatotoxic effects through oxidative stress, inflammation, and cellular apoptosis. This study evaluates the bio-efficacy of Allium cepa (onion) extract in protecting against lead-induced liver damage in albino rats. Rats received lead acetate and onion bulb extract for 1,7, 14 & 21 days and samples were collected for analyses. Key parameters assessed were liver function biomarkers (ALT, AST, ALP, ACP, LDH), and histopathological changes in liver tissue. Liver injury is manifested by elevated levels of enzymes, as well as histopathological alterations, including massive hepatocyte degeneration and increased collagen deposition. The results revealed that Allium cepa extract significantly reduced liver enzyme levels and notable improvements in liver architecture, including reduced necrosis and inflammation. These findings suggest that Allium cepa extract may serve as a natural therapeutic agent for mitigating lead-induced hepatotoxicity, paving the way for further research into its clinical applications. This study highlights the potent hepatoprotective properties of Allium cepa, attributed to its rich antioxidant and sulphur compounds. Presently, the main objective of the study is to establish herbal dietary supplements as protective agents against heavy metal-induced toxicity that are safe, affordable, and easily available.

Keywords: Lead toxicity; Allium cepa; Hepatotoxicity; Oxidative stress; Rattus norvegicus; Extract

Introduction

Lead (Pb) is a non-essential trace element widely used in industries, such as battery manufacturing and recycling. It is a toxic heavy metal pollutant and its worldwide emission rate is very high. Lead Poisoning is the oldest known and most widely studied work and environmental hazard. In the past few decades, globalization and industrialization increased the use of heavy metals like lead in our day-to-day activities. According to NIOSH (National Institute for Occupational Safety and Health) more than ten million persons all around the world, are potentially exposed to lead in the workplace. In the last decade, this field had been prime attention for researchers (Lin and Aarts, 2012; Jarup, 2003).

Lead is an abundant, ubiquitous, dangerous, and important toxic environmental contaminant of global concern due to its significant role in modern industry and it is still being used recklessly. The total annual emission of lead by motor vehicles and industrial plants alone is more than half a million tons throughout the world and it can persist in the environment for 150 – 5000 Years. (Flora et al., 2008). Lead is exposed to living systems through air, water, soil, food, and consumer products. Common sources are lead acid batteries, paints & pigments, lead wires & pipes, metal recycling plants, foundries, smelting plants mines, radiation shields, ammunition, surgical equipment, jet –

engines, ceramic glazes, glass, plastic, rubber, welding, printing, toys, cosmetics, and solid waste combustion plants (Patrick, 2006). Children are more susceptible to lead poisoning. Young children absorb higher percentages of ingested lead: around 40-50% compared to 10% in adults. There are approximately ten million children aged between 1-5 years with blood lead levels above 5 µg/dl, the reference level (CDCP, 2012; Needleman, 2004).

Lead is a potent, systemic poison that serves no known useful function once absorbed by our body. Occupational and daily use of lead in houses is unavoidable. Lead poisoning accounts for about 0.6% of the global burden of disease (WHO, 2009). In 2004, the National Toxicology Program's Report on Carcinogens listed "Lead and Lead Compounds as "R" (reasonably anticipated to be human carcinogens). According to USEPA, only 15 µg/l lead is permissible in drinking water. FDA has set an action level of 0.5 µg/ml lead in consumer products. Today, paint intended for residential use is limited to 0.06% lead content. The use of lead solder and other lead-containing materials in connecting household plumbing to public water supplies has been banned by the EPA. Lead's toxic effects can occur at very low concentration levels in blood according to the U.S. Centers for Disease Control: for children 10 µg/dl and adults 25 µg/dl. Recent research indicates that lead is associated with neurobehavioral damage at blood levels of 5 µg/dl and even lower.

Absorption of lead occurs through inhalation and ingestion route or occasionally through skin contact. Lead from the atmosphere or soil ends up in groundwater or surface water. So drinking water is a potential source of lead exposure. About 15–50% of lead is exposed through the ingestion route and 95% of it enters blood stream representing a very high absorption rate. Lead inside the body interferes with a variety of body processes and thus becomes toxic to many organs and tissues including the liver, kidneys, bones, nervous system, and reproductive system. Interference with the nervous system causes behavioral changes resulting in neuropsychiatric disorders. The Centers for Disease Control (CDC), 2011 set the standard elevated blood lead level for adults to be 25µg/dl. However, for children, the number is set much lower at 5µg/dl. Lead is toxic to wildlife also.

In the late 19th century, lead toxicity was widely acknowledged as a neurotoxic that builds up in soft tissues and bones. It damages the nervous system, disrupts the activity of biological enzymes, and causes illnesses ranging from behavioural disturbances to brain damage. Additionally, it impacts the cardiovascular, renal, and general health systems (Boskabady et al., 2018). We evaluated lead exposure-induced liver damage based on liver function, including liver enzymes (AST, ALT, LDH and ALP). Chronic lead poisoning is the outcome of a cumulative effect caused by the human body's inability to quickly eliminate lead after exposure. Within four to six weeks, almost 99 percent of the lead in the blood enters erythrocytes and spreads to different degrees throughout the brain, liver, renal cortex, lungs, teeth, bones, and other tissues and organs (Hohnadel et al., 1973). Lead exposure over an extended period of time is associated with a number of malignancies, liver damage, cardiovascular illness, neurological diseases, and osteoporosis. Lead can also impact other bodily tissues (Alissa & Ferns, 2011). Although there has been much research on the connection between lead exposure and neurological problems, little is known about how lead exposure affects hepatotoxicity. We assessed how lead affected liver function. Histopathology and liver enzymatic markers were used to evaluate liver function.

Allium cepa, belonging to the Liliaceae family is a perennial herb with the stem in the underground bulb. It is a worldwide commonly cultivated crop known for a range of medicinal properties viz. anti-inflammatory, antioxidant, hypolipidemic, cardio-protective, anti-diabetic, hepato-protective, nephroprotective, and anticancer etc. (Chakraborty et al., 2022). Onion consists of a number of well-known vitamins (B2, C, and B1), microelements (selenium and potassium) phenols and flavonoids acting as antioxidant and phyto-protective agents. (Foo and Tristani, 2011)

Material and methods

Animal model

Eighty adult male Wister albino rats of almost the same age, weighing about 100 ± 10 g, were obtained from inbred colonies. Rats were housed under standard environmental conditions (25°C temp. and 12 hrs light/dark cycles) in well-ventilated cages, fed on standard rat chow and provided tap water *ad-libitum*. All the animal care and experiments were performed according to guidelines of institutional animal ethical committee (360/01/9/CPSEA/2001) under GLP.

Chemicals

The chemical selected for study was Lead acetate, which was of analytical grade purity and obtained commercially from Merck, India. Its LD_{50} was estimated by Log dose/Probit regression line

method and found to be 44.00 g/kg body weight by oral route. Sub-lethal dose ($LD_{50}/10$) was dissolved in 10 ml distilled water. (Finney, 1971)

Herbal extract

Bulb extract of *Allium cepa* was taken for experimental protective studies. For this purpose the outer scaly leaf of *A. cepa* was removed, the bulb thoroughly rinsed in a glass bowl containing tap water and finally in distilled water, then fleshy leaves were cut into pieces. 500 g of cut fleshy leaves in 250 mL distilled water was blended with an electric blender and filtered with a clean white cloth to obtain the fresh aqueous extract of *A. cepa*. The fresh aqueous filtrate extract was poured into a 100 mL Pyrex conical flask and stored in the refrigerator at -4°C until used. The extract was analyzed by Gas Chromatography/Mass Spectrometry. (Ajayi and Akinsanya, 2023)

Experimental design

All rats were assigned randomly into four groups with twenty rats each *viz.* acute (1d) and sub-acute (7, 14, and 21 ds). Sets were further divided into 4 groups – control (given water only), Lead treated, Allium + Lead treated and Allium treated. The designated sets/group and their respective doses are shown in Table–1.

At the end of 1st, 7th, 14th and 21st days of the experiment, the rats of respective sets were sacrificed to dissect their livers. All the livers were cut in two parts for histopathology and biochemical estimations respectively.

Biochemical estimation

One part of each liver was homogenized in 4°C cold physiological saline solution (pH = 7.4) in glass-Teflon potter homogenizer and centrifuged separately at 12000rpm for 1 hr. at 4°C. The clear supernatant was used for further experiments. Biochemical markers, the metabolic enzymes – AST, ALT, ALP and ACP and LDH were assessed by standard laboratory methods (Babson and Babson, 1973; Hilman, 1971; Reitmann and Franklin, 1957; Bessey et al., 1946).

Histopathological analysis

The other part of each liver was excised further in small pieces, fixed in 10% NBF (Neutral Buffer Formalin) solution for 24 h and washed with 70% ethanol. Tissues were then dehydrated using alcohol series from 70% to 100% alcohol and embedded in paraffin using an embedding machine. Paraffin blocks were sectioned using a rotary ultramicrotome, distributed onto glass slides, and then dried overnight. Slides were observed and photomicrographed (x400) under a light microscope after being stained with hematoxylin and eosin (H&E) dyes and mounting. (Palipoch and Punsawad, 2013)

Statistical analysis

The results were computed statistically using one-way ANOVA followed by SNK test for intergroup comparison and expressed as Mean ± SEM. The values were signified at 0.05 level. (Glantz, 1992)

Results

Table 2 shows that hepatic levels of metabolic enzymes (transaminases, phosphatases, and dehydrogenase) increase significantly ($p < 0.05$) after acute and sub-acute lead intoxication, when compared with control. Administration of Allium cepa extract before lead intoxication, significantly ($p < 0.05$) reduces the elevated level of transaminases within 7 days, while that of phosphatases and dehydrogenase within 21 days of treatment respectively. Further no changes were exhibited by the extract alone.

Liver sections of control and *A. cepa* treated rats show normal hepatic architecture with normal-sized central vein and sinusoids with intact strings of hepatocytes and Kupffer cells (Figure 1, 2). Whereas the sections of lead treated group show damaged architecture with congested sinusoids and central vein and degenerated hepatocytes (Figure-3). Hepatic sections of the group treated with lead and *A. cepa* both show distorted architecture with lesser congested sinusoids and central vein and moderate to little degenerated hepatocytes (Figure-4).

Discussion

One of the largest and most vital organs in the human body, the liver plays a key role in drug metabolism and detoxification. Worldwide, there is an increase in liver disease-related illnesses and deaths. Primordial form (inorganic) lead is often eliminated with urine, although ingested lead can also be released into bile, stomach fluid, saliva, and ultimately expelled through feces (Alhusaini et

al., 2019). Although lead poisoning in the neurological system has been studied the most in comparison to other systems, lead can permeate throughout the body and have hazardous effects.

Table 1. Acute and Sub-acute daily doses of Lead acetate and *Allium cepa* extract for *Rattus norvegicus*

Groups Sets	Group I (Control)	Group II (Lead treated)	Group III (*Allium* treated)	Group IV (*Allium* + Lead treated)
Set : 1 Acute (1d)	Water	4.4 mg/kg b.wt	500 mg/kg b.wt	500 mg/kg b.wt + 4.4 mg/kg b.wt
Set : 2 Subacute (7ds)	Water	0.63 mg/kg b.wt	500 mg/kg b.wt	500 mg/kg b.wt + 0.63 mg/kg b.wt
Set : 3 Subacute (14ds)	Water	0.31 mg/kg b.wt	500 mg/kg b.wt	500 mg/kg b.wt + 0.31 mg/kg b.wt
Set : 4 Subacute (21ds)	Water	0.21 mg/kg b.wt	500 mg/kg b.wt	500 mg/kg b.wt + 0.21 mg/kg b.wt

Hepatocytes contain a well-developed enzymatic system related to the metabolism of proteins, fats, carbohydrates, and xenobiotics. These enzymes are either present in cytosol or attached to biological membranes viz. plasma membrane, mitochondrial membrane, and lysosomal membrane (Martini, 1989). ALT is a cytosolic enzyme, while AST has two isozymes one being cytoplasmic and the other being bound to mitochondrial membrane. ALP is found firmly attached to plasma membrane and ACP is present within lysosomes (Luxton, 1999). ALT and AST, located in cytoplasm are specific indicators of cellular necrosis. They are released in circulation in response to cellular damage, thus increasing their level (Dama et al., 2011). ALT appears to reflect hepatic disease more specifically than AST and others, because of biological location of the enzyme. However, the assessment of AST and ALT along with ALP may reflect injury to liver (Ayalogu et al., 2001).

Table 2: Hepatic metabolic Enzyme Level of *Rattus norvegicus* after Lead acetate and *Allium cepa* extract Treatments

Hepatic Enzyme (IU/L)	Treatment days	Control	Lead treated	*Allium* + Lead treated	*Allium* treated
ALT	Acute (1 d)	74.33±1.20	84±1.00*	81±1.00*	73±0.58^{NS}
	Subacute(7 ds)	77.33±0.88	87±1.15*	80±1.00^{NS}	76±0.58^{NS}
	Subacute(14ds)	76±1.1	98±1.00*	79±1.15^{NS}	74±1.15^{NS}
	Subacute(21ds)	77±1.00	105±1.00*	79±0.58^{NS}	75±1.53^{NS}
AST	Acute (1 d)	111.33±1.45	119.33±1.20*	118.67±0.88*	109.33±1.20^{NS}
	Subacute (7 ds)	110±1.15	123±2.52*	115.33±1.77^{NS}	109±1.15^{NS}
	Subacute 14ds)	112.33±1.20	137.67±1.45*	116±1.53^{NS}	110±1.15^{NS}
	Subacute(21ds)	109±1.15	183±1.00*	113±1.33^{NS}	107±1.00^{NS}
ALP	Acute (1 d)	177.67±0.88	212.33±0.33*	202±0.58*	176±0.58^{NS}
	Subacute (7 ds)	174±0.58	208.33±0.88*	196±1.15*	171.83±0.44^{NS}
	Subacute(14ds)	174.4±0.76	217.2±2.31*	189.33±2.6*	174±1.15^{NS}
	Subacute(21ds)	181.67±0.88	221.67±1.45*	186±1^{NS}	183±.058^{NS}
ACP	Acute (1 d)	243.67±0.88	266±0.58*	254.33±1.10*	243±1.15^{NS}
	Subacute (7 ds)	241.33±0.88	268.67±0.67*	252.67±0.88*	240±0.58^{NS}
	Subacute(14ds)	242±1.53	273±0.58*	245.83±1.59^{NS}	239±1.00^{NS}
	Subacute(21ds)	244.33±0.88	288±0.58*	246.67±0.88^{NS}	242.33±0.88^{NS}
LDH	Acute (1 d)	83.33±0.61	142.73±1.46*	123.5±0.8*	81.83±0.9
	Subacute (7 ds)	83.67±0.67	148.66±1.45*	109.7±1.2*	83.33±0.8
	Subacute(14ds)	85.67±0.67	152.67±1.45*	96.83±0.6*	84.90±0.6
	Subacute(21ds)	87.53±0.52	156.1±1.10*	89.67±0.88	87.33±0.88

According to certain research, lead can potentially impact metabolism and cause liver illness. Actually, one of the primary organs where most metals accumulate after exposure is the liver (Fang et al., 2021). Some distortion in liver tissue includes damage to the membrane of the hepatocyte and organelles leading to swelling, damage, and necrosis (Al-Dbass, 2012; Lee et al., 2009). In this study photomicrograph of Allium + Lead treated group exerts the rejuvenating effect of A. cepa extract because of its antioxidant ingredients. Lead attacks functional macromolecules like lipids, proteins, and nucleic acids by causing inflammation, apoptosis, free radical production, and the depletion of antioxidant molecules (Abdel-Hamid et al., 2020). Its toxicity primarily stems from its capacity to substitute Pb^{2+} for other bivalent cations like Ca^{2+}, Mg^{2+}, and Fe^{2+} as well as monovalent cations like Na^{+}. This ultimately disrupts cellular signaling, protein folding, enzyme

regulation, maturation, ionic transport, oxidant-antioxidant balance, and inflammatory responses (Boskabady et al., 2018).

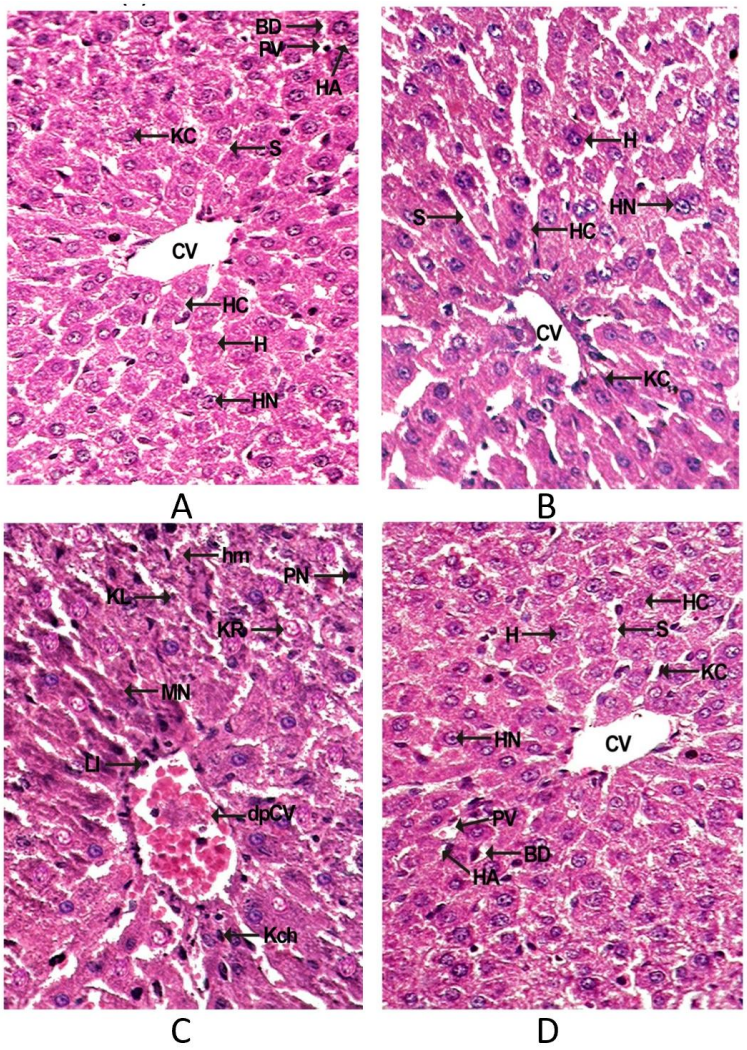

Fig. (A-D). Photomicrograph of hepatic sections of various treatment groups showing central vein (CV), Kupffer cells (KC), sinusoids (S), Polyhedral hepatocytes (H) with prominent nucleus (HN) in the cord-like arrangement (HC) and portal triad containing portal vein (PV), Hepatic artery (HA) and bile duct (BD) (X 400) **A.** Hepatic section of control rats **B.** Hepatic section of allium treated rats **C.** Hepatic section of lead acetate treated rats **D.** Hepatic section of allium + lead treated rats.

It is not entirely clear how lead causes liver disease. Lead poisoning is believed to be primarily caused by oxidative stress. By causing oxidative stress, oxidative damage to cellular lipids, proteins, and DNA, as well as inflammatory indicators in the liver, lead may harm the liver (Chen et al., 2019) It is therefore supposed that lead may cause metabolic disorders in the liver while affecting liver function. Any hepatoprotective drug's effectiveness depends on its ability to either lessen the negative effects or repair the normal liver histology and physiology that the hepatotoxin has harmed. It is evident from the results that *A. cepa* extracts were able to reduce significantly all the elevated biochemical parameters due to lead-induced hepatotoxicity and this was collaborated by the results of histopathological studies. *A. cepa* (onions) have antioxidant potential due to the presence of high amounts of organosulfur compounds, polyphenols, and flavonoids which are natural antioxidants. *A. cepa* and its constituents, especially quercetin, show anti-inflammatory effects (Chakraborty et al., 2021). The mechanism of action could be its membrane-stabilizing effect and antioxidant properties that could prevent the release of intracellular enzymes. (Ozougwu and Eyo, 2014). It might also inhibit the lipid peroxidation of hepatic microsomal enzymes (Chattopadhyay, 2003). Another possible mechanism is that the active ingredient in *A. cepa* allyl propyl disulphide could have increased the level of Glutathione which binds to toxic metabolites and increased their excretion rate from the body.

Conclusion

Lead-induced hepatotoxicity is a major health challenge worldwide. The breakdown of the antioxidant defense system because of excessive free radicals resulting from hydrocarbon

breakdown can lead to the destruction of the cell and tissue architecture. This might be responsible for the histopathological and biochemical changes observed in this study. In most cases lead toxicity is preventable and prevention strategies may be individual (measures taken by families to avoid exposures), public (measures through laws and nationwide policies that ban lead in products or reduce allowable levels in water or soil) and preventive medicines. Although a number of preventive medicines and chelators are available to overcome lead toxicity, they have side effects of their own. The present study suggests a herbal preventive measure provided through dietary supplements, a commonly used condiment – *Allium cepa* or Bulb onion. *Allium cepa* is a medicinal plant used for the management of ailments/diseases and as culinary spices. Their pharmacological potential ranges from hepatoprotection, immunomodulation, antimutagenic, antioxidant antibacterial, anticarcinogenic, antifungal, hypoglycemic, hyperglycemic and anti-atherosclerotic. *A. cepa* extract positively modulates the lead hepatotoxicity by attenuating ROS.

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