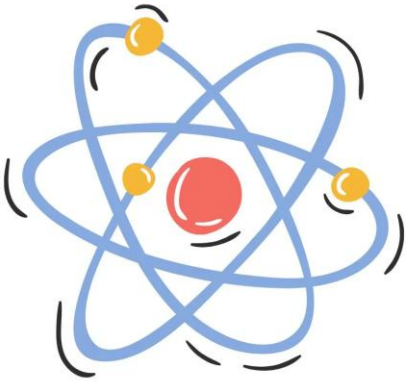

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Abstract: *Heavy metal toxicity is a major health problem in the world since it has caused extensive pollution in the environment and prolonged human exposure. Due to prolonged human exposure and widespread environmental pollution, heavy metal poisoning is a serious global health concern. Lead, cadmium, mercury, and arsenic are metals that build up in the body and are hazardous primarily through oxidative stress, inflammation, and apoptosis. Damage to endogenous antioxidant systems and excessive generation of reactive oxygen and nitrogen species lead to lipid peroxidation, protein oxidation, DNA damage, and progressive organ failure. Although chelation therapy is commonly used to reduce metal burden, its long-term usage is limited by low specificity, security concerns, and inability to prevent oxidative tissue damage. Due to their multi-targeted antioxidant, anti-inflammatory, and cytoprotective properties, polyherbal formulations are now regarded as potentially useful treatment methods. Additionally, polyherbal combinations have been shown in Wistar rat models to rescue antioxidant enzyme activities, restore low glutathione levels, control essential molecular pathways in adulthood, such as Nrf2, NF-KB, and MAPK, and protect important organs including the brain, liver, and kidney. This paper summarizes the cytotoxic effects of heavy metals in relation to oxidative stress and discusses the benefits, drawbacks, and potential of polyherbal formulations as a preventative measure. The findings suggest that polyherbal techniques may be utilized in the future as an alternative or supplement to lessen toxicity caused by heavy metals.*

Keywords: *Oxidative Stress; Chelation Therapy; Organ Toxicity; Heavy Metals; Mu ate in Antioxidants: Wistar Rats; Polyherbal Preparation.*

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1. Introduction

Heavy metal toxicity has become a serious global public health and environmental concern due to increased industrialization, urbanization, mining, agricultural activities, and poor waste management (**Khrushch et al., 2023**). Heavy metals, such as lead, cadmium, mercury, and arsenic, are persistent environmental contaminants that wind up in ecosystems and biological systems because they do not biodegrade. In contrast to acute poisoning, early diabolical heavy metal exposure can often result in overt cellular damage, which is difficult to detect and treat successfully (**Martin, 2013**). The extensive exposure and high biological half-life of the heavy metals are very significant contributors to the global burden of disease. It is also essential to understand the mechanisms behind toxicity in order to develop safer and effective therapeutic interventions. Human contact is primarily through contaminated foods, drinking water, air, and workplace environments. Long-term exposure has been linked to multisystem toxicity, which affects the neurological, hepatic, renal, cardiovascular, and reproductive systems, even at lower levels.

Unlike acute poisoning, early exposure to toxic heavy metals can often result in overt cellular damage that is difficult to identify and effectively cure (Martin, 2013). The heavy metals' high biological half-life and widespread exposure are major causes of the world's disease burden. Understanding the mechanisms underlying toxicity is also essential for developing safer and more successful therapeutic strategies.

1.1 Global Burden of Heavy Metal Toxicity

One of the greatest issues in the world and especially in the developing and industrializing nations is the exposure to heavy metals especially where there might be weak environmental laws, or those laws that are not properly followed. Exposure is primarily due to industrial effluents, mining activities run off, recycling of electronic wastes, pesticides use and groundwater contamination **(Mrozik et al., 2021)**. In the view of international health reports, there are millions of innocent people systematically exposed to poisonous metals which induce more morbidity and deaths.

Children, pregnant women, older adults, and workers at the industry are vulnerable populations because of their vulnerabilities and long exposures. Neurodevelopmental disorders have been strongly linked to heavy metals like lead and mercury whereas cadmium and arsenic have been linked to damage of renal system, osteoporosis, cardiovascular diseases, and carcinogenesis **(Chapman, 2008)**. The socioeconomic and healthcare impact of heavy metal toxicity is

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challenging in the long term, and this argument might serve as an indication of the severity of the issue in question and the necessity of successful preventive interventions and treatment options that might allow alleviating the adverse effect of the condition on the system.

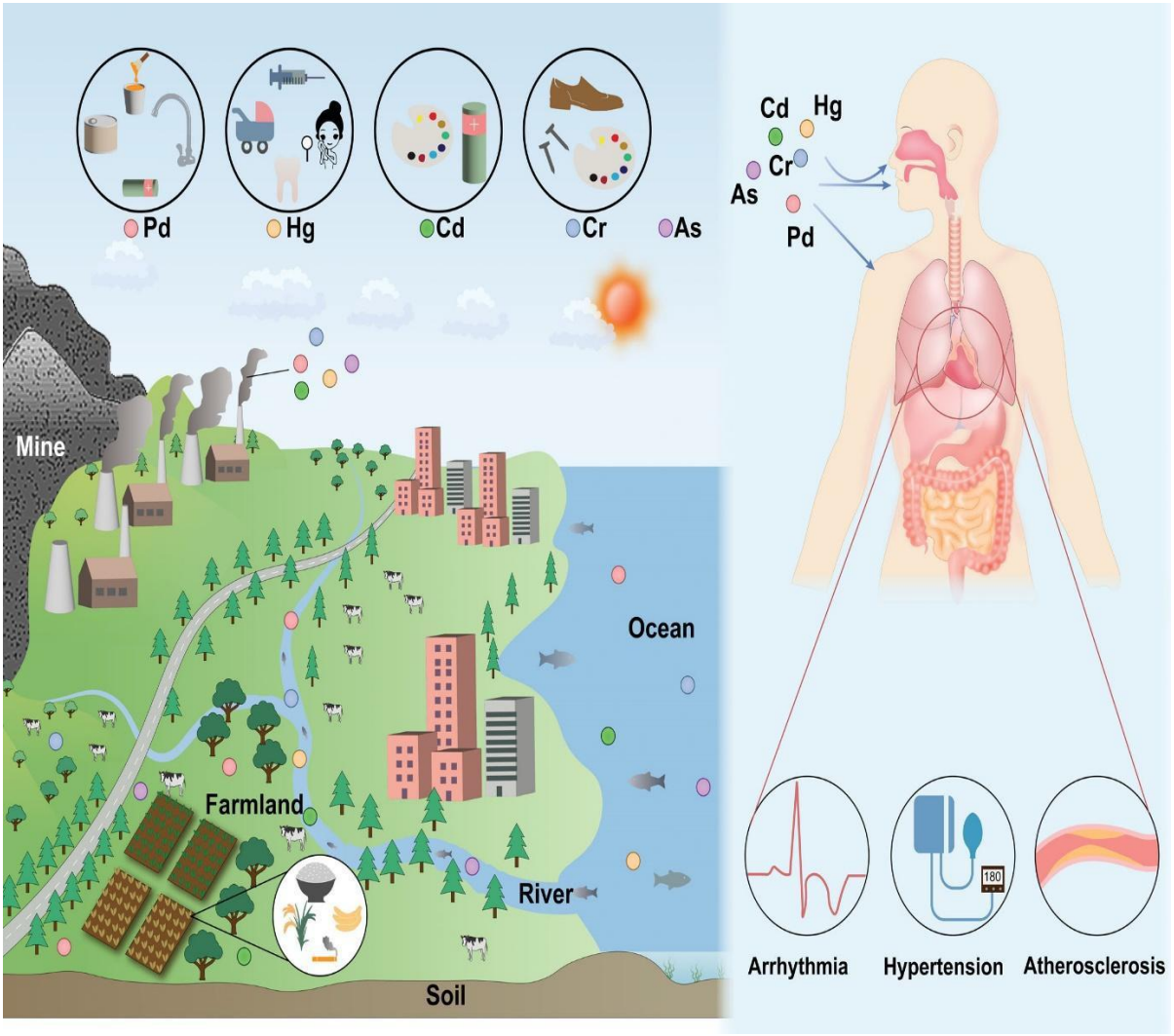

Figure 1: Environmental sources of heavy metals and major routes of human exposure.

This figure illustrates key sources such as industrial emissions, mining activities, agricultural chemicals, contaminated water, food chains, and occupational exposure, highlighting the global burden of chronic heavy metal toxicity.

1.2 Oxidative Stress as a Central Mechanism of Toxicity

Oxidative stress is generally considered one of the most commonly used molecular pathways of toxicity of heavy metal **(Sharma et al., 2012)**. The heavy metals stimulate the excessive production of reactive oxygen species and reactive nitrogen species either directly by redox

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cycling or indirectly by impairment of mitochondrial functionalities and antioxidant enzymes. At the same time, they weaken endogenous antioxidants like glutathione, as well as block major antioxidant enzymes, resulting in cellular redox homeostasis imbalance.

The consequences of this oxidative imbalance include lipid peroxidation of cellular membranes, structural and enzymatic proteins oxidation, dysfunction of the mitochondria, and DNA injury **(Bansal & Bilaspuri, 2010).** Continuous oxidative stress triggers inflammatory responses and apoptotic processes that eventually cause injury to tissues and organ failure. The central experimental rationale behind the use of antioxidants in the treatment of heavy metal poisoning rests with the central role of oxidative stress in the poisoning, which offers a solid scientific rationale in the use of antioxidants that result in the simultaneous arrest of various pathways.

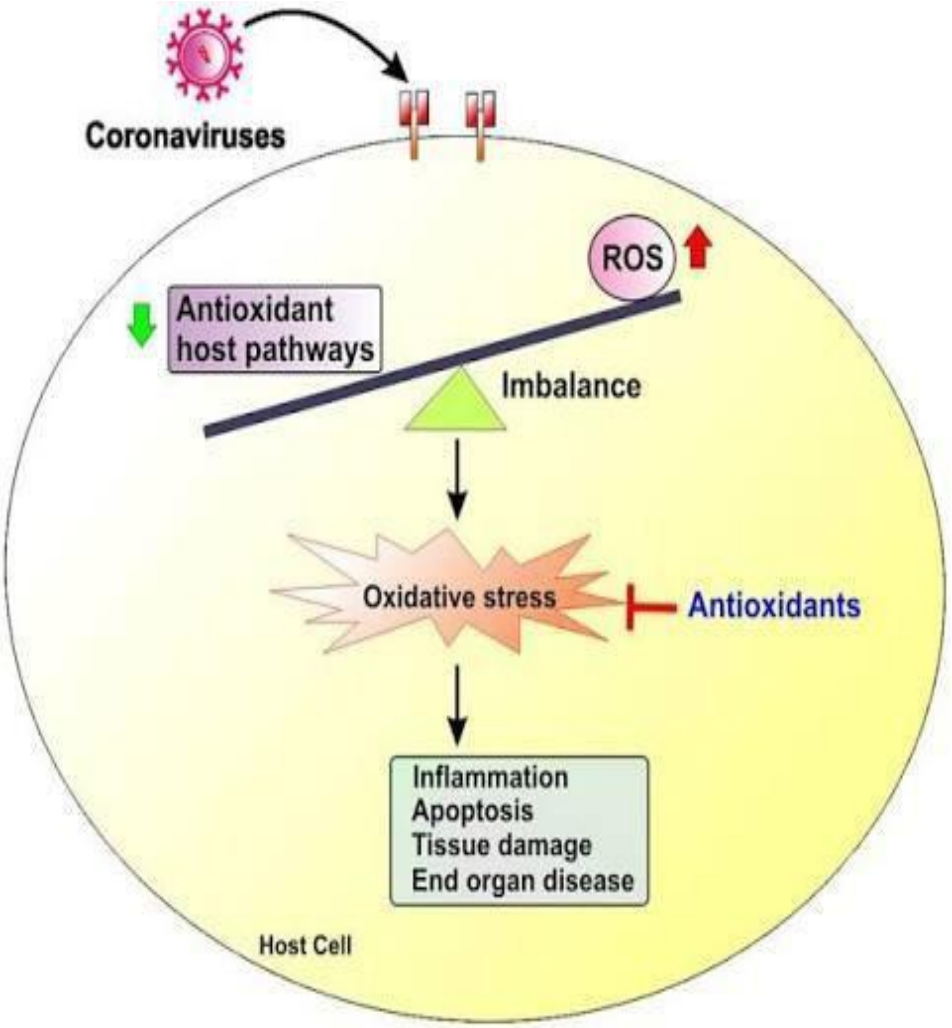

Figure 2: Mechanism of heavy metal–induced oxidative stress and cellular damage.

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The figure depicts excessive reactive oxygen species generation, antioxidant depletion, mitochondrial dysfunction, lipid peroxidation, DNA damage, inflammatory signaling, and apoptosis leading to organ toxicity.

1.3 Limitations of Conventional Chelation Therapy

The therapy of heavy metal poisoning still depends more on chelation therapy which comprise of using chelating agents like dimercaptosuccinic acid, dimercaprol and ethylenediaminetetraacetic acid **(Yap, 2016) .**These substances function by binding metal ions and increasing the body's elimination of them. While chelation therapy may be useful in severe and acute cases of metal poisoning, it has less of a clinical impact in situations of low-dose and chronic exposure. Chelation therapy has a limited clinical significance in cases of chronic and low-dose exposure, despite the fact that it can be useful in acute and severe cases of metal poisoning.

The long-term and widespread use of chelation therapy is restricted by a number of disadvantages. Because cheating agents typically have poor selectivity, they eliminate essential trace elements like iron, zinc, and copper, which interferes with regular physiological functions. Additionally, there have been negative consequences include allergies, gastrointestinal issues, hepatotoxicity, and nephrotoxicity (**Perfect et al., 2010**). Furthermore, chelation therapy is mostly utilized to aid in the elimination of metals without completely addressing the oxidative stress, inflammation, or tissue damage that have already been brought on by heavy metal exposure.

What is more, the repeated administration is often needed because of redistribution of metals of deep tissue compartments which exposes more danger to develop toxicity and patient non-compliance. These constraints suggest that there is a necessity to adopt substitute or complementary therapeutic measures that can offer wider protection against the cellular and organ damages associated with heavy metal.

1.4 Rationale for Polyherbal Therapeutic Approaches

The rationale of polyherbal therapeutic methods is that multi-targeted and synergic effects on most medicinal plants would be achieved by combining several medicinal plants, which would provide high efficacy in comparison to mono-herbs preparations. In the context of heavy metal toxicity, polyherbal formulations are particularly useful since metal-induced damage is complex, multifactorial, and linked to oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis.

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Flavonoids, phenolic acids, alkaloids, terpenoids, and tannins are examples of bioactive phytochemicals present in medicinal plants, many of which have potent anti-inflammatory, antioxidant, and cytoprotective properties (**Salehi et al., 2020**). In addition to protecting vital organs like the liver, kidneys, and brain, polyherbal preparations can also increase endogenous antioxidation, reduce reactive oxygen species, and control inflammatory cascades.

When compared to conventional chelation therapy, polyherbal approaches provide a number of advantages, such as decreased toxicity, improved long-term safety, and the capacity to restore redox balance rather than merely eliminate metal ions. The growing body of research on the protective and antioxidant qualities of medicinal plants provides a solid foundation for the study of polyherbal preparations as additional or substitute strategies for managing oxidative stress caused by heavy metals.

2. Heavy Metals of Toxicological Concern

Heavy metals are a class of elements with high atomic weight and density that, even in small amounts, have harmful effects on biological systems. These are particularly lead and cadmium, which are widely distributed in the environment, have long-lasting biological effects, and are strongly linked to organ damage caused by oxidative stress. Over time, some cumulative toxicity will result from the metals entering the body from contaminated food, drinking water, and air poisoning or from occupational exposure.

2.1 Lead (Pb)

Lead is among the best-investigated toxic heavy metals that are still a significant social health issue on the global agenda. The main mediators of lead's harmful effects are oxidative stress and interference with the redox balance of cells. Lead disrupts antioxidant defense systems by inhibiting sulfide mutases like superoxide dismutase, catalase, and glutathione peroxidase, in addition to depleting intracellular glutathione content. This imbalance results in excessive production of reactive oxygen species, which causes lipid peroxidation, protein oxidation, mitochondrial dysfunction, and damage to the human environment. After being absorbed, lead spreads to other tissues like the liver, kidney, brain, and bones, where it may stay for decades.

Lead's harmful effects are primarily mediated by oxidative stress and disruption of cells' redox equilibrium. In addition to depleting intracellular glutathione, lead interferes with antioxidant

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defense systems by blocking sulfide mutases such as glutathione peroxidase, catalase, and superoxide dismutase. This imbalance leads to an excess of reactive oxygen species, which damages DNA and causes lipid peroxidation, protein oxidation, and mitochondrial dysfunction.

Neurotoxicity, cognitive impairment, anemia, renal dysfunction, hepatotoxicity, and cardiovascular abnormalities are among the clinical signs associated with lead exposure. Lead exposure is a proven paradigm for evaluating antioxidant and protective therapies since it is a durable biomarker that causes oxidative stress-associated tissue damage in experimental settings, particularly in Wistar rats (Matei et al., 2021).

2.2 Cadmium (Cd)

Cadmium is a very harmful environmental pollutant whose biological role in a person is not known. Some of the very big sources of cadmium are industrial activities, phosphate fertilizers, smoking cigarettes, foodstuffs, and polluted water sources. Cadmium has a half- life that is usually very high and it is usually several decades and may be progressive which means that over a period of time it may accumulate in essential organs, particularly the kidneys and the liver.

Oxidative stress was highly related to cadmium toxicity, but cadmium himself is not a redox-active metal. It causes oxidative damage indirectly through disturbance of mitochondrial activity, prevention of antioxidant enzymes, and enhancement of glutathione depletion **(Belenichev et al., 2024)**The effects of this include the higher levels of lipid peroxidation, protein damage, and the stimulation of inflammatory and apoptotic pathways.

The long-term effects of cadmium include nephrotoxicity, hepatotoxicity, bone demineralization, endocrine interference and carcinogenesis. Cadmium toxicity exposes the rat models in Wistar to consistent oxidative and histopathological progression, which renders it an excellent experimental technique to study metal-induced toxicity and assess the protective effect of polyherbal formulation with high antioxidant content.

2.3 Mercury (Hg)

Mercury is a very toxic heavy metal, which can be found in the form of elements, inorganic, and organic forms, and their toxicokinetic and toxicodynamic characteristics differ. Humans are primarily exposed to mercury through industrial effluents, coal combustion, and dental

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consumption of tainted fish and seafood, particularly when it involves organic mercury compounds such as methylmercury. Due to its high lipid concentration, mercury readily passes through cellular membranes like the placental barrier and the bloodbrain **(Chain, 2012).**

The effects of oxidative stress and compromised cellular antioxidants are closely linked to the pathophysiology of mercury. Mercury's strong affinity for sulfhydryls causes reduced glutathione to break down and antioxidant enzymes to become inactive. This results in increased lipid peroxidation, mitochondrial dysfunction, and excessive generation of reactive oxygen species.

Because oxidative stress causes neuroinflammation and apoptosis, particularly in neuronal tissues, exposure to mercury has been clinically linked to neuro-toxicity, cognitive impairments, tremors, kidney defects, and immunotoxic reactions. In Wistar rat experiments, exposure to mercury produces marked oxidative stress, behavioral abnormalities, and histopathological changes in the brain, liver, and kidneys (**Yancheva et al., 2022**). These characteristics make mercury a viable toxicant when evaluating the protective role of antioxidant and polyherbal interventions.

Clinically, exposure to mercury has been linked to immunotoxic responses, tremors, kidney abnormalities, neurotoxicity, and cognitive impairments. Mercury exposure causes significant oxidative stress, behavioral abnormalities, and histological alterations in the brain, liver, and kidneys in Wistar rats used in experiments (**Yancheva et al., 2022**). Because of these characteristics, mercury can be considered a toxicant when evaluating the protective effect of antioxidants and polyherbal remedies.

2.4 Arsenic (As)

Arsenic is a common environmental pollutant likely to be found in groundwater, soil, and foodstuffs especially in areas that are naturally contaminated. Occupational exposure is not the primary route of human exposure, but may also serve as a supplement to ingestion of contaminated drinking water and food. Arsenic is toxic whether in an inorganic or organic form of the poison though inorganic arsenic is the most dangerous.

The effects of arsenic toxicity are mainly achieved by the involvement of oxidative stress, inflammation, and cellular signaling pathways interference **(B. Chen et al., 2024)**. Arsenic

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encourages the occurrence of reactive oxygen, inhibiting mitochondrial respiration, and anti oxidant enzyme activity, leading to lipid peroxidation, protein oxidation and DNA damage. Long-term effects on inflammatory and apoptotic pathways also result in long-lasting tissue damage.

Experimental and epidemiological surveys have associated arsenic exposure to skin lesions, cardiovascular disorders, neurotoxicity, diabetes, hepatic and renal dysfunction and high risk of cancer. Arsenic exposure is reliable in causing oxidative stress, inflammatory reaction, and structural damage of various organs in Wistar rat models. These effects give good experimental ground in the study of antioxidant-based and polyherbal interventions to reduce the toxicity caused by arsenic **(Mohiuddin, 2019).**

3. Mechanisms of Heavy Metal–Induced Oxidative Stress

The toxicity that is caused by heavy metal is mediated in large by the oxidative stress condition which is a disequilibrium between the formation of reactive oxygen and nitrogen species and the ability of endogenous antioxidant protection mechanisms to counter it. Heavy metals alter the cellular redox homeostasis by a variety of actions such as directly producing free radicals, damaging mitochondrial activities, affecting the release of antioxidant molecules and also damaging antioxidant enzymes**(Fulda et al., 2010)**. All these actions culminate to molecular damage, inflammation, and cell death which eventually causes dysfunction of organs.

3.1 Reactive Oxygen and Nitrogen Species Generation

Excessive production of reactive oxygen species and reactive nitrogen species is one of the leading agents of heavy metal toxicity. Redox active metals (mercury and arsenic) have the potential to directly take part in redox cycling reactions, resulting in the sustained generation of superoxide anions, hydrogen peroxide and hydroxyl radicals. Non-redox active metals, such as cadmium and lead, are indirect inducing factors of oxidative stress through disturbance of electron transport chains in mitochondria and damage to the fermentation of antioxidant enzymes.

The heavy metals further increase nitric oxide production and trigger the development of reactive nitrogen species like peroxynitrite by the mechanisms of interaction with the nitric oxide synthase pathways. A buildup of these excessively reactive species overwhelms cellular antioxidant defenses leading to oxidative damage to lipids, proteins and nucleic acids **(Li et al., 2023)**. Unremitting increase of reactive oxygen and nitrogen species further boosts the

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inflammatory signaling pathways enhancing cellular damage and tissue atrophy.

3.2 Lipid Peroxidation and Membrane Damage

One of the considerable effects of oxidative stress caused by a heavy metal is lipid peroxidation, which is one of the pathological processes of cell membranes. Reactive oxygen species invades polyunsaturated fatty acids in phospholipid bilayers causing a chain reaction in the formation of lipid peroxides and by-products which are toxic like malondialdehyde and 4-hydroxynonenal. These wastes affect the integrity and fluidity of membranes affecting the normal functioning of the cells.

Disruption of the cell membranes and organelle membranes leads to impairment in the ion transport, distortions in the membrane-bound enzyme action, and the deterioration of the mitochondrial activity. Lipid peroxidation helps in the exaggerated membrane permeability, swelling of a cell, and eventual cell have been found in the crucial organs like the liver, kidneys, and brain. Bio elements of lipid peroxidation are repeatedly found to be at high levels in experimental models of heavy metal exposure and serve as an important indicator of oxidative damage and a target of antioxidant-based therapeutic interventions.

3.3 Protein Oxidation and Enzyme Dysfunction

Oxidative stress due to heavy metals is a very serious event that causes protein oxidation and is one of the main causes of cell dysfunction **(Farrugia & Balzan, 2012)**. The production of reactive oxygen and nitrogen species that occur as a result of heavy metal exposure causes insult to the amino acid side chains resulting in the creation of carbonyl groups, oxidation of the sulfhydryl residues, and structural changes in proteins. These oxidation reactions alter the protein structure, destabilize, and disrupt the biologic function.

The direct binding of heavy metals to functional groups of proteins is also possible, especially the thiol and imidazole groups which causes the inactivation of the enzyme. Oxidative modification is particularly prone to key metabolic and antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, participating energy metabolism enzymes, and so on. The dysfunction of enzymes breaks the cellular homeostasis and increases the oxidative stress, and deteriorates thedetoxification systems.

The build-up of oxidized and misfolded proteins also causes additional burden to proteolytic mechanisms and results in cell stress and the activation of pro-inflammatory and apoptotic

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signaling pathways **(Rashid et al., 2023)**. Protein oxidation is also a significant cause of tissue damage and dysfunction of organs as it is repeatedly seen in experimental models of heavy metal exposure, including a protein carbonyl content increase and enzyme inactivity.

3.4 DNA Damage and Genotoxicity

One of the serious and irreparable outcomes of oxidative stress caused by heavy metal is DNA damage. Oxygen and nitrogen radicals formed during metal contact act on nucleic acids, leading to base modification, single and dual strand breaks, DNA protein crosslinking and rearrangement of chromosomes. These genotoxic activities disrupt the genomic integrity and disturb genomic replication and transcription.

The heavy metals also affect the repair of DNA by inactivating repair enzymes and interfering with the signal pathway during the maintenance of genomes. Moreover, metal interactions with DNA or substitution of menteel wayside]metal cofacors by some metals in DNA-binding proteins directly increase the genetic instability. Chronic DNA damage can lead to mutations, altered gene expression, cell cycle arrest, and apoptosis. Prolonged exposure to heavy metals has been strongly associated with an increased risk of cancer, birth defects, and degenerative diseases. Heavy metal exposure consistently induces genotoxic biomarkers such as the formation of micronuclei and DNA strand breaks in Wistar rat models, identifying oxidative DNA damage as a crucial toxicality mode and a crucial target of antioxidant-induced therapy.

4. Endogenous Antioxidant Defense System

Endogenous antioxidant defense systems are crucial for maintaining cellular redox equilibrium and shielding biological systems from oxidative damage. The creation of reactive oxygen and nitrogen species and their neutralization by antioxidant systems are in equilibrium in the body. Heavy metal interaction, however, upsets this equilibrium by producing more free radicals and weakening antioxidant defenses, leading to oxidative stress and cellular damage.

Enzymatic antioxidants are the first line of defense against oxidative stress and are also most vulnerable to the inhibitory effects of heavy metals. The antioxidant defense system is an enzymatic-non-enzymatic structure that exists in a coordinated mode to eliminate reactive species, eliminate cellular injury, and maintain cellular integrity. Enzymatic antioxidants are one such group that are both the most vulnerable to the inhibitory effects of heavy metals and the first line of defense against oxidative stress.

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4.1 Enzymatic Antioxidants

Enzymatic antioxidants are special types of proteins that react catalytically with the oxygen radicals and prevent their occurrence in cells. These enzymes are of great significance in restricting the oxidative damage during normal and pathological conditions. The heavy metals disrupt the activity and expression of these enzymes by fixing to the enzyme active sites, substituting the essential metal cofactor, and undergoing oxidative alterations and thus, inhibiting cellular defense mechanisms.

4.1.1 Superoxide Dismutase (SOD)

One of the most significant antioxidant enzymes is superoxide dismutase that prompts the process of the dismutation of the superoxide radicals to hydrogen peroxide and the molecular oxygen. It is the first step during a detoxification of the reactive oxygen species. SOD is found in numerous isoforms which are found in the cytosol, mitochondria and the extracellular spaces hence, they ensure a wide spread protection of superoxide damage.

The activity of SODs is severely influenced by exposure to heavy metal, which disrupts the cofactor of the metals i.e. copper, zinc, manganese, required as a prerequisite to facilitate the action of the enzymes. SOD inhibition causes the accumulation of superoxide radicals and propagation of oxidative stress as well as cellular macromolecules destruction. All the experimental models on the toxicity of lead, cadmium, mercury and arsenic report a decrease in the SOD activity.

4.1.2 Catalase (CAT)

The other significant key enzyme that inhibits formation of highly reactive hydroxyl radical is catalase which changes hydrogen peroxide into water and oxygen. Catalase is primarily localized in peroxisomes and its absence is important in the preservation of cells against oxidative damage caused by hydrogen peroxide. The heavy metals influence both the functionality of catalase as they bind directly to the enzyme, also, it influences the oxidation of the protein structure of the enzyme. The depletion of catalase activity results in accumulation of hydrogen peroxide and, therefore, oxidative stress and the intensification of lipid peroxidation and malfunctioning of mitochondria. Catalase inhibition mainly happens in those tissues that have already been subjected to heavy metals particularly the liver and the kidneys.

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4.1.3 Glutathione Peroxidase (GPx)

Glutathione peroxidase is an antioxidant enzyme, the structure of which is reliant on selenium and catalytically eliminates hydrogen peroxide and lipid peroxides to less toxic products with reduced glutathione as the substrate. Use of GPx in oxidative damage prevention of cell membranes and redox balance is very crucial.

The destabilization of the activity of GPx and the decrease in the level of intracellular glutathione and interference with the availability of selenium, a crucial element to enzyme production and performance, are such effects of heavy metals. Reduced GPx activity raises lipid peroxidation and membrane instability that contributes to the damage of tissues. The experimentally treated Wistar rats have also repeatedly demonstrated lesser activity of GPx at the conclusion of exposure to heavy metal, which has been underscored by that it is a bio marker of oxidative stress and that as a therapeutic purpose of antioxidants treatment.

4.2 Non-Enzymatic Antioxidants

The part of the protective mechanism against oxidative stress in the cell is non-enzymatic antioxidants as well, which cannot be isolated. They act by direct scavenging of reactive oxygen and nitrogen species, metal chelation, and stimulating the activity of antioxidant enzymes unlike the enzymatic antioxidants. They play a very significant role in redox homeostasis in the intracellular milieu particularly in toxic insult context such the one in the case of heavy metal exposure.

Reduced glutathione is the most classical non-enzymatic antioxidant used and the most functional antioxidant within the cell. It works free-standing, as well as in collaboration with the activity of enzymatic systems to protect the cells against oxidative damages.

4.2.1 Reduced Glutathione (GSH)

The reduced glutathione is a tripeptide molecule (glutamate, cysteine and glycine) and constitutes one of the central points of control in cell redox homeostasis. Additionally, GSH maintains cellular enzyme-bound essential proteins in the reduced state, neutralizes reactive oxygen and nitrogen species that are immediate and direct to the radicals as well as

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detoxification reactions, is a substrate of glutathione peroxidase, and is necessary for the restoration of other antioxidants. It is crucial for the replenishment of other antioxidants and serves as a substrate for glutathione peroxidase.

In one way or another, exposure to heavy metals results in a significant reduction in intracellular GSH levels. Four metals—lead, cadmium, mercury, and arsenic—are highly attracted to the sulfhydryl group, which causes it to bind itself and eat the glutathione directly. Additionally, heavy metals destabilize enzymes involved in the synthesis of glutathione, which contributes to the oxidative conversion of GSH to the oxidized glutathione form, thereby impairing cells' antioxidant capacity. Due to their strong attraction to the sulfhydryl group, four metals—lead, cadmium, mercury, and arsenic—directly bind to and consume glutathione. Furthermore, heavy metals that contribute to the oxidative conversion of GSH to the oxidized glutathione form destabilize enzymes involved in the synthesis of glutathione, hence impairing the antioxidant capability of cells.

GSH is a well-established biomarker of oxidative stress, and a decrease in it increases the risk of oxidative damage, lipid peroxidation, mitochondrial diseases, and apoptosis. In experimental models, exposure to heavy metals has been shown to restore the reduced levels of glutathione to their initial levels using Wistar rats. GSH is a well-known indicator of oxidative stress, and when heavy metals are exposed to experimental models using Wistar rats, the decreased levels of glutathione have been seen to return to their initial levels.

The reduction of the levels of glutathione is hence deemed a priority in the antioxidant and polyherbal therapeutic approaches of reducing the toxicity of heavy metals.

5. Experimental Models in Wistar Rats

The experimental models that are based on animals play a role in the elucidation of the toxicological impact of the heavy metals as well as establishing the efficacy of the therapeutic intervention. The toxicology and pharmacology studies in most other live animals apply the Wistar rats because they exhibit excellent predictability to toxicants, and their use is appropriate in biochemical, molecular, and histopathological experimentalations. The Wistarrats experimentation has been a good tool in the road to discovering the fundamental pathways regarding oxidative stress injury to body organs (done by heavy metals) and the inhibitory effect of the treatment which includes antioxidant-modified elements.

5.1 Rationale for the Use of Wistar Rat Models

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The most common rodent strains that have been used in the experimental part of toxicology also include the Wistar rats that are epigenetically consistent and can easily be handled and reasonably predictable to environment and chemical toxins. Their metabolic routes, the antioxidant defence mechanism, and the mechanism of their organ systems are similar to the human being hence can be employed as a good model in the study of the toxicity induced by the heavy metals.

Its size is a relatively large organ of the Wistar rats with the help of which in-depth biochemical, molecular, and histopathological researches are not complicated. Further, it is also possible to compare studies by having an absolute range of reference values of oxidative stress biomarkers, antioxidant enzymes and inflammatory mediators. These properties make the Wistar rats a highly appropriate animal model in the ascertainment of dose dependent and tissue toxicity of the polyherbal preparations besides therapeutic efficacy of the polyherbal preparations in the laboratory environment.

5.2 Methods for Induction of Heavy Metal Toxicity

All experiments that involve heavy metal toxicity are triggered in the norm by subjecting the Wistar rats to any of the metals including lead, cadmium, mercury or even arsenic in a programmed experimental procedure. Based on the study design and suitability of the study to human exposures, oral, intraperitoneal or subcutaneous route is commonly employed with metals. The oral route of administration is more traditionally desired to be closer in its forms of exposure to the environment and diet.

It has evolved and has both short and long-term toxicity, the models of acute and sub-chronic or chronic exposure can do the same to check the exposure. Toxicity induction is identified by biochemical alteration such as an increase of lipid peroxidation, a decrease of the antioxidant enzyme activities, loss of glutathione as well as the signs of inflammation. The stimulation of metal-induced injury is positive as well in cases where histopathological screening of an organ (liver, kidneys, brain and heart) is done.

Such experimental models have provided a good base on which one can analyze the mechanism of oxidative stress and ascertain the protective action of polyherbal formulations containing antioxidants as opposed to the action of the heavy metals treatment inducing toxicity.

5.3 Behavioral and Functional Assessments

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The experiments also determine the toxicity of heavy metal on the Wistar rats as its results are dependent on the behavioral and functional measures since it can offer the functional comparison to the biochemical and histopathological changes. The assumption sees that lead, mercury, and arsenic are extremely toxic to the central nervous system, motor functions, cognition and emotive behaviour therefore, behavioural analysis is a tricky indicator of neurotoxicity and general malpractice within the system.

General behavioral tests contain open field tests which are used to evaluate the presence of locomotor activity, exploratory behavior, elevated plus maze tests and light-dark box tests are used to evaluate the presence of anxiety-like behavior, and the maze based tests e.g. Morris water maze or Y-maze examined to evaluate learning and memory functions. The tests of rotarod and grip strength are also widely accepted so as to gauge the motor coordination and strength of the neuromuscular system especially where the models have been used with neurotoxic metals.

Functional tests are not neurobehavioral tests but they test the functioning of certain organs. A renal functioning is determined by measuring the serum creatinine, urea and the output of the urine and liver functioning is determined by measuring liver enzyme tests. In accordance with the organization of the experiment, the cardiovascular and metabolic parameters can be followed. An excellent measure of protective efficacy is post-therapeutic outcome of a positive outcome, in terms of behavior change and functional performance. Rats of the Wistar species were used in experiments using polyherbal formulation supplementation in an effort to provide a biochemical and histological analysis to augment a comprehensive analysis of these compounds' therapeutic potential against heavy metals that damage organs and induce oxidative damage.

6. Polyherbal Formulations: Concept and Scientific Basis

Polyherbal preparations are an important aspect of traditional medicine and are receiving growing scholarly attention as therapies for complex multifactorial disorders. Polyherbalism is a concept based on the strategic assembly of many medicinal plants with the goal of increasing efficacy, safety, and therapeutic coverage. Polyherbal formulations can be used to treat heavy metal toxicity, which is caused by a variety of pathogenic processes such as oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis.

Polyherbal substances have been empirically discovered to have the ability to sustain a number of molecular pathways simultaneously and can give a holistic approach to illness treatment. This

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multi-component technique is ideal for the complicated toxicity of heavy metals. This multi-component method is appropriate for the complicated toxicity of heavy metals.

6.1 Principles of Polyherbalism

Polyherbalism is based on the fact that a combination of such medicinal plants with complementary pharmacological effects would achieve better curative effects than a single herb. Plants contain different bioactive compounds, and these compounds affect different targets thus enhancing the efficacy in minimizing the side effects.

In polyherbal preparation, the selection of herbs is based on the compatibility of herbs in oil drugs based on their pharmacodynamic activity, antioxidant effects, organ-protective effect and safety. It is because proper formulation facilitates the balanced dosing, optimum bioavailability and reduced toxicity. The emphasis on integrating synergies within the scope of traditional knowledge systems has been emphasized over decades of time, and the mentioned tendency is sustained by the current pharmaceutical research, experimental, and molecular studies.

6.2 Synergistic and Multi-Targeted Effects

Synergism is one of the scientific assumptions of the polyherbal formulation efficiency. The resulting interaction is synergetic where the effect of a series of herbs is more expensive than the effect of the herbs as separate components. The multi-targeted approach is essential in case of co-morbidities in oxidative stress, inflammation, and cell dysfunctions are possible and when the presence of these factors is noted in heavy metal toxicity.

The polyherbal preparations have the capability to simultaneously remove reactive oxygen and nitrogen species, induce indigenous antioxidant reaction, alter inflammatory developing elements, stabilize mitochondrial formation and regulate apoptosis. Such multi-pathway modulation increases positive therapeutic outcome and provides general defense against cell and organ damage by the heavy metal.

6.3 Advantages over Single-Herb Therapy

Polyherbal formulations provide several advantages over single herb treatments, especially in the management of complicated toxicological diseases. Single herbs are connected with a limited range of pathways, which may be insufficient to overcome the multifactorial impact of heavy metal toxicity. Polyherbal mixtures, on the other hand, have a longer pharmacological and

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therapeutic effect.

Other benefits include reduced toxicity due to modest doses of individual herbs, great safety during long-term usage, and improved stability and biocompetency of the active compounds. Polyherbal preparations also minimize the likelihood of developing resistance to therapy and have a systemic influence on the many body systems. These benefits make polyherbal methods promising as an alternative or supplement to traditional therapies in reducing oxidative stress conditions caused by heavy metals.

7. Evidence of Polyherbal Protective Effects

Experiments on Wistar rat models have shown that polyherbal extracts, which are high in antioxidants and cytoprotective phytochemicals, have a significant effect on reducing biochemical, functional, and structural changes caused by toxic metal exposure. Many research on Wistar rat models showed that polyherbal extracts high in antioxidant and cytoprotective phytochemicals had a substantial effect on reducing biochemical, functional, and structural changes produced by toxic metal exposure. All these protective effects can be primarily attributed to the restoration of redox balance, the regulation of inflammatory processes, and the preservation of cell integrity of different organs.

7.1 Restoration of Antioxidant Status

One of the most consistent effects of research conducted to evaluate polyherbal interventions is the restoration of the antioxidant defenses systems altered by exposure to a heavy metal. Polyherbal extract has been claimed to raise the effect on lipid peroxidation by reducing it and enzymatic antioxidants activity such as superoxide dismutase, catalase, and glutathione peroxidase. In addition to that, reports of the replacement of the low levels of glutathione have also been reported so that, this implies that there is an enhancement of the redox homeostasis of the cell.

Combination of the phytoconstituents results in the good capacity of catching reactive oxygen and nitrogen species and metal ion chelation in addition to the stabilization of its functioning in mitochondria. Substitution of the antioxidant condition comes in center stage to prevent oxidative damage and disease development in the heavy metal toxicity model.

7.2 Organ-Specific Protection

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Polyherbal preparations exert selective organ protection which targets tissues that fall prey most to accumulation of heavy metals and oxidative damages. They have turned out to be of great protective effect to the vital organs like the liver, kidneys and the brain in case of any research experiment.

7.2.1 Hepatoprotective Effects

The liver has a major location of the metabolism and detoxification of the heavy metals and the liver is therefore highly susceptible to the oxidative damage. Polyherbal preparations also have been found in a study to reduce high levels of liver enzymes, improve the activity of antioxidants enzymes and decrease lipid peroxidation of tissues of the liver. These are associated with the improvement of the hepatic architecture and reduced inflammatory infiltration indicating that it is a good surveillance of the metal caused hepatotoxicity.

7.2.2 Nephroprotective Effects

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7.3 Histopathological Evidence

The histopathological evidence of the structural effects demonstrates the effects of polyherbal preparations as protective agents. Heavy metal-exposed Wister rat tissues exhibit common modifications such as altered cellular degeneration, inflammatory infiltration, necrosis, and architectural destruction. Polyherbal treatment of animals has also been shown to significantly

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minimize such degenerative alterations, restoring normal structure to the tissues of the liver, kidney, and brain.

The association between histological improvement and biochemical normalization provides more evidence that polyherbal formulations are protective. Richard Dawson and Riley Emnall support these findings of polyherbal solutions' therapeutic potential in reducing lead metal-Stereolithic oxidative stress and organ system failure.

8. Molecular Pathways Involved in Protection

Polyherbal products exhibit preventative action against heavy metal induced toxicity through the alteration of different molecular signaling mediating oxidative stress, inflammation and survival of cell. Heavy metals also disrupt cellular signal networks that leads to long-term oxidative damage, inflammation and apoptosis. These related systems can be managed simultaneously by all-embraced cellular protection because of the multi-component structure that is capable of controlling these interrelated systems.

8.1 Nrf2/Keap1 Antioxidant Signaling Pathway

One of the controllers participating in the cell antioxidant defense process is the nuclear factor erythroid 2related factor 2 (Nrf2). The Kelch-like ECH-associated protein 1 in most cases directs Nrf2 to the proteasome degradation cycle and localizes it in the cytoplasm. When oxidative stress occurs, Nrf2 becomes dissociated with Keap1, trans-locates to the nucleus, and initiates the expression of antioxidant and cytoprotective gene expression.

The exposure of heavy metals disrupts Nrf2 signaling that results in suppressed expression of antioxidant enzymes such as superoxide dismutases, catalases, glutathione peroxidases and heme oxygenases-1. It is discovered that polyherbal preparations activate the Nrf2 pathway resulting in the enhancement of antioxidant enzyme activity, and redox regulator. It is possible to trigger the Nrf2 in such a way so it is necessary to reduce the oxidative stress and promote the cell resistance to the stress generated by heavy metals.

8.2 NF-κB–Mediated Inflammatory Pathway

Major transcription factors involve nuclear factor kappa B that controls immune and inflammatory reaction. NF- 0B signalling induced by the oxidative stress due to heavy metal

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stimulates the increase of pro-inflammatory cytokines, chemokines and adhesion molecules. This is chronic inflammation and tissue damage after sustained unremitting stimulation of this pathway.

The action of polyherbal formulations in the anti-inflammatory effect is active by inhibiting the activity of the NF- -kB and the expression of the inflammatory responses. Polyherbal treatments prevent the period of cellular injury connected to inflammation and the pathway of dysfunction of the organs initiated by heavy metal exposure in its progress through inhibiting the activity of oxidative stress and NF-KB activation.

8.3 MAPK/ERK Signaling Cascade

Mitogen-activated protein kinase signaling pathway of which the extracellular signal regulated kinases are involved has a role to play in regulating cell proliferation, differentiation, stress responses as well as apoptosis. They induce gene expression and cell dysfunction changes through the activation of MAPK signaling pathways through the activation of the production of oxidative stress-dependent mechanisms by heavy metals.

Polyherbal preparations are also found to control the MAPK/ERK signaling pathway by subduing oxidative injury and a redestination of usual phosphorylation descriptions by the kinases. Control of this pathway is regulated to ensure the improvement of cell survival, reduction in inflammatory response and mitigating tissue injury. MAKP/ERK signaling modulation is another point with which it is appropriate to incorporate the structuring of the range of pathways that yield positive outcomes under polyherbal dosages.

8.4 Regulation of Apoptosis (Bax/Bcl-2 Pathway)

Apoptosis is the most outstanding process of tissues damage by heavy metal. Mitochondrial dysfunction and heavy metal-induced oxidative stress changes the ratios towards pro- apoptotic signalling by raising the expression of Bax and reducing the concentration of anti- apoptotic Bcl-2. The outcome of the imbalance is permeabilization of mitochondrial membranes, cytochrome c release and death pathways through caspase dependencies.

Polyherbal extracts are claimed to have the ability to regulate apoptotic signalling through restoration of a Bax/bcl-2 ratio to cell survival. Polyherbal therapies inhibit the undue apoptosis and structural integrity of the tissues through the alleviation of oxidative stress and stabilization of the mitochondria work. One of the most significant molecular processes in which polyherbal

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formulations can help in the protection of apoptotic products due to heavy metals is in Apoptotic pathway regulation.

9. Comparison with Conventional Chelation Therapy

The most common medical method that is used in the treatment of heavy metal toxicity is a chelation therapy. It entails the intake of chemical substances that bind the metal ions and help them to be expelled out of the body. Although chelation therapy is still useful in acute poisoning, it was not useful in chronic low-level heavy metal exposures. An evaluation of the effectiveness of the chelation therapy and polyherbal approaches reveals significant discrepancies in the effectiveness, the safety and the mechanism of action.

9.1 Role of Dimercaptosuccinic Acid (DMSA)

Dimercaptosuccinic acid is the most common of the oral chelating agents, which are used in the treatment of lead and other heavy metal poisonings. Sulfhydryl groups are found in DMSA to bind with metal ions to form stable complexes which are excreted mostly via the kidneys. Because of oral bioavailability and low toxicity in relation to older chelators, DMSA is more often the pre-eminent treatment preference in the clinical environment, especially in children with lead poisoning.

It has been proven in experimental research that DMSA is effective in the reduction of blood and tissue metal in an acute exposure model. Nevertheless, its treatment effect is predominantly connected with eliminating metals and is not directly involved in the treatment of oxidative stress and inflammation, as well as the destruction of the tissues caused by heavy metals. Therefore, some cellular damage can remain and be observed after chelation therapy.

9.2 Limitations and Adverse Effects of Chelation

Although chelation therapy is effective in the reduction of metals, it has a number of weaknesses and side effects. In most situations, chelating agents are not selective and may have unintentional affinity for essential trace elements such as zinc, copper, and iron, resulting in micronutrient shortages. It has been linked to side effects such as gastrointestinal discomfort, nephrotoxicity, hepatotoxicity, and allergic responses, particularly when used long-term or often.

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Furthermore, chelation therapy can cause metal redistribution from deep tissue compartments into the bloodstream, resulting in future treatment cycles. This increases the risk of toxicity and reduces patient compliance. Notably, chelation therapy has no antioxidant or anti-inflammatory effects and will not restore oxidative or cellular damage.

On the other hand, polyherbal formulations offer a broader therapeutic content that includes reducing oxidative stress, controlling inflammation, and encouraging tissue repair. They are also safer when taken over an extended period of time. These variations highlight the potential significance of polyherbal therapies as supplemental or alternative approaches in the treatment of toxicity caused by heavy metals.

10. Safety, Toxicity, and Standardization Issues of Polyherbal Formulations

While polyherbal preparations have received more attention as treatment possibilities for heavy metal-induced toxicity, concerns about safety, toxicity, and standardization remain pressing. The biological effect of medicinal plants varies depending on a number of parameters, including plant species, geographic origin, harvesting conditions, processing methods, and formulation composition, despite society's perception that these plants are harmless. The concentration and activity of bioactive components, as well as their efficacy and safety, can all be affected by variations in these parameters.

The pharmacokinetics of herbs and surgeons may alter as a result of interactions with herbs in polyherbal formulations. Negative consequences include hepatotoxicity, nephrotoxicity, or even interactions with common medications might result from improper dosage, overuse, or poor quality. Therefore, in order to identify safe dose levels and adverse safety profiles over an extended period of time, a comprehensive toxicological assessment that includes acute, sub-chronic, and chronic toxicity testing is required.

Standardization is a major problem in the production of polyherbal formulations. This is because there are a number of active ingredients that cause issues with reproducibility and quality control. Pre-testing production methods, defining batch-to-batch consistency, and identifying and measuring market constituents were all necessary for standardization. High analytical techniques, including as spectroscopy and chromatography, are increasingly being employed to preserve the stability, purity, and quality of polyherbal products.

The issues of safety, toxicity, and standardization must be addressed in order to take into account the scientific validation and clinical translation of the polyherbal formulations. Preclinical studies, adherence to good manufacturing processes, and regulatory inspections are necessary to

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ensure that polyherbal remedies are long-term safe and effective in managing the oxidative stress caused by heavy metals.

11. Limitations of Current Research

Even though there is solid experimental support that polyherbal formulations have a protective effect on heavy metal-induced oxidative stress, there are certain research limitations that prevent the applicability of such research to the clinical practice. One of the major drawbacks is that preclinical trials were mostly conducted using animal models, specifically Wistar rats. The clones of human toxicokinetic and pharmacodynamic reactions may not be closely solved by these models, despite the fact that they have been highly helpful in providing mechanistic insights.

The lack of a standard experimental design in many investigations is another limitation. It is difficult to compare polyherbal formulations directly due to differences in heavy metal type, dose, exposure duration, administration method, and composition. Furthermore, the phytochemical content of polyherbal extracts is not adequately described by a number of studies, which limits replication and the capacity to comprehend the mechanism.

The safety of long-term usage and the dose response margin are further limited by the lack of pharmacokinetic studies on herbal components in relation to heavy metals. Furthermore, most studies do not evaluate long-term toxicity, functional recovery, or behavioral outcome; instead, they focus on short-term biochemical and histologic effects.

Another significant gap in the current research is the absence of well-designed clinical trials. Polyherbal therapy systems must rely on standardized formulations, validated biomarkers, and long-term safety studies in order to go to the clinical level. To increase the evidence base for polyherbal therapy in the treatment of heavy metal-induced toxicity, these weaknesses can be addressed by carrying out rigorous experimental design and translational research.

12. Future Perspectives

The research on polyherbal formulations in the management of heavy metal-induced oxidative stress in future must consider enhancing the translational relevance and applicability of research in clinical practice. In order to improve the repeatability and comparability of the studies, it is necessary to use standardized polyherbal preparations with well-known phytochemical profiles. The identification of bioactive marker compounds and the

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determination of dose-response relationships will also help to increase the reliability of formulations and therapeutic consistency. Modern molecular and omics-based solutions, such as proteomics, metabolomics, and genomics, could also help to further understand the multi-target aspects of polyherb-herb interactions, find new molecular targets, and gain a better understanding of the mechanisms involved in therapy. Determining the dose-response relationships and identifying the bioactive marker molecules will also contribute to improving formulation dependability and therapeutic consistency.

The multi-target characteristics of polyherbal solutions may also be better understood with the help of cutting-edge molecular and omics-based approaches including proteomics, metabolomics, and genomes. The techniques can be utilized to better understand pathway modification at the level of the antioxidant and cytoprotective activities, identify new molecular targets, and elucidate herb-herb interactions. Combining the methods will also aid in improving understanding of the mechanisms underlying therapy.

Future interactions between traditional medicine practitioners, pharmacologists, and regulatory bodies will be crucial to translating polyherbal therapies into evidence-based intervention. In order to fill in the knowledge gaps, rigorous experimental and clinical studies should be conducted to support the formation of safe, efficient, and standardizable methods of polyherbal use to reduce heavy metal-induced oxidative stress and related cases of organ damages. In particular, clinical trials should be properly managed to confirm the effectiveness and eventual pharmacokinetic analyses. Notably, human subjects who may be exposed to heavy metals should be used in clinical trials to verify the efficacy and safety of polyherbal formulations. Transforming polyherbal medicines into evidence-based interventions will need future interactions between pharmacologists, regulatory agencies, and practitioners of traditional medicine.

In order to fill in the information gaps, thorough experimental and clinical research should be conducted to assist the development of safe, effective, and standardized polyherbal usage techniques to lessen oxidative stress caused by heavy metals and associated organ damage instances.

13. Conclusion

One of the important pathways in the pathogenesis of heavy metals, such as lead, cadmium, mercury, and arsenic, which cause progressive cell damage and dysfunction in multiple organs,

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is oxidative stress induced by heavy metals. Experimental studies using Wistar rat models have consistently demonstrated that oxidative stress, inflammation, and apoptosis are primarily implicated in mediating the toxicity caused by metals. While conventional methods of chelation therapy have proven beneficial in reducing metal overload, their ability to address oxidative and tissue-level platform. Experimental research using Wistar rat models has consistently demonstrated that oxidative stress, inflammation, and apoptosis are the main mechanisms causing the damage induced by metals. While traditional chelation therapy techniques have shown promise in reducing metal overload, they are limited in terms of safety and their capacity to address oxidative and tissue-level platforms.Because they combine the multitasking of multiple pathological pathways, polyherbal preparations offer a promising treatment approach. Polyherbal strategies protect against cellular and organ damage caused by heavy metals through the reinstatement of antioxidants, the control of inflammatory signals, and the control of apoptosis. Their strengths over single-agent treatment include their multi-targeted and enhanced safety profile and their long-term prospective usage. By restoring antioxidants, regulating inflammatory signals, and preventing apoptosis, polyherbal methods guard against heavy metal-induced cellular and organ damage. Their advantages over single-agent treatment include their multi-targeted and improved safety profile, as well as their potential long-term use.

perhaps if the majority of the evidence that is now available is preclinical, the growing number of experimental findings supports the need for additional study on polyherbal formulations as a supplement to or perhaps alternative treatment for the symptoms of heavy metal toxicity. To guarantee that the therapeutic potential of the therapy is completely realized, standardization, mechanistic validation, and clinical translation investigations of the therapeutic potential of polyherbal methods in combating heavy metal-induced oxidative stress are required in the future.

14. Conflict of Interest

The authors assert that they do not have any conflicts of interest when it comes to publication of this review article.

15. References (APA Style)

1. Khrushch, O., Moskalets, V., Fedyk, O., Karpiuk, Y., Hasiuk, M., Ivantsev, N., Ivantsev, L., & Arjjumend, H. (2023). Environmental and psychological effects of Russian war in Ukraine. *Grassroots Journal of Natural Resources*, *6*(1), 37–84. https://doi.org/10.33002/nr2581.6853.060103
2. Martin, M. L. (2013). Spirituality, Medical Science and Health: the Spiritual effects of

https://doi.org/10.71058/jodac.v10i0710

a sense of entitlement in the Ministry of Healing in the Christian Church. In *Unisa Institutional Repository (University of South Africa)*. https://doi.org/10.13140/rg.2.2.17737.34400

3. Mrozik, W., Rajaeifar, M. A., Heidrich, O., & Christensen, P. (2021). Environmental impacts, pollution sources and pathways of spent lithium-ion batteries. *Energy & Environmental Science*, *14*(12), 6099–6121. https://doi.org/10.1039/d1ee00691f
4. Chapman, T. L. (2008). *Genetic heavy metal toxicity: Explaining SIDS, Autism, Tourette's, Alzheimer's and Other Epidemics*. iUniverse.
5. Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany*, *2012*, 1–26. https://doi.org/10.1155/2012/217037
6. Bansal, A. K., & Bilaspuri, G. S. (2010). Impacts of oxidative stress and antioxidants on semen functions. *Veterinary Medicine International*, *2011*, 1–7. https://doi.org/10.4061/2011/686137
7. Yap, S. H. K. (2016). *Optical microfiber sensor for heavy metal ion detection*. https://doi.org/10.32657/10356/68936
8. Perfect, J. R., Dismukes, W. E., Dromer, F., Goldman, D. L., Graybill, J. R., Hamill, R. J., Harrison, T. S., Larsen, R. A., Lortholary, O., Nguyen, M., Pappas, P. G., Powderly, W. G., Singh, N., Sobel, J. D., & Sorrell, T. C. (2010). Clinical Practice Guidelines for the Management of Cryptococcal Disease: 2010 update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*, *50*(3), 291–322. https://doi.org/10.1086/649858
9. Salehi, B., Azzini, E., Zucca, P., Varoni, E. M., Kumar, N. V. A., Dini, L., Panzarini, E., Rajkovic, J., Fokou, P. V. T., Peluso, I., Mishra, A. P., Nigam, M., Rayess, Y. E., Beyrouthy, M. E., Setzer, W. N., Polito, L., Iriti, M., Sureda, A., Quetglas-Llabrés, M. M., . . . Sharifi-Rad, J. (2020). Plant-Derived Bioactives and Oxidative Stress-Related Disorders: A Key Trend towards Healthy Aging and Longevity Promotion. *Applied Sciences*, *10*(3), 947. https://doi.org/10.3390/app10030947
10. Al-Musharafi, S. K. (2016). Heavy Metals in Sewage Treated Effluents: Pollution and Microbial Bioremediation from Arid Regions. *The Open Biotechnology Journal*, *10*(1), 352–362. https://doi.org/10.2174/1874070701610010352
11. Matei, A., Caruntu, C., Tampa, M., Georgescu, S. R., Matei, C., Constantin, M. M., Constantin, T. V., Calina, D., Ciubotaru, D. A., Badarau, I. A., Scheau, C., & Caruntu, A. (2021). Applications of Nanosized-Lipid-Based drug delivery systems in wound

https://doi.org/10.71058/jodac.v10i0710

care. *Applied Sciences*, *11*(11), 4915. https://doi.org/10.3390/app11114915

12. Belenichev, I., Popazova, O., Bukhtiyarova, N., Savchenko, D., Oksenych, V., & Kamyshnyi, O. (2024). Modulating nitric oxide: Implications for cytotoxicity and cytoprotection. *Antioxidants*, *13*(5), 504. https://doi.org/10.3390/antiox13050504
13. Chain, E. P. O. C. I. T. F. (2012). Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. *EFSA Journal*, *10*(12). https://doi.org/10.2903/j.efsa.2012.2985
14. Yancheva, V., Georgieva, E., Velcheva, I., Iliev, I., Stoyanova, S., Vasileva, T., Bivolarski, V., Todorova-Bambaldokova, D., Zulkipli, N., Antal, L., & Nyeste, K. (2022). Assessment of the exposure of two pesticides on common carp (Cyprinus carpio Linnaeus, 1758): Are the prolonged biomarker responses adaptive or destructive? *Comparative Biochemistry and Physiology Part C Toxicology & Pharmacology*, *261*, 109446. https://doi.org/10.1016/j.cbpc.2022.109446
15. Chen, B., Yu, P., Chan, W. N., Xie, F., Zhang, Y., Liang, L., Leung, K. T., Lo, K. W., Yu, J., Tse, G. M. K., Kang, W., & To, K. F. (2024). Cellular zinc metabolism and zinc signaling: from biological functions to diseases and therapeutic targets. *Signal Transduction and Targeted Therapy*, *9*(1), 6. https://doi.org/10.1038/s41392-023-01679-y
16. Mohiuddin, A. (2019). Natural Foods and Indian herbs of cardiovascular interest. *Pharmacy & Pharmacology International Journal*, *7*(2). https://doi.org/10.15406/ppij.2019.07.00235
17. Fulda, S., Gorman, A. M., Hori, O., & Samali, A. (2010). Cellular stress responses: cell survival and cell death. *International Journal of Cell Biology*, *2010*, 1–23. https://doi.org/10.1155/2010/214074
18. Li, J., Li, L., Zhang, Z., Chen, P., Shu, H., Yang, C., Chu, Y., & Liu, J. (2023b). Ferroptosis: an important player in the inflammatory response in diabetic nephropathy. *Frontiers in Immunology*, *14*, 1294317. https://doi.org/10.3389/fimmu.2023.1294317
19. Farrugia, G., & Balzan, R. (2012). Oxidative stress and programmed cell death in yeast. *Frontiers in Oncology*, *2*, 64. https://doi.org/10.3389/fonc.2012.00064
20. Rashid, H., Jali, A., Akhter, M. S., & Abdi, S. a. H. (2023). Molecular mechanisms of oxidative stress in acute kidney injury: Targeting the LOCI by resveratrol. *International Journal of Molecular Sciences*, *25*(1), 3. https://doi.org/10.3390/ijms25010003