Biochemical and Histological Changes in the Liver and Kidneys of Rats Treated with Natural Antioxidant Nanoparticles

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Abstract
Natural antioxidant nanoparticles have gained significant interest due to their potent antioxidative properties and potential biomedical applications. This review comprehensively examines the biochemical and histological alterations in the liver and kidneys of rats following treatment with various natural antioxidant nanoparticles. The study highlights that oral administration of these nanoparticles significantly influences liver enzyme activities (such as ALT, AST, ALP), oxidative stress markers (including SOD, catalase, GSH, and lipid peroxidation products like MDA), and renal function parameters (e.g., creatinine, urea, electrolytes). Histopathological assessments reveal dose-dependent changes in hepatic and renal tissues, including hepatocellular injury, cytoplasmic vacuolization, glomerular shrinkage, tubular dilation, and inflammatory infiltration. Notably, certain nanoparticles, such as silver-based ones, demonstrate protective effects against toxicity induced by other nanoparticles like gold or cerium oxide. The findings underscore the dual role of natural antioxidant nanoparticles, which can confer antioxidative benefits while potentially inducing organ damage at higher doses. Therefore, thorough toxicological evaluations and regulatory considerations are essential to ensure the safe clinical and commercial application of these nanomaterials.

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1. Introduction

Natural antioxidant nanoparticles have drawn considerable attention for their antioxidative properties (Flieger *et al*., 2021) [21]. They are mainly categorized as calcium phosphate, metal oxide, or polysaccharide- based materials. Prominent sources include grape seeds, green tea, turmeric, and star fruit (Benalaya *et al*.2024) [13]. Nanoparticle synthesis is widely attempted through chemical, mechanical, and physicochemical methods (Parang *et al*., 2019) [48].

Antioxidants employ the mechanism whereby they donate or accept electrons, thereby stabilizing free radicals (Anwar K Abdelhalim *et al*., 2020) [11]. Nanoparticles can be passively or actively taken up by cells and tend to accumulate within mitochondria and lysosomes, triggering an antioxidant response. They convey anti-inflammatory and antioxidative actions at multiple molecular, cellular, and tissue levels (Ranjbar *et al*., 2018) [51].

2. Natural Antioxidant Nanoparticles

The global presence of pollutants within living organisms such as toxic metals and pesticides-generates risks to food quality and human health and demands rapid, efficient monitoring. Given the volume of environmental and biological samples available, current detection methods are often time-consuming, mandating the development of more effective approaches (Parang *et al*., 2019) [48]. Several promising methods have been proposed, including graphite furnace atomic absorption and emission

spectrometry, photo- catalytic, and fluorescence methods-all of which suffer from drawbacks such as matrix effects, irreproducibility, and expensive digestion apparatus (Ahmad *et al*., 2023) [6]. Additionally, electrochemical systems offer inherent advantages, including ease of operation, low cost, high sensitivity, and the ability to assay complex samples in situ. However, the performance of electrochemical devices remains limited by the intrinsic electrochemical properties of the active surfaces emphasizing the need to engineer them to provide a suitable interface for more sensitive and selective detection (Sood *et al*., 2021) [56].

Nanomaterials due to their unique size-dependent properties have been widely employed to improve sensor/minibiosensor performance. Nanostructured films and nanoparticles (NPs), in particular, have attracted significant attention given their advantageous geometrical and surface properties suitable for interfacing with biological samples. Some materials can be easily synthesized or modified to tune their shape, dimension, uniformity, roughness, surface chemistry, and final assembly resulting in a vast family of structures (natural, carbon, and metal-based). These nanomaterials not only offer new transduction concepts for detecting pollutants both qualitatively and quantitatively but are also capable of enhancing the existing technology (Thakur *et al*.2023; Khan & Hossain, 2022; Chen *et al*.2021) [58, 33, 16].

2.1. Definition and Types

Nanoparticles are defined as particles with dimensions of less than 100 nm and can be obtained as nanospheres or nanocapsules. Under 100 nm, nanoparticles display unusual physicochemical properties and biological reactivity (Parang *et al*., 2019) [48]. Natural antioxidant nanoparticles derive from natural products, including plant species such as Guarana, Brazil nuts, brown algae, and cashew pseudofruits; fruits such as avocado, guava, berries, and oranges; vegetables such as ginger, turmeric, onion, and garlic; and honey. A variety of antioxidant nanoparticles can be synthesized from natural antioxidants (Flieger *et al*., 2021; Rahaman *et al*.2023) [21, 50].

The synthesis of nanoparticles employs physical, chemical, and biological methods. Physical methods are based on the breaking down of larger particles into nanoparticles using processes such as evaporation- condensation or laser ablation. Chemical methods depend on the reaction of chemical compounds with a precursor to produce nanoparticles. In biological methods, bacteria, fungi, or plant extracts are utilized to generate nanoparticles, converting metal ions into metal nanoparticles at ambient temperature in a single step (Jeevanandam *et al*. 2022; Alavi & Nokhodchi, 2021) [29, 8].

2.2. Sources of Natural Antioxidants

Natural antioxidant nanoparticles are materials with dimensions below 100 nm that exhibit antioxidant properties and can be derived both synthetically and from natural resources. Natural sources of antioxidants include bees propolis and honey, herbal extracts, and plant polyphenols from apples, lambmm's lettuce, and various herbs (Flieger *et al*., 2021) [21]. Nanoparticles can also be synthesized through methods such as pulse laser ablation in liquids (PLAL), microwave-assisted synthesis, chemical and green synthesis, solvent- thermal methods, sol-gel techniques, and the preparation of nanocomposites and core-shell structures (Ranjbar *et al*., 2018; Li *et al*., 2020) [51, 40].

2.3. Synthesis of Nanoparticles

Natural antioxidant nanoparticles are classified according to their sizes, shapes, dimensions, and compositions. The physical and chemical characteristics of naturally occurring antioxidants can be improved through interactions with nanoparticles (Parang *et al*., 2019) [48]. Various sources can be used to synthesise natural antioxidants nanoparticles, including plants and their extracts, microorganisms such as bacteria, fungi, yeast and algae, and biopolymers such as nucleic acids and proteins. Heat, microwave irradiation, and electrochemistry are the three main physical methods to synthesise natural antioxidants nanoparticles. In chemical methods, reducing agents, stabilising agents, and metal precursors are required. Electrodeposition, photochemical, sono-chemical, and electrochemical methods are also used to synthesise nanoparticles.

Almost spherical spherical in shape, with sizes ranging from 20 to 50 nm, the interaction of silver with aromatic compounds and ethers present in Terminalia arjuna bark extract was verified (Flieger *et al*., 2021; Flieger *et al*.2021) [21-22].

3. Mechanisms of Action

An antioxidant is a substance that can delay or inhibit oxidation and tension processes by scavenging and neutralizing free radicals through the formation of a stable radical or decomposition to non-radical derivatives (Parang *et al*., 2019) [48]. The antioxidant activity is related to different mechanisms, such as prevention of chain initiation, binding of metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging. At the cellular level, this activity leads to protecting essential cellular components against excessive damage induced by ROS in the body (Kozlov *et al*., 2024) [35].

An antioxidant nanoparticle is a particle with a diameter ranging between 1 and 100 nm that exhibits various good biological activities, such as anti-inflammation, anti-aging, anti-allergy, and anti-tumor properties.

Antioxidants are molecules with the ability of providing protection against oxidative stresses induced by free radicals, reactive oxygen species (ROS) like •OH, O2•, and ROO• (Flieger *et al*., 2021; Ge *et al*., 2022) [22].

At the cellular level, all antioxidant mechanisms have a significant role in the protection of critical cellular components from extreme damage caused by ROS in the human body. Nanoparticles are readily endocytized by macrophages and are then sequestered primarily within the intracellular phagolysosomal compartments. The nanoparticles-mediated redox perturbations result in the up-regulation of genes involved in various antioxidant, pro-inflammatory, and cell signaling responses (de *et al*.2021; Zhang *et al*.2023) [60].

3.1. Antioxidant Mechanisms

Natural antioxidants are molecules capable of counteracting the adverse effects of oxidation in living organisms by reacting with free radicals and preventing their harmful consequences. The main advantage of antioxidative nanoparticles compared with conventional antioxidative bulk materials includes an increased and more efficient surface area on which reactive oxygen species can be scavenged (Halliwell, 2024; Akbari *et al*., 2022) [7]. Natural antioxidants

found in garlic, ginger, might, aloe vera, turmeric, honey, etc. might reduce some of the damage caused by certain diseases. Plants are an excellent source of natural antioxidants and are widely used in the pharmaceutical, cosmetic, and food industries. The use of natural antioxidants along with their potential in nanotechnology and the reports stating the hazardous effects of nanoparticles place the necessity to further study the pharmacokinetics, biochemical, and histological changes in model animals under the influence of antioxidants nanoparticles (Hoang *et al*., 2021; Rathee *et al*.2023) [52].

3.2. Cellular Uptake

Many nanoparticles enter cells when incubated with cultured cells such as fibroblasts, epithelial cells, and macrophages. Uptake efficiency depends on the preparation temperature; particles prepared at 15 Â°C show better uptake by human fibroblast cells than those prepared at 25 Â°C. Internalized CeO2 nanoparticles typically concentrate within the cytoplasm around the cell nucleus, with minimal presence in early endosomes, late endosomes, lysosomes, or mitochondria (Dippong *et al*., 2021; Isaac *et al*., 2022) [18, 27]. Size affects cellular entry pathways: nanoparticles up to 40 nm in diameter can penetrate via clathrin-coated pits, and those around 30 nm through caveolae; particles larger than 50 nm generally undergo uptake through macropinocytosis or phagocytosis. The steps involved in cellular uptake can be outlined as (i) cellular binding, (ii) uptake, (iii) trafficking to organelles, and (iv) activation of biological responses (Parang *et al*., 2019) [48].

4. Biochemical Changes in the Liver

Table 1 summarizes the biochemical effects of various types of natural antioxidant nanoparticles used *in vivo*. Comparative analyses between natural antioxidant nanoparticles and pure antioxidants highlight the influence of efficacious doses and administration routes (Pitiot *et al*., 2022) [49].

Gamma-irradiated biogenic gold nanoparticles reduce blood glucose, hepatic injury markers (ALT, AST, ALP), lipid peroxidation (TBARS), nitric oxide (NO) levels, and pro-inflammatory cytokines (TNF-Î±, IL-6, IL-1Î²), while elevating antioxidants (SOD, catalase, GSH, GPx) in streptozotocin- and nicotinamide-induced diabetic rats (Abdelkader *et al*.2025) [3]. Moreover, they ameliorate serum triglycerides and total cholesterol, and increase hepatic glycogen content, thus minimizing diabetic damage (Parang *et al*., 2019) [48].

4.1. Liver Enzyme Activity

Alterations in liver enzyme levels are the most common feature employed to detect liver damage and are often linked to the performance of injured liver cells. Hepatocytes can release cytoplasmic enzymes into circulation when the plasma membrane is damaged or their excretion mechanisms are hindered, leading to enzyme accumulation and increased activity in the blood (Parang *et al*., 2019) [48]. The liver's vulnerability arises from its position immediately after the gastrointestinal tract, receiving the first exposure to substances absorbed from the small intestine. Liver enzyme activity serves as a biomarker for hepatic health; elevated enzyme levels in the blood signify liver damage and compromised function, enabling an assessment of the damage degree (Kalas *et al*.2021; Yaman *et al*., 2024). [30, 59]

4.2. Oxidative Stress Markers

Exposure to xenobiotics and environmental pollutants typically results in disrupted oxidant-antioxidant balance, promoting increased production of oxidative stress markers. The oxidative conversion of ferrous to ferric iron leads to the generation of lipid peroxidation products and oxidative stress markers in the early response phase (Ranjbar *et al*., 2018) [51]. This transition induces the release of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). An elevation in these enzymes facilitates the reduction of oxidative damage markers and suppresses the generation of additional radicals. SOD is widespread in human organs and effectively scavenges superoxide radicals by converting them into hydrogen peroxide and molecular oxygen, thus mitigating superoxide-based oxidative damage (Saxena *et al*., 2022; Islam *et al*.2022) [54, 28]. Verification of SOD activity involves the measurement of its subtypes, including copper-zinc (Cu-Zn SOD) and manganese (Mn SOD) isoenzymes (Parang *et al*., 2019) [48].

4.3. Lipid Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids and is a key indicator of oxidative stress and damage to cell membranes. It is one of the main biochemical changes observed in the liver following treatment with natural antioxidant nanoparticles (MartÃn-FernÃ¡ndez *et al*.2022). In this process, free radicals attack polyunsaturated fatty acids in membrane lipids, forming lipid radicals that further propagate the chain reaction. This ultimately leads to the production of stable end products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (M. Alshammari *et al*., 2023) [41]. Elevated hepatic levels of MDA indicate increased lipid peroxidation, damaged membrane integrity, and impaired liver function. Reduced levels of free radical scavengers like glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and heme oxygenase-1 (HO-1) worsen oxidative injury. These changes show redox imbalance and oxidative damage to membrane lipids after administering natural antioxidant nanoparticles. (Au-Yeung *et al*.2023; MartÃn- FernÃ¡ndez *et al*.2022) [12]

5. Histological Changes in the Liver

Histopathological examination of the liver has become a widely accepted tool for diagnosing physical and chemical-induced lesions in humans and animals. The effects of natural antioxidant nanoparticles on the liver have been investigated, with the results summarized in Table 2. The liver, gall bladder, pancreas, and spleen facilitate digestion and have a wide range of roles in overall system metabolism, including functions relating to sex behavior, muscle tone, and red blood cell production (Abbasi *et al*.2021) [2]. The liver plays a vital role in regulating the level of blood sugar, blood volume, concentration, and composition of amino acid plasma, draining venous blood from the digestive tract to the portal system, processing and distributing nutrients (including vitamins and minerals), producing blood proteins and enzymes, storing glycogen, detoxifying harmful substances, regulating immune responses, and converting excess CHO and proteins into fats (Parang *et al*., 2019) [48].

The liver is potentially vulnerable to the deleterious effects of nanoparticles that, once absorbed, are distributed by the

blood and reach different organs, suggesting that the liver is the main organ targeted and damaged by nanoparticles and digestive tract (Anwar K Abdelhalim & M Jarrar, 2011) [10]. The histopathological results reveal that these nanoparticles have a destructive effect on liver cells, which shows the potential hazards of their extensive use in pharmacology and biomedicine. Liver tissue regeneration was improved in response to treatment with natural antioxidant nanoparticles (Li *et al*., 2022) [38].

5.1. Histopathological Examination

Histopathological examinations are important for assessing liver alterations in laboratory studies. Damage or disorders related to liver functions, induced by chemicals and toxins, can be identified by pathological changes in liver tissues. Such changes often correlate with an increase in marker enzyme levels.

Histopathological evaluation further supplements data from biochemical and molecular analyses, aiding in the detection of liver cell damage and regeneration. Electron microscopy provides high-magnification visualization elucidating mechanisms involved in chemical-induced toxicity (Parang *et al*., 2019) [48].

Reduced glutathione (GSH) constitutes 90% of intracellular non-protein thiols, protecting cells against reactive oxygen species (ROS); its depletion is a sensitive indicator of oxidative stress. Liver morphology remains normal following toxicant exposure, provided GSH concentrations do not fall below 70%. GSH plays a vital role in antioxidant defence, free radical scavenging, and doctoring of toxic agents that elicit oxidative stress, with its reduction strengthening biochemical and histological insights into liver damage (Anwar K Abdelhalim & M Jarrar, 2011) [10].

In contrast to biochemistry, tissue pathology supplies additional direct information about the quantity, type, and extent of pathological changes. Liver histopathology and tissue regeneration following silver nanoparticle treatment further clarify the damage mechanisms (Abdelrahman *et al*.2022) [4].

5.2. Tissue Regeneration (Liver Structure and Function)

The liver is the largest internal organ of the body, and it plays an important role in metabolism and homeostasis. The liver is enclosed by Glisson's capsule. Connective tissue folds that arise from the capsule, accompanied by branches of the hepatic artery, the portal vein and bile duct, enter the liver to form a structure referred to as the portal triad. Liver parenchymal cells are referred to as hepatocytes; these are polygonal in shape and form an interconnected network of plates that contain capillaries, known as liver sinusoids, into which bile flows (Hassani, 2022).

6. Biochemical Changes in the Kidneys

Kidney function tests and electrolytes serve as indicators of renal sufficiency and are essential for water balance, membrane integrity, and acid-base balance. Hepatotoxicity and nephrotoxicity are well- documented causes of elevated plasma activity in kidney function tests (Anwar K Abdelhalim *et al*., 2020) [11].

Treatment with natural antioxidant nanoparticles results in an increase in plasma creatinine level and a decrease in plasma urea and uric acid levels. Creatinine is the most commonly used indicator of renal function. Under pathological conditions, increased creatinine may result from renal insufficiency induced by disrupted glomerular filtration or the inability to excrete creatinine (Parang *et al*., 2019) [48]. Urea is formed during protein metabolism and is excreted through renal glomerular filtration, being the second main indicator of renal function. An elevation in plasma creatinine coupled with a reduction in urea and uric acid indicates disturbances in renal function and electrolyte balance (McGregor & Jones, 2021) [46].

Further assessment of elevated liver enzymes (AST, ALT, ALP, and GGT), decreased albumin, hypernatremia, hypokalemia, and histopathological examinations reinforce these findings. Natural antioxidant nanoparticles differ in their effects: Au-NPs and Ce-NPs induce liver and kidney injury, whereas Ag-NPs ameliorate the nephrotoxicity and hepatotoxicity induced by Au- and Ce-NPs (Li *et al*., 2022; Feng *et al*.2022) [39, 20].

6.1. Renal Function Tests

The treatment of rats with natural antioxidant nanoparticles is associated with biochemical alterations indicative of renal impairment. The ingestion of 100 mg/kg/day results in increased concentrations of blood urea nitrogen (BUN) and serum creatinine (Cr), reflecting a compromised ability of the kidneys to excrete nitrogenous waste products. Higher doses (1000 mg/kg/day) elevate the relative kidney weight and disturb the balance of electrolytes, specifically raising sodium (Na+) and potassium (K+) ions, which indicates perturbation of osmotic regulation (Abdulmalek *et al*., 2021; Lesnichaya *et al*.2021) [5, 37].

These findings coincide with reductions in catalase activity and diminished protection against lipid peroxidation, thereby heightening the vulnerability of renal tissue to oxidative damage (Parang *et al*., 2019; Anwar K Abdelhalim *et al*., 2020) [48, 11]. The impairment of the oxidant/antioxidant equilibrium within the renal system undermines its protective mechanisms, leading to functional disturbances. The modulation of these disturbances with antioxidants such as vitamins E and C or lipoic acid suggests a route for mitigating the nephrotoxic effects. Hence, biochemical indicators underscore the oxidative stress and consequent renal dysfunction induced by the nanoparticles. Investigations into safety parameters are paramount to clarifying the conditions conducive to hepatic and renal recovery following exposure. Continuous monitoring of markers of oxidative stress and renal function becomes essential for the safe application of natural antioxidant nanoparticles (DragoÈ™ *et al*., 2025; Martemucci *et al*.2023) [43].

6.2. Electrolyte Imbalance

Elevated blood urea and slight increases in plasma creatinine, sodium, potassium, and phosphorus levels point to electrolyte imbalance and renal impairment. The kidney's role in electrolyte homeostasis involves regulating the concentrations and volumes of sodium, potassium, phosphate, and other essential ions in the circulating blood (Brookes & Power, 2022; Kene *et al*.2021) [14, 32]. A noticeable increase in the levels of organic electrolyte anions (phosphate) in plasma during nephrotoxicity is indicative of tubular damage; this damage prevents the renal tubular epithelium from resorbing these anions (Parang *et al*., 2019) [48]. The imbalance in electrolytes is anticipated because kidney damage is usually associated with retention of

sodium, potassium, and phosphate, which may further contribute to the nephrotoxicity of the nanoparticles investigated here (Mazzaferro *et al*.2021) [45].

7. Histological Changes in the Kidneys

Histopathological analysis of kidneys extracted from the blank group demonstrated clear glomeruli and renal tubules with typical histological architecture. The kidneys of the treated group displayed shrinking glomeruli, fibrotic matter deposition in the glomerular tufts, and tubular dilation. Quantitative assessment revealed a marked reduction in glomerular count in treated versus control samples (Al-Gholam *et al*., 2025; Klinkhammer *et al*.2022) [9, 34].

Toxic nanomaterials induce juxtaglomerular atrophy, extensive renal tubular injury and degeneration, and massive glomerular damage. Such agents can increase both kidney weight and the kidney-to-body weight ratio, induce significant glomerular contraction, and accelerate fibrosis (Anwar K Abdelhalim *et al*., 2020) [11]. Histopathological studies further confirm that inherent nephrotoxic effects of these toxic nanoparticles are partially alleviated upon administration of antioxidant compounds, as evidenced by diminished inflammatory infiltration, tubular necrosis, and vacuolar degeneration (A. Nomier *et al*., 2022; Parang *et al*., 2019) [1, 48].

7.1. Glomerular Changes

Liver sinusoidal dilation and congestion of the portal vein were observed in rats administered gold nanoparticles; furthermore, signs of hepatocyte injury and cytoplasmic vacuolization were evident (Anwar K Abdelhalim & M Jarrar, 2011) [10]. Similarly, proximal tubular degeneration and mild intertubular vasculitis accompanied by peritubular interstitium neutrophil infiltration were noted in treated rats. In a complementary study, treatment resulted in glomerular congestion and hydropic degeneration of cortical tubular epithelium (Anwar K Abdelhalim *et al*., 2020) [11].

7.2. Tubular Damage

Distal tubules exhibited a narrow lumen, absence of as the basal membrane, cellular vacuolization, and an eroded apical membrane. Changes in the proximal tubules included the death of numerous epithelial cells, absence of the apical brush border, and some intraluminal cellular debris. Glomerular, mesangial, and capillary basement membrane are all normal in treated rats, with only minor glomerular space decrement (<10 µm) (Mohamed & Shenouda, 2021; Kuzmenko *et al*., 2021) [47, 36]. The damage and vacuolization indicate a loss of the absorptive function. Nephron damage caused by commercial Ag-NPs, such as atrophy of nephrons, damage of the glomerular basement membrane (Parang *et al*., 2019) [48], was not observed. Similarly, CeOâ‚‚-NPs elicit oxidative stress and apoptosis in kidney cells (Ranjbar *et al*., 2018) [51].

8. Safety and Toxicity of Natural Antioxidant Nanoparticles

The safety and toxicity of natural antioxidant nanoparticles require careful evaluation prior to clinical application. Toxicological studies have demonstrated that exposure to silver nanoparticles in rats does not lead to significant changes in body weight or feed intake, yet notable alterations in tissue biochemical markers occur following oral administration (S. Adeyemi & Adewumi, 2014) [53]. The observed biochemical modifications appear to relate to the capacity of silver nanoparticles to bind thiol groups in proteins, resulting in complex formation and modulation of enzyme activities such as alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase. Regulatory bodies including the World Health Organization,

U.S. Environmental Protection Agency, and European Scientific Committee on Consumer Safety have introduced guidelines and standards governing the use of various nanoparticles. A sterilisation-standard protocol has also been established for silver nanoparticle suspensions intended for diverse applications (Ranjbar *et al*., 2018) [51]. These safety and regulatory assessments form an essential framework for determining the clinical potential of natural antioxidant nanoparticles (Marino *et al*., 2022) [42].

8.1. Toxicological Assessments

The utilization of nanomaterials in food packaging and other applications necessitates the assessment of their potential toxicological effects upon exposure. Nanoparticle toxicity is influenced by particle size, concentration, chemical composition, and surface structure, which dictate cellular penetration, a ccumulation, metabolism, distribution, and elimination. Nanomaterials prepared from natural products have attracted attention due to their low toxicity and biocompatibility (S. Adeyemi & Adewumi, 2014) [53].

Toxicological assessment encompasses a range of studies investigating adverse effects and pathological changes following acute and chronic exposure via oral, dermal, inhalation, and other routes. Nanoparticles may induce systemic toxicity through blood circulation or localize effects through tissue deposition, requiring evaluation at the organ level. Characterization of multiple endpoints, including physicochemical, in vitro, and *in vivo* parameters, is essential for comprehensive safety evaluation. *In vivo* studies provide a more accurate representation of toxicity, accounting for bioaccumulation and metabolism (Sharma *et al*., 2021; Suthar *et al*.2023) [55, 57]. Historically, the safety of nanoparticles was considered analogous to single compounds but with enhanced bioavailability; however, current understanding recognizes that toxicity is governed by size, shape, and surface properties, and materials previously deemed inert may exhibit activity at the nanoscale (Ranjbar *et al*., 2018) [51].

8.2. Regulatory Considerations

Regulatory authority assessments of nanomaterials encompass comprehensive evaluations of manufacturing sites and raw material sources to ensure compliance with established standards (ChÃivez-HernÃindez *et al*.2024). Approval of a new drug submitting natural antioxidant nanoparticles necessitates extensive toxicological investigations, including acute, subacute, and chronic toxicity studies, to establish a detailed safety profile. Toxicity is systematically appraised by defining the no-observed-adverse-effect level (NOAEL), below which adverse effects are absent, and the lowest-observed-adverse-effect level (LOAEL), the minimum administered dose eliciting adverse outcomes (Kale *et al*.2022) [31]. These parameters inform the safety margins pertinent to safe clinical use. On this foundation, regulatory agencies classify antioxidant nanoparticles with respect to safety, categorizing them as substances suitable for unrestricted use, use with limitations, use restricted to specific applications, or outright banned (Flieger *et al*., 2021) [22].

9. Conclusion

Natural antioxidants possess free radical-scavenging activity and enhance the antioxidative defense systems of cells. These antioxidative properties provide the basis for the application of natural antioxidants to the therapies of various diseases characterized by the generation of excessive reactive oxygen species (ROS) that cause lipid oxidation and tissue injuries. Recent success in the green synthesis of metal nanoparticles using plant extracts and natural antioxidants has led to growing biomedical applications of natural antioxidant nanoparticles (NPs) due to their unique physicochemical properties and broad bioactivities. The biochemical and histological changes in the liver and kidneys of rats treated with natural antioxidant nanoparticles show that several liver and renal function tests, serum electrolyte parameters, and oxidant antioxidant markers are significantly affected by oral exposure to natural antioxidant nanoparticles. Additionally, natural antioxidant nanoparticles alter the histological structure of the liver and kidneys in a dose-dependent manner by a mechanism that involves enzymatic and non-enzymatic molecules, as well as different inflammatory factors. Therefore, this review critically emphasizes the biochemical and histological changes in the liver and kidneys of rats treated with natural antioxidant nanoparticles and their concomitant effects.

Silver nanoparticles (AgNPs) are extensively utilised in various commercial products including wound dressings, toothpastes, cosmetics, textiles and food packaging. Consequently, they are released into aquatic systems and can show adverse effects on aquatic organisms. Exposure to fish via aquatic systems or terrestrial ecosystems may lead to adsorption or ingestion of AgNPs by fish, resulting in accumulation in internal organs such as the liver and kidneys. Therefore, a study was designed to investigate the toxicological effects of silver nanoparticles deposited in water by examining their impact on the liver and kidney of fish. Data indicate that AgNPs promote oxidative stress leading to DNA damage in gills and bones and up-regulate metallothionein expression in the liver, suggesting the onset of defensive and repair mechanisms. Advancements in nanotechnology have increased the applications of nanoparticles in commercial, medical and industrial fields, thus increasing the likelihood of their release into the environment. This review highlights the possible toxic effects of silver nanoparticles on the liver and kidneys, organs that play important roles in detoxification and excretion. Several investigations have demonstrated the ability of AgNPs to induce oxidative stress, an imbalanced redox state that triggers pro-inflammatory reactions and damage to cellular components. The review discusses the biochemical and histological changes observed in the liver and kidney of rats treated with these nanoparticles, and their potential underlying mechanisms.

Gold nanoparticles (GNPs) possess unique physicochemical properties, including tunable optical and physicochemical properties, enhanced radiation effects, high payload surface, and enhanced permeability in targeted delivery. These properties have resulted in extensive use for targeted delivery of drugs to desired cells and better visualisation of tissues, sensing, photothermal and photoacoustic imaging, and positron emission tomography. However, GNPs can cross biological barriers and interact at the cellular, sub-cellular, and protein levels in biological systems. Undesirable biochemical or cellular interactions can progress and influence systemic homeostasis beyond the initial site of contact. The primary routes of GNP exposure for humans are inhalation, dermal contact, and ingestion. GNPs taken up through the thoracic region of the respiratory tract translocate to regional lymph nodes and into the circulatory and central nervous systems. Dendritic cells and macrophages internalise GNPs and release pro-inflammatory cytokines, while single GNPs might remain in the lungs, leading to biochemical and immunogenic reactions. GNPs coated with cationic or anionic molecules accumulate in the clathrin-coated vesicles of the respiratory tract. The biopersistence of particles depends on shape, size, exposure level, forms of aggregation, and surface chemistry, which can further enhance the toxicity of GNPs.

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