Environ. Eng. Res. 2025; 30(4): 240624
https://doi.org/10.4491/eer.2024.624

pISSN 1226-1025
eISSN 2005-968X

Research

Ameliorative effects of ginger extract on ZnO and TiO_2 nanoparticle – induced toxicity in liver and kidneys of rats

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Received October 31, 2024 **Revised** December 21, 2024 **Accepted** December 26, 2024

ABSTRACT

Recently, the use of ZnO and TiO_2 nanoparticles (NPs) in commercial and industrial products has increased. However, concerns have been raised regarding their possible negative effects on human health. Limited studies have been conducted on co-exposure toxicity of ZnO and TiO_2 in rats, so the consequences of their co-exposure have not yet been reported. Recently, ginger has gained considerable attention owing to its ability to improve human health. The aim of this study was to examine effects of ginger extract on serum biochemical, hematological, and histological alterations induced by ZnO and TiO_2 alone and in combination in rats. Seventy adult Sprague-Dawley rats were divided into seven groups, with 10 rats in each group. Distilled water played the role of vehicle for the control groups. ZnO NPs and TiO_2 NPs significantly reduced hemoglobin, platelets, and red blood cells, while increasing white blood cells, aspartate, alkaline phosphatase, alanine aminotransferase, urea, and creatinine levels. Histological analysis revealed time-dependent liver and kidney damage. Simultaneous exposure to both NPs initially resulted in less damage; however, more damage occurred later. Ginger significantly reduced these effects, suggesting that ginger extract may be a potential biocompound for shielding and improving liver and kidney functions against NP-induced toxicity.

Keywords: Hepatotoxicity, Nanoparticles, Nephrotoxicity, Oral route, Titanium Dioxide, Zinc Oxide

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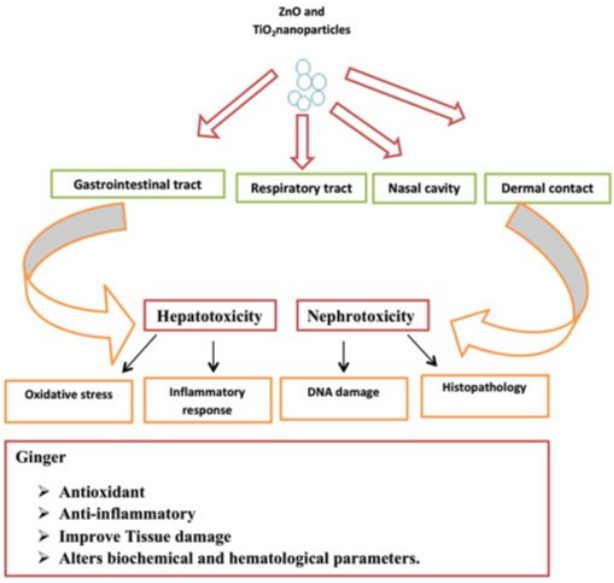
Graphical Abstract



1. Introduction

Nanoparticles (NPs) vary in size from 1 to 100 nm. Owing to their special qualities, nanomaterials are utilized extensively in consumer products such as electronics, antibacterial materials, cosmetics, and medications [1]. There are several routes by which the human body can inhale NPs, such as direct exposure to the skin, entry through the digestive tract, arterial injection, respiratory pathways, and implantation. The absorbed NPs are then transported to vital organs through the lymphatic and circulatory systems, where they interfere with biological molecules and cause physiological systems to malfunction [2]. Because physiological homeostasis is maintained by multiple interconnected systems in the human body, unexpected NP invasion can disrupt cell signaling under normal conditions, disrupt organ and cell functions, and cause pathological diseases [3]. NPs can interact with most cellular components, such as proteins, mitochondria, and DNA. They produce reactive oxygen species (ROS), which impair the ability of cells to perform diverse functions [4].

Zinc oxide nanoparticles (ZnONPs) are frequently utilized as active components in daily applications, such as toothpaste, food materials, sunscreen, food additives, and several medications, because of their remarkable antibacterial properties and ultraviolet (UV) light absorption [5]. This has led to concerns about the degree of their probable harmfulness, including their genotoxic, cytotoxic, and pro-inflammatory effects [6]. Concerns about the unexpected and detrimental health impacts of ZnONPs have been expressed in the scientific and public communities [7].

High concentrations of ZnONPs have been proven to be toxic, even though the US Food and Drug Administration has listed them as "generally recognized as safe" (GRAS) substances [8]. The long-term safety of low quantities of ZnONPs has not yet been elucidated [9]. The liver and kidneys are the primary organs for ZnONP buildup in preliminary studies [10]. ZnONPs primarily cause oxidative stress, which inhibits antioxidant systems in kidney and liver cells [11]. ZnONPs can trigger different cell death pathways and cause renal and hepatic inflammation [12].

Titanium dioxide nanoparticles (TiO_2NPs) are also used in medical and industrial fields as well as in food colors [4]. The brightness and high refractive index of TiO_2NPs render them white; therefore, they are used in skincare products, water treatment agents, sunscreens, and cosmetics [13]. Because TiO_2NPs provide opacity to products, they are used to produce paints, cosmetics, toothpaste, plastics, and other pharmaceutical products [14]. Human exposure to TiO_2NPs has increased because of their widespread manufacture and use in various media and pathways [15]. TiO_2NPs can be ingested directly by humans as nanomedicines or nanofoods. There is growing concern regarding the potential health impacts of TiO_2NP exposure [16].

ROS may increase sharply as a direct result of TiO_2NPs entering cells by inducing a respiratory burst and ROS regeneration cycle [17]. This can lead to genetic damage, inflammation, and damage to mitochondrial function and structure, ultimately resulting in cell autophagy, apoptosis, or necrosis [18]. An increasing amount of data indicates that TiO_2NPs cause nephrotoxicity and liver function impairment, among other harmful health effects. The toxicity of TiO_2NPs was recently revealed in published data. Liu et al. found that red blood cells (RBCs), lymph nodes, brain, liver, and lungs were all sites of absorption and accumulation of TiO_2NPs [19]. An in vitro investigation revealed that renal proximal cell death was triggered by high concentrations of TiO_2NPs [20]. Additionally, TiO_2NPs may induce nephric inflammation and impairment, which could be due to oxidative stress [18].

The liver is the part of the body involved in detoxification, nutritional storage, secretion, digestion, and biosynthesis. Exposure to different NPs can cause hepatotoxicity, which is defined as harmful consequences of nanoparticles exposure or liver damage [21]. Excessive use of these chemicals results in the massive production of free radicals, lipid peroxidation, structural and functional destruction of membranes, and, ultimately, lethal toxicity to hepatic cells [22]. The kidneys are the primary organs for the secretion of both endogenous and foreign chemicals. The kidney filters out all hazardous substances and metabolites, which are eliminated through the urine. NPs cause renal toxicity directly in the kidney through a process called biotransformation in the proximal tubular cells or indirectly through the liver by producing toxic metabolites that have nephrotoxic effects and cause acute renal failure [23].

Ginger, *Zingiber officinale* Roscoe, is a member of the family Zingiberaceae, and it is widely used as a spice to improve food flavor. It is a common medicinal herb containing flavonoids and other polyphenols with anti-inflammatory, antioxidant, antidiabetic, hypolipidemic, and anticarcinogenic properties. Because ginger contains gingerols, shogaols, zingerone, gingerdiol, and zingiberene, it possesses antioxidant properties [24]. Numerous studies have demonstrated the hepatoprotective and nephroprotective properties of ginger. These effects are attributed to increased levels of liver and kidney biomarkers, decreased inflammation, increased antioxidant activity, and prevention of toxicant-induced lipid peroxidation [25].

Based on these observations, we investigated the potential adverse effects of the combined use of ZnONPs and TiO_2NPs, which have not yet been reported. The primary goal of this study was to assess the hepatorenal toxicity of ZnONPs and TiO_2NPs in rats. Furthermore, we aimed to evaluate whether ginger, a natural antioxidant and anti-inflammatory agent, can alleviate NP-induced hepatorenal damage. In addition, hematological, biochemical, and histological analyses were performed to evaluate the *in vivo* toxicity ZnONPs and TiO_2NPs in rats, with a special focus on their hepatotoxic and nephrotoxic effects.

2. Materials and Methods:

2.1. Chemicals

ZnONPs were obtained in the form of a buffer solution of saline phosphate at 50 wt.% from Sigma-Aldrich Co. (USA). Analytical grade TiO_2NPs were purchased from Sigma-Aldrich Co. Prior to the start of the trial, a pilot study was conducted to identify the initial dosage of each NP that showed hazardous effects on rats without mortality. Based on the pilot study and previous studies, 300 mg/kg of ZnONPs [26] and 50 mg/kg of TiO_2NPs [27] were selected.

Fresh and high-quality ginger rhizomes were purchased from a local market in Lahore, and a botanist from the Lahore College for Women University's Botany Department verified their authenticity. The rhizomes were washed thoroughly and air-dried. The dried rhizomes were finely ground using an electrical grinder. A dose of 200 mg/kg body weight each day was administered to each rat in the form of 0.5 mL of a ginger suspension made from powdered ginger and distilled water. The lowest dosage of ginger used was consistent with the average daily intake of people, as found in a survey [28].

2.2. Animals

Three-week-old Sprague-Dawley rats of both sexes weighing 250–260 g were purchased from the National Institute of Health, Islamabad, Pakistan. The rats were kept in spacious cages made of polypropylene and stainless steel with wood shavings, typical pellet meals, and water. At least one week before the experiment, the animals were kept in a well-ventilated room with a 12 h artificial light/dark cycle, constant temperature of 23 ± 2°C, and relative humidity of 60–70%. After one week of acclimatization, the rats were weighed and randomly assigned to the control and experimental groups, with each treatment group consisting of 10 rats.

2.3. Experimental Design and Dosage

Seventy adult Sprague-Dawley rats were randomly assigned to seven groups, with 10 rats in each group. An electrical balance was used to weigh the NPs in grams, and a magnetic stirrer was used to mix them with water. The animals were orally administered NPs via gavage.

Group I – Control group: Rats in this group were provided with water daily via oral gavage.

Group II – Experimental group I (E1): Rats in this group were orally administered ZnONPs (300 mg/kg body weight) daily.

Group III – Experimental group II (E2): Rats in this group were orally administered TiO_2NPs (50 mg/kg body weight) daily.

Group IV – Experimental group III (E3): Rats were orally administered ZnONPs (300 mg/kg body weight) and TiO_2NPs (50 mg/kg body weight) daily.

Group V – Experimental group IV (E4): Rats were orally administered ZnONPs (300 mg/kg body weight) simultaneously with ginger extract (200 mg/kg body weight) daily.

Group VI – Experimental group V (E5): Rats in this group were orally administered TiO_2NPs (50 mg/kg body weight) simultaneously with ginger extract (200 mg/kg body weight) daily.

Group VII – Experimental group VI (E6): Rats in this group were orally administered ZnONPs (300 mg/kg body weight) and TiO_2NPs (50 mg/kg body weight) simultaneously with ginger extract (200 mg/kg body weight) daily.

All experiments with laboratory animals were performed in compliance with the guidelines approved by the University Ethics Committee.

2.4. Blood Sample Collection and Tissue Preparation

The animals in each group were anesthetized with ether and then dissected after an overnight 8 h fasting period at the end of the 4^{th} and 8^{th} weeks of the experiment. To obtain blood samples, the anesthetized rats were subjected to cardiac puncture, and blood was collected into heparinized and non-heparinized tubes for hematological and biochemical analyses. Their hands and limbs were fixed with pins, and scissors were used to cut off the skin. Blood samples were collected in non-heparinized tubes, left to clot at room temperature for 20 min, and centrifuged for 15 min at 3000 rpm to extract the serum. The clear supernatant and serum were

separated for estimating biochemical parameters such as ALT, AST, ALP, total protein, albumin, urea, and creatinine levels. The liver and kidneys were separated, and the adhering blood was removed by washing with cold physiological saline (0.9% NaCl) and drying between filter papers. The kidney and liver tissues were fixed in 10% neutral buffered formalin, and the samples were dehydrated by passing through increasing grades of ethyl alcohol, cleaned in xylene, and then embedded in paraffin. The embedded tissues were sectioned (5–6 μm thickness) using a rotary microtome (Leica RM2235; Leica Microsystems, USA)). For microscopic examination, the sections were stained with hematoxylin and eosin (H&E).

2.5. Statistical Analysis

Datasheets were created using Microsoft Excel. The data were statistically analyzed using one-way ANOVA. Differences were considered statistically significant when the p value was less than or equal to 0.05. The data were analyzed using SPSS version 22.

3. Results and Discussion

3.1. General Observations

None of the treated groups experienced any mortality. When the rats were administered individual NPs, the only noticeable alteration was a slight reduction in furring. After 20 days, food consumption increased in the ZnONP-treated group, and this trend persisted in the other experimental groups until the end of the experiment. Food consumption was lower in the MIX group than in the other groups.

3.2. Hematological Parameters

The results are displayed in Table S1; a significant decline in hemoglobin (HB), red blood cells (RBC), and platelets (PLT) levels was observed in the ZnONP-treated group at 4 and 8 weeks when compared with the control groups. However, an increase in white blood cells (WBC) levels was detected in this group when compared with the control groups. In the TiO_2NP-treated group, a significant decline in hemoglobin (HB), red blood cells (RBC), and platelets (PLT) levels was observed in the 4^{th} and 8^{th} week; however, white blood cells (WBC) levels increased during the experiment. The MIX group showed a significant decline in hemoglobin (HB), red blood cells (RBC), and platelets (PLT) levels and an increase in white blood cells (WBC) level during the 60 days of the experiment when compared with the control group.

However, concurrent usage of ginger extract along with ZnONPs resulted in a gradual increase in hemoglobin (HB), red blood cells (RBC), and platelets (PLT) levels when compared with the groups treated with ZnONPs alone. A significant decrease in white blood cells (WBC) count was detected in the ZnO + ginger extract-treated group. According to Table S1, TiO_2NP dosage along with ginger extract caused a significant increase in hemoglobin (HB), red blood cells (RBC), and platelets (PLT) levels and a significant decrease in white blood cells (WBC) count when compared with the groups treated with TiO_2NPs alone. In the MIX group, concurrent treatment with ginger resulted in a significant increase in hemoglobin (HB), red blood cells (RBC), and platelets (PLT) levels and a decrease in WBC count when compared with the groups treated with combined NPs alone.

3.3. Biochemical Parameters

Table S2 presents the mean results of biochemical parameters such as Alanine transaminase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), creatinine, and urea in the control and treated groups at 4 and 8 weeks. The mean results showed a slight increase in Aspartate aminotransferase (AST) levels in the ZnONP, TiO_2NP, and MIX groups at 4 weeks when compared with the control group. A significant increase in Aspartate aminotransferase (AST) levels was detected in all treated groups at the end of 8 weeks. Similarly, Alanine transaminase (ALT) and Alkaline phosphatase (ALP) levels were significantly higher in the NP-treated groups. Comparison between Alanine transaminase (ALT) and Alkaline phosphatase (ALP) in the ZnONP, TiO_2NP, and MIX groups revealed that the Alkaline phosphatase (ALP) concentration increased as the Alanine transaminase (ALT) concentration increased. This is due to the localization of both enzymes in the cell. ALT is present in the cytoplasm, whereas Alkaline phosphatase (ALP) is located under the cytoplasmic membrane; therefore, in the case of any cell destruction, more Alanine transaminase (ALT) and Alkaline phosphatase (ALP) are released into the bloodstream (Fig. 1).

According to the findings of this study, NP administration caused a significant increase in urea and creatinine levels in the NP-treated groups when compared with the control group. In the Mix group, in which the animals were treated with both NPs, the maximum increase in all enzymes was observed (Fig. 1). Administration of ginger along with NPs caused a significant decrease in enzyme concentrations when compared with the groups treated with NPs alone (Table S2).

Fig. 1 is about the graphical presentation of the biochemical parameters difference of liver and kidneys at the end of 4^{th} and 8^{th} weeks of treatment.

3.4. Histopathological Results

The findings of the current study revealed that ZnONPs induced major alterations in the renal tissues after 60 days of exposure. Normal renal cortex and nephritic tubules were observed in the kidney sections of the control group (Fig. 2a). Arrows in the figure point normal sized renal cells without any congestion and obstruction. Liver sections from the control group revealed normal structures. As it can be seen from the figure, there is normal hepatic portal triad, central vein, and hepatocytes (Fig. 2b). Arrows point that there are normal sized hepatocytes and central vein without any infiltrations. The group treated with ZnONPs revealed pathological changes in the kidney, including enlargement of the collecting tubules and proliferation of inflammatory cells between the collecting tubules. Arrows point the inflamed renal cells (Fig. 3a). In the liver, ZnONP-induced pathologies included aggregation of inflammatory cells around blood vessels and proliferation of Kupffer cells. As it can be seen from figure (arrows) inflamed kupffer cells (Fig. 3b).

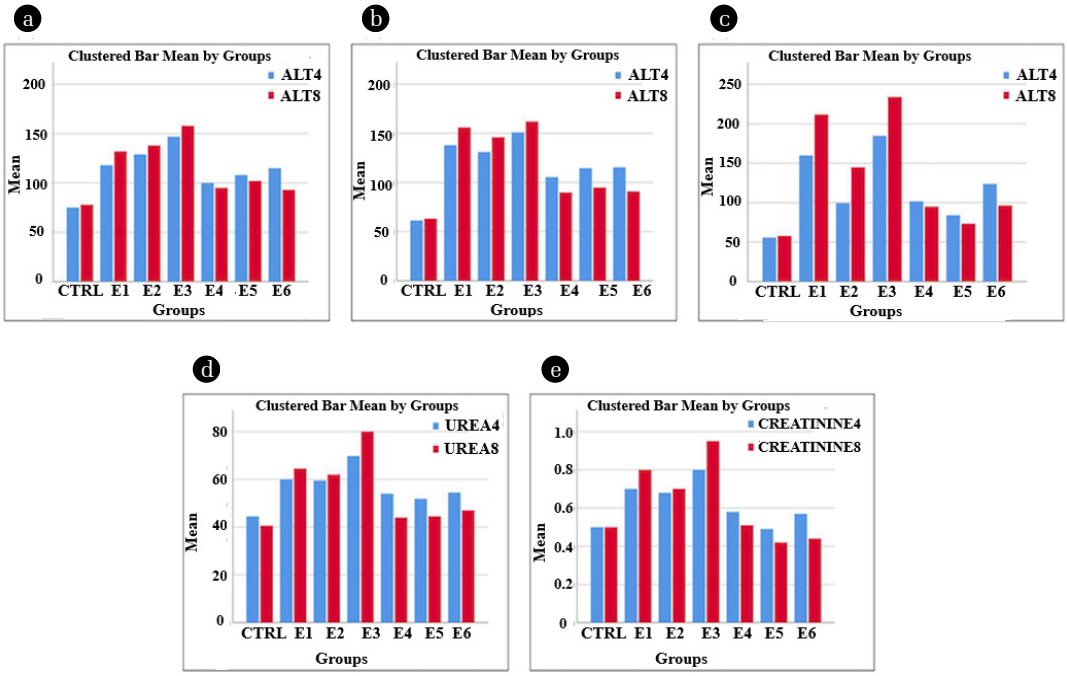


Fig. 1. Hepatic and renal functional markers aspartate, alanine aminotransferase, alkaline phosphatase (AST, ALT, and ALP, respectively) (a, b, c), Urea, and Creatinine (d, e) in the serum of the control and treated rats after 4 and 8 weeks of administration

Major histopathological changes were observed in the kidney and liver tissues of the TiO_2NP-treated group. The renal histological analysis showed inflammation and dilation of Bowman's capsule as well as detachment of junctions between the glomeruli and renal tubules. As arrows point the dilation of the Bowman's capsule of treated group (Fig. 4a). When the animals were exposed to TiO_2NPs orally, these NPs entered the liver through blood circulation. The NP aggregates were taken up by Kupffer cells and most likely ended up in the phagolysosomes found in the hepatic sinusoids and portal tract. The hepatic histological analysis revealed lesions and dilation of the central vein (arrows) (Fig. 4b).

Major histopathological changes were observed in the Mix group treated with both ZnONPs and TiO_2NPs. Kidney sections from this group showed congestion of blood vessels with inflammatory cells, obstruction, and necrotic patches in the renal tubules, as

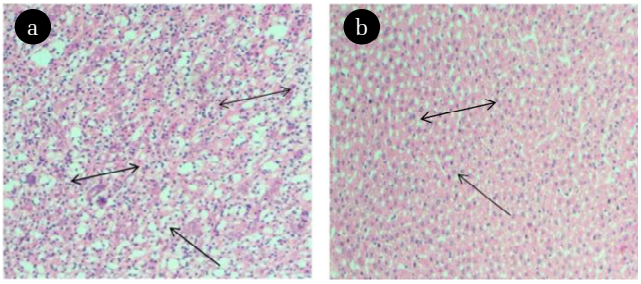


Fig. 2. Section of rat (a) kidney showing normal structures i.e normal cortex and tubules and (b) liver of the control group showing normal structure i.e hepatic portal triad, and uniform shaped hepatocytes. H&E stain 400X.

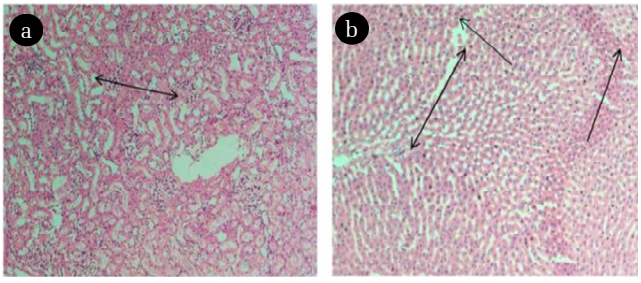


Fig. 3. Section of rat (a) kidney and (b) liver treated with ZnONPs showing infiltration of inflammatory cells and (a) between renal tubules and enlargement of collecting tubules (b) around blood vessels and proliferation of Kupffer cells. H&E stain 400X.

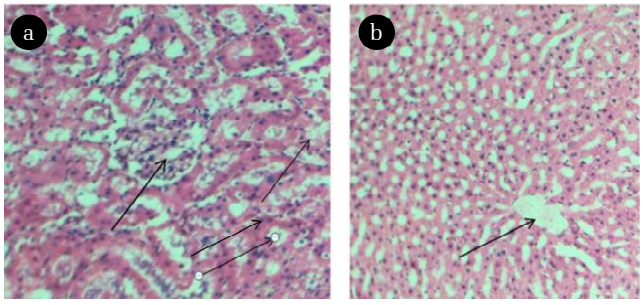


Fig. 4. Section of (a) kidney and (b) liver in rats treated with TiO_2NP_s demonstrating (a) Bowman's capsule swelling and dilatation (arrow) and (b) inflamed Kupffer cells as well as congestion and obstruction of the central vein. H&E stain 400X.

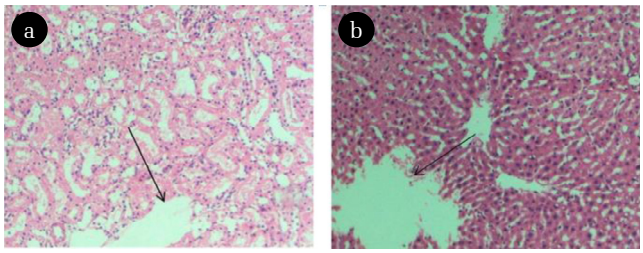


Fig. 5. Section of (a) kidney and (b) liver of rats treated with ZnONPs and TiO_2NPs showing (a) congestion of blood vessels, degeneration and necrosis of tubules, and (b) inflamed hepatocytes as well as congestion and obstruction of the central vein. H&E stain 400X.

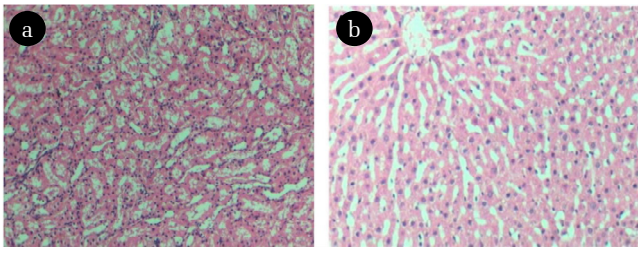


Fig. 6. Section of (a) kidney and (b) liver of rats treated with ZnONPs along with ginger showing (a) ameliorative role of ginger with respect to normal-sized collecting tubules and less congestion of blood vessels and (b) ameliorative role of ginger with respect to normal-sized hepatocytes and less congestion in the central vein. H&E stain 400X.

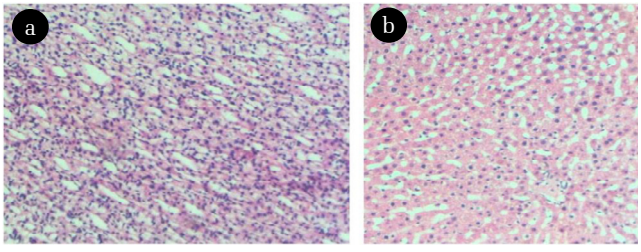


Fig. 7. Section of (a) kidney and (b) liver of rats treated with TiO_2NPs along with ginger showing (a) ameliorative role of ginger with respect to normal-sized collecting tubules and less congestion of blood vessels and (b) normal architecture. H&E stain 400X.

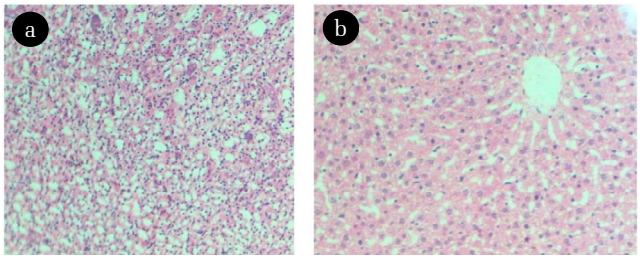


Fig. 8. Section of (a) kidney and (b) liver of rats treated with ZnONPs and TiO_2NPs along with ginger demonstrating (a) normal hepatocytes as well as less congestion and obstruction of the central vein and (b) normal-shaped hepatocytes, central vein, and portal tract. H&E stain 400X.

arrow points the degeneration and necrosis in the renal tubule (Fig. 5a). The liver sections of this group showed inflamed hepatocytes with mild vacuolation, apoptosis, and congestion of the central vein with inflammatory cells associated with sinusoidal dilation arrows (Fig. 5b).

Kidney sections of the rats treated with ZnONPs along with ginger for 8 weeks showed an ameliorative effect of the ginger extract: the collecting tubule returned to its normal size and fewer inflammatory cells from the collecting tubules, although some pathological effects, such as necrotic areas, were still observed (Fig. 6a). The liver sections of this group showed remarkable improvement in pathological changes induced by ZnONPs, including normal-sized hepatocytes and reduced congestion of the central vein, as there were fewer inflammatory cells than the ZnO-treated group alone (Fig. 6b).

Microscopic examination of the kidney structures of rats treated with TiO_2NPs and ginger revealed minimal cellular infiltration and normal-sized collecting tubules and renal tissues (Fig. 7a). The liver sections of this group showed normal hepatocytes with less congested central veins and a histological picture similar to that of the control group (Fig. 7b).

Analysis of kidney sections treated with both NPs (ZnONPs and TiO_2NPs) and ginger showed some patches of obstruction in the renal tubules and no congestion around the blood vessels with normal structures (Fig. 8a). Liver sections from this group showed normal hepatocytes with less congested central veins, very few infiltrations, and a clear portal tract (Fig. 8b).

3.5. Discussion

TiO_2NPs and ZnONPs have become the most commonly used NPs in industrial and biomedical applications, causing a steady increase in the risk these particles pose to human health through oral and dermal absorption, inhalation, and injection [29]. Thus, it is necessary to assess the potential effects of both NPs alone and in combination. Consumers and employees may be overexposed to ZnONPs, TiO_2NPs, or both, either intentionally or mistakenly [30]. The aim of this study was to determine the *in vivo* toxicity of ZnO and TiO_2 and ameliorative role of ginger in rats. Because the kidneys and liver are susceptible to NP toxicity, we primarily examined these organs [31].

The results of the current investigation showed that ZnONPs and TiO_2NPs separately and in combination induced a decline in RBC, Hb and PLT counts and an increase in WBC count as compared to the control group. Mix group, in which the rats were treated with both NPs, was affected to the greatest extent. The decline in Hb levels in the NP-treated group may be a result of the ability of the NPs to inhibit the bone marrow from producing new blood cells, which in turn reduces blood Hb levels and blood cell counts. ZnONPs and TiO_2NPs can cause hemolysis of RBCs, which reduces hemoglobin levels. These results are consistent with those of [21], who reported that both NPs separately or in combination reduces Hb levels. According to the current study, both NPs significantly decreased RBC counts when compared to the control group. NPs cause a decline in the blood cell count because they increase oxidative stress, impede cell activity, reduce cellular antioxidant levels, and stimulate antimitotic properties. The main reason why RBCs are destroyed by NPs is that they

produce free radicals. This may be due to the capacity of NPs to cross cell membranes, even in cells such as RBCs that are not engaged in phagocytosis, thereby causing their death.

The findings of this study showed that the PLT counts of all the groups treated with NPs were significantly lower than those of the control group. This may be due to the capacity of NPs to absorb platelets (PLTs) and cause blood clots. Rats developed thrombocytopenia following NP exposure, as indicated by a significant reduction in the PLT count. The bone marrow contains PLT-producing cells called megakaryocytes. In the bone marrow, the megakaryocytes are broken down into smaller PLTs. The death of megakaryocytes may result in a decrease in blood PLT count. The current study revealed an increase in WBCs counts in the NP-treated groups. WBCs are vital defense cells that enhance the immune system's response to NPs. Because they are phagocytic cells, their numbers increased after NP exposure. This may be due to the reason that, when NPs enter the lymphatic system, they cause lymph node inflammation, which consequently results in WBC elevation.

ZnO and TiO_2 nanoparticles are widely employed in everyday items like paints, sunscreens, food packaging, cosmetics, and medications. Because of their multiple applications, humans are being exposed to these nanoparticles more frequently, which raises serious concerns about their toxicity. Because of their dose-dependent toxicity, safer experimental designs are possible. The ability of ZnO and TiO_2 nanoparticles to cause oxidative stress, inflammation, and organ toxicity makes them an excellent model for researching ameliorative effects of natural products like ginger. Other metal oxides such as arsenic exposure is a major concern in occupational settings it is less pertinent to research on contemporary, nanotechnology-driven materials. Because it is extremely poisonous and carcinogenic, its use is limited. At low doses, it is extremely toxic and can cause serious, irreversible side effects such neurotoxicity, skin lesions, and cancer. The preventive benefits of products like ginger extract may be overshadowed by arsenic toxicity, which is less controllable [32].

Similarly, copper oxide nanoparticles are frequently more cytotoxic as compared to ZnO or TiO_2. At comparatively low concentrations, their strong reactivity can cause substantial oxidative stress, DNA damage, and cell death. Because of this, CuONPs may not be as appropriate for controlled trials because its effects could outweigh the protective mechanisms under investigation, like those offered by antioxidants like ginger extract [33]. Although AgNPs are a useful model for studying toxicity caused by nanoparticles, studies evaluating ameliorative interventions such as ginger extract are less appropriate for them due to their high and complex toxic potential, environmental concerns, and lower prevalence in consumer products when compared to ZnO and TiO_2 nanoparticles. For such studies, ZnO and TiO_2 offer more regulated and broadly applicable models [34].

The effectiveness of any protective agent or drug mostly relies on its ability to mitigate adverse effects or restore normal physiological function disrupted by foreign drugs. Reduced levels of RBC, Hb and PLT and increased levels of WBC are valuable indicators of the degree of hepatocellular and renal injury. Reduced levels of RBCs, Hb, and PLT and increased levels of WBC were recorded in groups II, III, and IV. In groups V, VI, and VII, these factors were reversed when compared with the NP-treated group, and the values were similar to those of the control group. The propensity of these enzymes to rebound to almost normal levels in stages V, VI, and VII is a definite indicator of the antihepatotoxic and antinephrotoxic properties of ginger.

For kidney function analysis, blood urea and creatinine levels were measured in the control and experimental groups. The findings of the current study revealed increased Urea and Creatinine levels in the ZnONP, TiO_2NP, and Mix groups. These alterations may be due to potential renal damage. The NPs caused a potential increase in the levels of urea, uric acid, and creatinine due to renal damage and ginger reversed these effects due to its antioxidant properties.

Time-dependent variations in all variables were confirmed by the histological observation of tissue sections in all experimental groups. According to the findings, the liver exhibits typical histopathological changes, such as pyknosis with focal necrotic regions, sinusoidal dilatation and congestion, aggregation of inflammatory cells around blood vessels, and proliferation of Kupffer cells in the NP-treated groups. After 4^{th} and 8^{th} week of dissections, the kidneys of the NP-treated groups displayed intratubular inflammation, brush boundary loss, tubular necrosis, dilated urine gap, abundant mesangial cells, and glomerular atrophy and loss. Renal alterations were more apparent in the ZnONP-treated groups, suggesting that ZnONP primarily accumulated in the renal tissues. In contrast, numerous pathologies in hepatic tissues were induced by TiO_2NPs, such as fatty changes, bile duct proliferation, and pigmentation, all of which indicated that the liver had experienced oxidative stress due to lipid peroxidation and chronic inflammation.

Although more severe than NPs alone, the Mix group also showed progressive liver and kidney damage. The NPs caused the same hepato-renal alterations. The results showed that administering ginger to the rats improved the NP-induced histopathological alterations in the liver and kidney tissues. This demonstrated the effectiveness of ginger in preventing hepatotoxicity and renal toxicity. The present study showed that ginger prevented the fibrosis and necrosis caused by long-term ZnO and TiO_2 poisoning in rats. Although the exact mechanism of action is unknown, it may be related to the known anti-inflammatory, anti-necrotic, and ant fibrotic properties of ginger. The ameliorative effects of ginger ethanol extract on CCl_4 and acetaminophen-induced liver toxicity in rats and the influence of ginger on hepatic damage. Numerous histological alterations were induced by CCl_4 and acetaminophen, and serum ALT, AST, and ALP activities were increased. According to the liver histological analysis, ginger extract was shown to have a protective effect on CCl_4 and acetaminophen-induced damage.

4. Conclusions

In conclusion, our findings demonstrated that ZnONPs and/or TiO_2NPs when given orally changed biological indices, such as hematological and biochemical parameters, and caused tissue damage in rats. The findings of this study suggest that the consumption of ginger maintains the integrity of the liver and kidney tissues and protects them against damage caused by NPs. ZnONPs were found to be more toxic than TiO_2NPs. Moreover, TiO_2NPs caused

more damage to the liver than did ZnO NPs, which mostly harmed the kidneys. Furthermore, compared with the individual effects of NPs, the combined exposure to ZnONPs and TiO_2NPs caused less significant disruptions, suggesting a somewhat antagonistic activity. However, at the end of the trial, there was a synergistic effect on most of the variables. To determine the mechanisms underlying co-exposure to these NPs and draw conclusions about their synergistic/antagonistic effects, more studies involving varying concentrations of both NPs are necessary. Such studies can provide important information that can be used to create policies and rules that guarantee proper precautionary measures for the consumption of such NPs, either alone or in combination, in a variety of applications. This analysis may reveal the better understanding of nanoparticle toxicity i.e how these nanoparticles differently affect vital organs of the body that may help to direct toxicity assessments in future. This study will help to develop protective strategies to mitigate the negative impacts of nanoparticles on human health. Ginger extract may be an efficient and economical method to lessen the negative effects of exposure to nanoparticles and it may be beneficial for people who are regularly exposed to nanoparticles via their environmental or occupational settings. As a result, ginger and its bioactive compound could turned into medication to prevent nanoparticles induced toxicity. This study will provide a framework for assessing other natural products in comparable toxicity situations. This research will provide avenues for nutritional, regulatory, and pharmacological remedies and it will connect Nanotechnology, toxicology and natural product research as well. It will be beneficial to public health and increase the safe application of nanoparticles in a variety of industries.

Authors Contributions

A.W. (PhD student) Conceptualized, conducted all experimentation and wrote the original manuscript. S.S. (Professor) Supervised and revised the edited manuscript. S.N. (Associate Professor) Wrote and reviewed the figure designs. S.T. (Associate Professor) Wrote and reviewed the data. R.A. (Associate Professor) Wrote and revised the manuscript. P.A. (Associate Professor) Revised and edited the draft. M.H. (Associate Professor) Wrote and revised the final draft. Y.J.C. (Associate Professor) Wrote and revised the draft. Y.-K.P. (Professor) Supervised and revised the edited manuscript.

Conflict-of-Interest Statement

The authors declare that they have no conflict of interest.

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