Research Article

Effect of Broccoli Seed Extract and ZnO Nanoparticles on the Liver and Kidney of Male Mice

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Abstract | The study evaluated the effect of Zinc oxide (ZnO) nanoparticles mixed with broccoli seed extract (BSE) on liver and kidney function, as BSE exhibits protective, antioxidant, and anti-inflammatory effects on various organs, including the liver and kidneys. Twenty-four male albino Swiss mice were divided into four groups: C group served as control, Br group was injected with BSE, Zen group was injected with nano ZnO, while the Zen+Br group was injected with a mixture of BSE and nano ZnO. The study shows the protective role of broccoli seed extract against ZnO nanoparticle–induced hepatic and renal toxicity in mice. In the group exposed to ZnO nanoparticles, results demonstrated a significant increase ($P<0.05$) in disturbances in liver and kidney function, accompanied by significant necrosis in both organs. In contrast, broccoli seed treatment alone significantly ($P<0.05$) maintained normal biochemical and histological structures, while its combination with ZnO nanoparticles significantly reduced ($P<0.05$) the severity of the observed alterations. These findings indicate that BSE can protect the liver and kidneys from the harmful effects of ZnO nanoparticles by stabilizing the body's biochemistry and protecting the structure of these organs, suggesting that it could be a natural therapeutic candidate for nanoparticle-induced injury.

Keywords | ZnO nanoparticles, Broccoli seed extract, Mice, Liver, Kidney, Toxicity

Received | January 06, 2026; **Accepted** | March 24, 2026; **Published** | July 11, 2026
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Citation | Mohammed IK, Ibraheem MR, Mohammed A, Ali SMM (2026). Effect of broccoli seed extract and ZnO nanoparticles on the liver and kidney of male mice. J. Anim. Health Prod. 14(3): 1076-1081.
DOI | https://dx.doi.org/10.17582/journal.jahp/2026/14.3.1076.1081
ISSN (Online) | 2308-2801

INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica*), a cruciferous vegetable, is widely recognized for its high content of effective compounds, like glucosinolates, sulforaphane, phenolics, and flavonoids. These phytochemicals are known to exert antioxidant, anti-inflammatory, anticancer, and hepatoprotective effects in both *in vitro* and *in vivo* models (Fahey *et al*., 1997; Yan and Yan, 2023). Broccoli extract serves as a powerful protective agent for both the liver and kidneys by enhancing the bod natural antioxidant defenses and reducing inflammatory damage. Clinical and experimental data suggest that its bioactive compounds can mitigate the toxic effects of heavy metals, such as lead and arsenic, while also supporting organ function in metabolic conditions like diabetes (Raeeszadeh *et al*., 2022). Among its parts, broccoli seeds are especially rich in glucoraphanin, a precursor to sulforaphane, which induces phase II detoxification enzymes and helps protect cells against oxidative damage (Baralic *et al*., 2024). Meanwhile, the use of zinc oxide nanoparticles (ZnONPs) has expanded in biomedical and environmental fields due to their magnetic properties, low cost, and biocompatibility. However, emerging evidence raises

concerns regarding their nanotoxicity, especially when administered systemically. Their small size allows them to cross biological barriers, leading to possible accumulation in vital organs such as the liver, kidneys, lungs, and brain, which can result in oxidative stress, inflammation, and even cellular damage (Eker *et al*., 2024; Aljabali *et al*., 2023). Zinc oxide nanoparticles induce significant toxicity in the liver and kidneys primarily through the generation of reactive oxygen species (ROS) and oxidative stress. Exposure leads to histopathological damage, including inflammation and tissue degeneration, and significantly alters biochemical markers of organ function. While the liver may show some capacity for gradual recovery depending on the exposure route, the toxic effects on the kidneys are often persistent and can lead to serious renal impairment. From the other point of view nanoparticle pharmacokinetics differs from conventional drugs due to their nanoscale size, surface properties, and composition. After administration, nanoparticles undergo absorption, distribution, metabolism, and excretion processes that are strongly influenced by particle size, shape, surface charge, and functionalization. Overall, controlling nanoparticle physicochemical properties allows modulation of pharmacokinetics to improve therapeutic efficacy and reduce toxicity (Blanco *et al*., 2015; Al-Ghareebaw, 2020). Zinc oxide nanoparticles may offer therapeutic or diagnostic advantages; their safe use must be balanced against possible risks. Therefore, investigating natural antioxidants, such as broccoli seed extract, for protective or synergistic effects in combination with nanomaterials is a growing research interest. Natural antioxidants could potentially mitigate nanoparticle-induced toxicity by reducing oxidative stress and modulating inflammatory pathways (Jiang *et al*., 2018).

This study aims to evaluate the histopathological and biochemical effects of broccoli seed extract, ZnO nanoparticles, and their combination on selected vital organs (liver and kidney) of white male mice. The outcome could help elucidate whether broccoli extract exerts a therapeutic role in the presence of metal-based nanomaterials.

MATERIALS AND METHODS

Nanoparticle solution

Zinc oxide nanoparticles (ZnONPs) were provided by Sigma-Aldrich, USA, with a size of about 30 nm and high purity. The nanoparticle was dissolved in distilled water to prepare a 10 mg/kg solution. 0.1 ml/mouse was injected intraperitoneally (Yaqoob *et al*., 2025).

Broccoli seed extract

The extract of broccoli was prepared by boiling crushed broccoli seeds in distilled water for 30–60 min, then filtering the extract through muslin cloth and then Whatman No. 1 filter paper. It was concentrated using a rotary evaporator or vacuum drying at less than 40°C to avoid thermal degradation, then sterilized the final extract by filtering it through a 0.22 µm syringe filter for sterility before intraperitoneal injection. Normal saline or PBS (pH ~7.2) was used to redissolve the dried extract for injection. The dosage utilized in this study was selected in consideration of earlier studies: Once a day for 14 days, 150 mg/kg body weight (Saxena *et al*., 2017).

Experimental animal and parameters

Twenty-four male albino Swiss mice, aged 8–10 weeks and weighing 22 ± 3 g, were obtained from the National Centre for Drug Control and Research in Baghdad. They were fed a complete diet and housed at the Biotechnology Research Center/ Al-Nahrain University (Razaq *et al*., 2015). All experimentations were performed according to the Animal Use Protocol of the Al-Nahrain University guidelines (Theiler *et al*. P.G./221). The experiment lasted about 2 weeks, and the animals were divided into 4 main groups, each with 6 mice. The first group was treated with phosphate buffer saline, considered as a control (C); the second group was given an intraperitoneal injection of 150 mg/kg body weight broccoli seed extraction (Br) suspension, while the third group was given 10 mg/kg body weight ZnO nanoparticles suspension (Zen); the last group was treated with a mixture suspension of both broccoli and ZnO nanoparticles (Zen+Br). After two weeks of treatment, blood was collected from their hearts, centrifuged at 3000 rpm for 10 minutes, and then the animals were sacrificed. The organs were rapidly taken out and preserved in 10% formalin for histological examination, the serum was stored at -20°C and later used to measure the levels of some biochemical indicators that led to liver and kidney functions like alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (g/dl) and urea (mg/dl) using ELISA kits (Orgmetric, Germany). The assays were carried out and biochemical data were analyzed according to the manufacturer's instructions.

Statistical analysis

Collected data were analyzed using GraphPad Prism version 8 (San Diego, CA, USA). Each experimental group included six mice. Multiple comparisons were performed using one-way ANOVA, followed by Tukey's post hoc test. Results are presented as mean ± standard error (SE), and $P < 0.05$ was considered statistically significant.

RESULTS

Broccoli and ZnO nanoparticles effects on liver function tests

Figure 1A presents AST levels, where no significant

difference was observed between the control and broccoli seed groups. However, a significant increase ($P < 0.05$) was observed in the ZnO nanoparticle group compared to the combination group (broccoli + ZnO nanoparticles), as well as the broccoli and control groups.

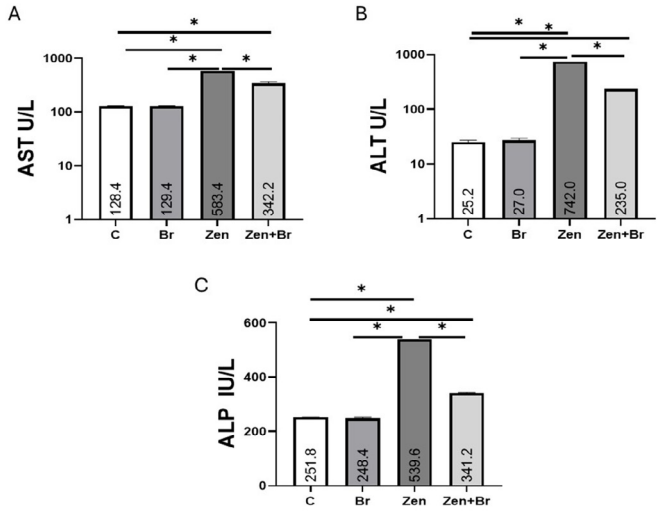

Figure 1: Effect of broccoli seed, ZnO nanoparticles, and their combination on liver function tests in mice. (A) AST, (B) ALT, and (C) ALP. Results are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA to determine significant differences ($P < 0.05$). C: control group; Br: broccoli seed group; ZnO: ZnO nanoparticle group; ZnO + Br: combination of ZnO nanoparticles and broccoli.

Figure 1B illustrates ALT levels, showing a significant decrease ($P < 0.05$) in the combination group compared to the ZnO nanoparticle group. Additionally, ALT levels were significantly higher in the ZnO group compared to all other groups ($P < 0.05$), while no significant differences were observed between the control and broccoli groups.

Figure 1C presents ALP levels, which showed a significant increase ($P < 0.05$) in the ZnO group compared to the other groups. In contrast, the combination group showed a significant decrease ($P < 0.05$) compared to the ZnO group. The broccoli group showed no significant difference compared to the control group.

Histopathological changes in the liver

Figure 2 shows various histomorphological changes in the liver. The control group showed normal liver architecture, characterized by a central vein and well-arranged cords of hepatocytes (Figure 2A). Similarly, the broccoli-treated group exhibited nearly normal histological features, with an intact central vein and organized hepatocyte cords (Figure 2B). In contrast, the ZnO nanoparticle-treated group showed focal areas of hepatic necrosis accompanied by inflammatory cell infiltration (Figure 2C). The combination group (broccoli + ZnO nanoparticles) demonstrated noticeable degenerative changes and the presence of apoptotic hepatocytes (Figure 2D).

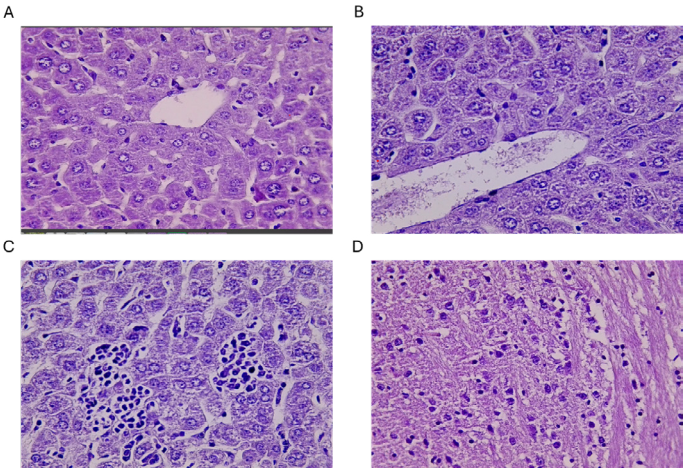

Figure 2: Histological changes in the liver. (A) Control group; (B) broccoli group; (C) ZnO nanoparticle group; (D) combination group (ZnO nanoparticles + broccoli).

Broccoli and ZnO nanoparticles effects on kidney function tests

Creatinine levels in both the control and broccoli groups were normal. Whereas the ZnO nanoparticle group increases creatinine concentration significantly ($P<0.05$). The combined group showed a lower concentration than the nanoparticle group ($P<0.05$; Figure 3A).

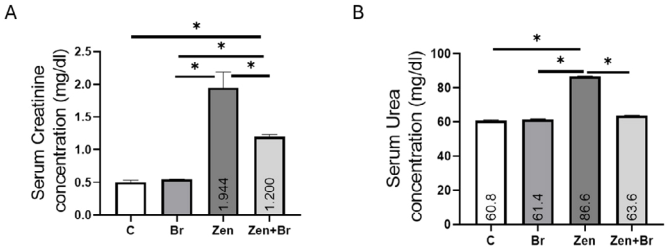

Figure 3: Effect of broccoli seed, ZnO nanoparticles, and their combination on kidney function tests in mice.(A) Creatinine and (B) urea. Results are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA to determine significant differences ($P < 0.05$). C: control group; Br: broccoli seed group; ZnO: ZnO nanoparticle group; ZnO + Br: combination of ZnO nanoparticles and broccoli.

Figure 3B showed urea concentration, which was elevated ($P<0.05$) in the ZnO group compared to all other groups in the study. The combination group shows a lower level ($P<0.05$) than the nanoparticle group.

Histopathological changes in the kidney

Histopathological examination of kidney tissues showed distinct changes among the study groups (Figure 4). The control group exhibited normal histological architecture of the glomeruli and renal tubules, including both proximal

and distal convoluted tubules (Figure 4A). The broccoli-treated group showed a nearly normal histological appearance, with intact glomeruli and renal tubules (Figure 4B). In contrast, the ZnO nanoparticle-treated group demonstrated degenerative changes in the renal epithelial cells of both proximal and distal convoluted tubules, with some cells exhibiting apoptotic features (Figure 4C). The combination group (ZnO nanoparticles + broccoli seed extract) showed mild degenerative changes and occasional apoptotic alterations in renal epithelial cells (Figure 4D).

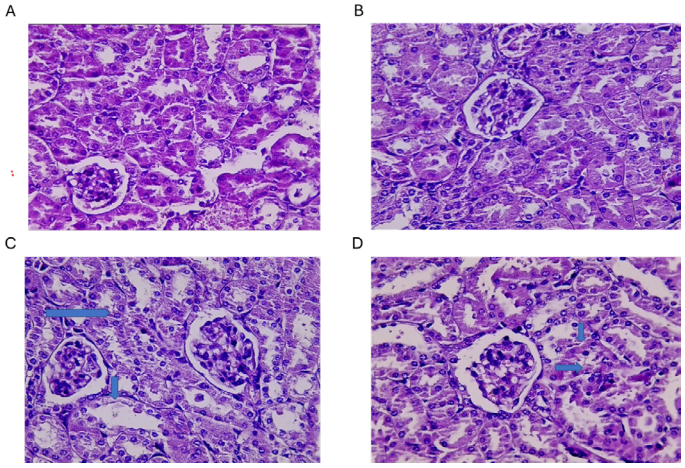

Figure 4: Histological changes in the kidney. (A) Control group; (B) broccoli group; (C) ZnO nanoparticle group; (D) combination group (ZnO nanoparticles + broccoli).

DISCUSSION

Broccoli contains glucosinolates that are metabolized into sulforaphane, a bioactive compound with potent biological effects. Sulforaphane enhances the expression of phase II detoxification enzymes in the liver, thereby facilitating the efficient metabolism and elimination of toxins (Yagishita *et al*., 2019). It also modulates the expression of genes involved in oxidative stress regulation, promoting increased glutathione production and improving metabolic profiles (Eve *et al*., 2020). Furthermore, broccoli has been shown to modulate the gut microbiota, reduce pro-inflammatory cytokines (IL-6 and TNF-α), increase anti-inflammatory IL-10 levels, and enhance antioxidant enzyme activities, including catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) (Lu *et al*., 2025). In liver tissue, broccoli reduces malondialdehyde (MDA) levels and increases non-protein sulfhydryl (NP-SH) content, thereby decreasing oxidative damage. Additionally, it lowers serum levels of GOT, GPT, ALP, GGT, and bilirubin, while histopathological findings confirm its hepatoprotective effects (Satomi *et al*., 2022). In animal models, broccoli has been shown to modulate genes and pathways associated with hepatic lipid accumulation, including the upregulation of lipid export and the downregulation of fatty acid uptake (Chen *et al*., 2016). Moreover, broccoli extract has demonstrated a dose-dependent protective effect, significantly reducing the number of damaged cells compared to groups treated with paracetamol alone (Hwang and Lim, 2014). The concurrent administration of broccoli extract with ZnONPs may exert synergistic beneficial effects, particularly in liver protection and in mitigating oxidative stress, inflammation, and toxin-induced damage (Mirzaei *et al*., 2025).

However, ZnONPs have been widely reported to induce nephrotoxicity, leading to structural and functional kidney damage, as evidenced by increased blood urea nitrogen (BUN), serum creatinine, uric acid levels, and, in some cases, proteinuria (Yan *et al*., 2012). In contrast, broccoli extract has been shown to reduce urea and creatinine levels, enhance antioxidant enzyme activities (SOD, CAT, and total antioxidant capacity), decrease lipid peroxidation, and alleviate histopathological damage (Raeeszadeh *et al*., 2022).

Despite these findings, studies investigating the combined effects of broccoli or its active compound, sulforaphane, with ZnONPs in kidney models remain limited. The underlying molecular mechanisms of kidney protection under co-exposure conditions are still not fully understood. It is suggested that antioxidant phytochemicals in broccoli may attenuate ZnONP-induced oxidative stress and reactive oxygen species (ROS) generation in renal tissues (Mao *et al*., 2023). In particular, sulforaphane activates the Nrf2 signaling pathway, enhancing phase II detoxification enzymes and reducing oxidative damage (Yoon *et al*., 2008).

Furthermore, liver enzyme activity is closely associated with overall physiological functions, including interactions with gut microbiota (Jouda *et al*., 2020). Herbal products have been widely recognized for their therapeutic potential in improving disease outcomes (Rashid *et al*., 2019). However, to date, there is a lack of comprehensive studies evaluating the combined effects of broccoli and ZnONPs on both liver and kidney functions within the same experimental model.

CONCLUSION

To sum up, the medical and food industries have shown a great deal of interest in zinc oxide nanoparticles (ZnONPs) as a way to eliminate or lessen the activity of microorganisms, but their application may be linked to possible liver and kidney toxicity. The toxicity can be countered by consuming broccoli, which is rich in sulforaphane, a bioactive substance that has been proven to lower the chances of developing some forms of cancer and has protective effects against oxidative their destruction.

ACKNOWLEDGMENT

Not applicable.

NOVELTY STATEMENT

This research article provides deep insight into the negative physiological roles of the zinc oxide nanoparticles on liver and kidney toxicity, which is alleviated via broccoli antiinflammatory effects.

AUTHOR'S CONTRIBUTION

IM and SM designed, conceptualization, reviewed the draft, and the last version of this manuscript. MI and AM did all the experiments and wrote the draft, revised and wrote the last version of this manuscript. AM, IM, MI, and SM have reviewed and approved the submitted version of the manuscript.

Data availability

The data is available from the corresponding author upon reasonable request.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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