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Possible Protective Effect of Basil Seeds and Their oil Against Zinc Oxide Nanoparticle-Induced Nephrotoxicity in Rats

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ABSTRACT

The objective of the research was to evaluate the potential safeguarding impact of basil seeds and ther oil on kidney damage caused by zinc oxide nanoparticles (ZnONPs) in male albino rats. Four groups of twenty-four male Sprague -Dawley rats each were formed. The rats in the first group (the control group) were fed a baseline diet. At the end of the trial involved administering 600 mg/kg of ZnO nanoparticles intravenously to the second, third, and fourth groups of rats, along feeding with a basic diet, 5% basil seed, and 5% basil oil, respectively. The experiment involved drawing blood samples, gathering internal organs, and weighing them at the end. A complete blood count was performed. The serum was separated for biochemical examination. Serum creatinine, uric acid, urea, zinc, calcium, magnesium, sodium, potassium, and the renal levels of malondialdehyde increased after injection of ZnONPs, as did reduced hemoglobin, creatinine clearance, zinc's renal activity, and antioxidant enzymes. On the other hand, the administration of basil seeds and their oil improved kidney functions and renal antioxidant enzymes. Therefore, basil seeds and their oil can potentially be preventive against ZnONPs-induced nephrotoxicity.

Keywords: ZnO nanoparticles; nephrotoxicity; oxidative stress; basil seeds; basil oil

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INTRODUCTION

New technologies have made it possible to lower material sizes (from 1 to 100 nm), which has produced creative nanomaterials. Numerous goods enabled by nanotechnologies have made their way into the international market in recent years. 1317 goods or product lines from over 30 nations were included in the Nanotechnology Consumer goods Inventory as of March 2011, an increase of over 521% (from 212 to 1317 products) from the Inventory's initial release in March 2006 **(Braakhuis *et al.*, 2014).**

Primary and aggregated particles that differ in size, shape, charge, crystallinity, chemical composition, and other properties make up nanomaterials. There will be much more variation in the future. It has been proposed that each of these traits influences the toxicity of nanomaterials **(De Jong *et al.*, 2008)**. When reduced to nanoscale, innocuous compounds in bulk form could become hazardous. Understanding the biological activity and possible toxicity of nanomaterials,

such as their proinflammatory, genotoxic, and cytotoxic effects, is therefore crucial **(Nel *et al.*, 2006; Service, 2007)**. Targeting inflammation for toxicological testing is necessary since inflammation-driven consequences, such as fibrosis, predominate the generally suggested pathogenic pathways launched by nanoparticles (NPs), membrane injury, oxidative stress, DNA damage, and apoptosis **(Mroz *et al.*, 2008; Lu *et al.*, 2009; Nel *et al.*, 2009).**

Zinc oxide (ZnO) is a white powder that is an inorganic substance. Generally, it is added to a wide range of products, such as paints, ointments, adhesives, sealants, pigments, plastics, glass, ceramics, rubber (e.g., tires for cars), lubricants, cement, toothpaste, sunscreens, food packaging, and other pharmaceuticals, batteries, ferrites, fire retardants, etc. The respiratory toxicant nano ZnO was identified as the source of metal fume fever, which manifested as myalgia, coughing, exhaustion, etc. Furthermore, investigations conducted on living cells demonstrated that exposure to nano-ZnO resulted in oxidative harm and triggered an inflammatory reaction in lung and vascular endothelial cells **(Gojova *et al.*, 2007; Lin *et al.*, 2009).** According to **Wang et al. (2008a)**, oral experience to ZnO nanoparticles has been shown to target the kidney, spleen, pancreas, heart, liver, and bone in animal tests, ZnO nanoparticles have a somewhat nephrotoxic effect and directly affect renal tissue's oxidative damage and inflammatory response **(Fadda *et al.*, 2012).**

Most people identify *Ocimum basilicum (Lamiaceae)* as a basil plant. The aerial portion of the plant has hypnotic, sedative, anti-TB, anti-epileptic, and antiviral properties; the leaves of the basil plant have analgesic, anti-inflammatory, hypotensive, anti-hepatotoxic, anti-hypercholesterolemia, anti-hyperglycemic, antiulcer, antioxidant, anticancer, and cardio-defensive properties **(Bariyah *et al.*, 2012; Fathiazad *et al.*, 2012; Ahmad *et al.*, 2015).**

Because traditional herbal therapy comes from a natural source and has fewer negative effects, it has been used extensively for thousands of years in both developed and developing nations **(Kamboj, 2012)**. Furthermore, using plants and their derivatives medicinally to treat a variety of illnesses shows promise (**Alomar, 2020).** Using in-vitro models, Ocimum basilicum seeds, for instance, exhibit antioxidant, a-glucosidase, and a-amylase inhibitory activities due to their peptide content (**Chaudhary *et al.*, 2016).**

Ocimum basilicum, sometimes known as basil, is a valuable medicinal plant in many traditional and folk medical systems. It contains a wide spectrum of essential oils, including nolelinoleic acid **(Pattanayak *et al*., 2010)**. Basil oil has antibacterial and blood-sugar-lowering effects and is used to treat kidney problems, gum disease, stomach spasms, colds, fever, vomiting, and other ailments. Additionally, it is believed to have a heart-affinity, lower blood pressure, and help the body adjust to new pressures and demands **(Hasan and AL-Saeed, 2018)**. **Alomar (2020)** also discovered that administering basil leaf extract to the kidneys ameliorated the biochemical and histopathological alterations brought on by TAA overdose. Hence, this study's aim wasto determine the protective impact of *Ocimum basilicum* seeds and oil on renal function in acute nephrotoxicity in male adult rats induced by zinc oxide nanoparticles.

MATERIALS AND METHODS

Materials:

1. The powdered seeds and oil of basil were purchased from MEPACO, an Arab Company for Pharmaceutical and Medicinal Plants located in Egypt.
2. We bought starch and soybean oil from the neighborhood market. We purchased cellulose, casein, vitamins, L-cysteine, minerals, dextrin, and choline chloride from Cairo, Egypt's Cairo Company for Chemical Trading.
3. The ZnO nanoparticles with a less than 100 nm particle size and a composition of more than 99.9% were acquired from Sigma-Aldrich in the United States.
4. A total of 24 male albino rats (belonging to the Sprague Dawley strain) were obtained from the Laboratory Animal Colony in Helwan, Cairo, Egypt. The rats had an average weight range of 150±10g.
5. kits were obtained from Alkan Company and Egyptian American Company for laboratory services.

Methods:

Preparation of the raw materials

To get rid of dirt and soil residues, the basil seeds were washed under running water. Next, it was air-draught oven dried at 40˚C, crushed into a fine powder, and stored in the dark glass bottle in a deep freezer (-16ºC) until additional investigation.

Experimental design

Twenty-four mature male Sprague Dawley strain albino rats weighing 150± 10g were kept in clean, well-ventilated cages for one week while they adjusted to a basic diet as per **Reeves et al. (1993)**. After that, they were randomly assigned to four groups. Every category included sexual animals. The rats in the first group (the control group) were fed a baseline diet. At the conclusion of the trial, the second group was given a single intraperitoneal (i.p). dose of 600 mg/kg of ZnO nanoparticles, as per **(Ahmad and Sharma, 2012)**. At the conclusion of the trial, the third group of rats was given a i.p. dose of 600 mg/kg of ZnO nanoparticles along with a basic diet and 5% powdered basil seed. At the conclusion of the trial, the fourth group of rats was given a i.p. dose of 600 mg/kg of ZnO nanoparticles along with a basic diet and 5% basil oil. Throughout the four weeks of the trial, daily records of the amount of food consumed and discarded were made. The rat's weight was also noted every week. The rats were fasted for a whole night following the final nZnO treatment, and then they were slaughtered. Blood samples were collected from each rat and centrifuged at 4000 revolutions per minute (rpm) for a duration of 10 minutes in order to separate the serum. The serum was meticulously separated and transferred into clean, dry Eppendorf tubes. Subsequently, the tubes were stored at a temperature of -20ºC to maintain their frozen state until they could be analyzed, following the methodology described by Schermer in 1967. Each rat was carefully dissected to extract its liver and kidneys, which were then cleansed of any sticky material using a saline solution (0.9%), dried using filter paper, and weighed. The kidney was removed right away to be examined biochemically. While the other kidney was kept in a 10% formalin solution for histopathological analysis, the other kidney was wrapped in dry,

clean aluminum foil and stored refrigerated until the antioxidant enzyme levels could be determined.

Biological parameters

Throughout the experimental duration, body weight was determined weekly. The body weight gain (BWG) was determined as per **Champman *et al*., (1959).**

Hematological Investigations

Blood samples treated with anticoagulant were analyzed using a hematological analyzer (Exigo Eos Vet, Sweden) to perform a complete blood count. The erythrogram included measurements such as red blood cell count (RBCs), hemoglobin (Hb) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and red blood cell distribution width absolute (RDWa). The leukogram consisted of lymphocytes (%), white blood cell count (WBCs), monocytes (%), and platelet count (PLT), as described **by Jain in 1986**.

Biochemical Analysis of Serum and Urine

For the analysis of serum and urine, various biochemical parameters were measured. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined following the methodology described by **Bergmeyer *et al*. in 1986**. Alkaline phosphatase (ALP) activity was assessed as per the procedure outlined by **Kind and King in 1954**. Albumin levels were determined using the method described by **Drupt in 1974**, while total protein levels were measured according to Sonnenwirth and Jaret in 1980. Urea nitrogen, uric acid, and creatinine levels in serum were estimated following the protocols described by **Patton *et al*. in 1977**, **Faulkner and King in 1976**, and **Fossati *et al*. in 1980**, respectively. Creatinine clearance (Cr Cl) was calculated based on the levels of serum creatinine (S Cr), urine creatinine (U Cr), and 24-hour urinary volumes.

Assessment of Oxidant/Antioxidant Activity in Kidney Tissue

The kidney tissue was extracted, homogenized, and centrifuged at 10,000 rpm for 20 min at 4°C. The resulting supernatant was used to measure the levels of various antioxidants. The levels of antioxidants were determined using a colorimetric method with a spectrophotometer Elisa (microplate reader Ryto2100 C) at wavelengths of 520 nm and 535 nm. Thiobarbituric acid-reactive substances (TBARS) technique was used to measure malondialdehyde (MDA) levels based on the methodology developed by **Misra and Fridovich in 1972**. Superoxide dismutase (SOD) activity was assessed using an assay, and catalase (CAT) activity was measured using a colorimetric assay, as outlined by **Sinha in 1972**. Glutathione (GSH) levels were determined following the protocol described by **Pablos *et al*. in 1995**.

Histopathological examination

Samples taken from the Kidney of the studied rats in different groups were fixed in formalin (10%). Afterward, they were washed under tap water and introduced in a bath containing serial

dilutions of graduated alcohol (methyl, ethyl, and absolute ethyl) used for dehydration. Samples were cleared in xylene and embedded in liquid paraffin at 56 °C. Next, sections of 4μm of thickness were cut, deparaffinized, and stained with Hematoxylin /Eosin stains for histopathological examination under a light microscope **(Bancroft & Gamble, 2008)**.

Statistical Analysis

The results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's test were employed to determine the significance of differences between mean values among different groups. A P value of 0.05 or less was considered statistically significant using SPSS version 20, as suggested by **Snedecor in 1969**.

RESULTS AND DISCUSSION

Biological evaluations

The results presented in Table 1 demonstrate the protective impact of basil seed and oil on relative organ weight and body weight gain in adult rats with renal injury induced by zinc oxide nanoparticles (ZnONPs). Our findings indicated that the mean value of BWG was significantly decreased in the positive control group (13.33 ± 1.49 g) compared to the negative control group (26.5 ± 1.32 g). In comparison to the negative control group (3.75 ± 0.19 g), the positive control group's relative liver weight (2.82 ± 0.25 g) significantly decreased. Furthermore, there was a significant diminish in the relative kidney weight (0.54 ± 0.05 g) between the positive control group and the negative control group (0.84 ± 0.14 g). When compared to control positive rats, treated rats fed on basil seed (5%) and basil oil (5%) exhibit substantial improvements ($P<0.05$) in BWG and relative organ weight. Research has shown that ZNONPs taken orally dissolve in the stomach, and because of their small size, Zn ions are subsequently absorbed into the bloodstream. Gastroenteritis caused by environmental pollutants such as zinc is a typical symptom following zinc consumption. Furthermore, chronic feeding of extremely high concentrations of zinc salts to mice has been shown to cause anemia, disturbance of energy metabolism, and growth retardation. These illnesses were said to be most likely caused by a decreased ability to absorb nutrients since they interfere with hunger and compete with other necessary elements like iron. This may be responsible for decreased body weight in the ZNONPs group. **(Piao *et al.*, 2003; Cho *et al.*, 2013).** Also, it has been reported that ZNP had concentration-dependent toxic impacts on some prime target organs such as kidneys, liver, and spleen inducing oxidative stress, necrosis, apoptosis, and other histological changes. This may explain the weight of the low organs **(Sharma *et al.*, 2011; Guan *et al.*, 2012).** These findings is in line with **(Heidai-Moghadam *et al.*, 2018),** who observed decreased body and kidney weights in ZNONPs-injected rats. In contrast, our results disagree with **(Mansouri *et al.*, 2015),** who found that liver weights were significantly increased in ZNP groups, which may be an outcome of WBC infiltration and RBC congestion and also ZNPs' accumulation in liver tissue.

The remarkable improvement in basil seed and oil groups can be attributed to their ability to promote appetite and alleviate maldigestion and malabsorption induced by ZNONPs, because it contains a variety of phytochemically active chemicals, including alkaloids, tannins,

saponins, anthraquinone, steroids, flavonoids, terpenoids, and cardiac glycosides, basil is regarded as one of the most significant sources of medicine and pharmaceuticals. Thus, it can be said that *Ocimum basilicum* includes minerals and bioactive substances that may improve the recovery process. Also, basil acts as a nephroprotective in treating various kidney ailments, such as drug-induced renal impairment, nephrocarcinoma, kidney and urethral stones, and a diuretic. This is attributed to these phytochemical compounds, natural antioxidants, and free radical scavengers that have been shown to protect against ZnONPs -induced oxidative damage **(Daniel *et al.*, 2011; Zaveri *et al.*, 2011).** Furthermore, basil seeds have been found to possess various pharmacological activities, including stomachic, antioxidant, antiviral, emmenagogue, antimicrobial, analgesic, antidiabetic, and anti-inflammatory effects. These seeds offer a range of health benefits, such as preventing type-2 diabetes, protecting the heart, acting as antioxidants and antimicrobials, and exhibiting anti-inflammatory, anticoagulant, antidepressant, and anticancer properties. Basil seeds are also highly nutritious, containing a substantial amount of proteins, including all essential amino acids except those containing sulfur, and tryptophan. They are rich in dietary fiber, both soluble and insoluble, and contain beneficial lipids, primarily linolenic and linoleic fatty acids. Additionally, basil seeds are a good source of essential minerals like potassium, calcium, and magnesium. They also contain phenolic compounds, including vicentine, orientine, and rosmarinic acid. **(Calderón Bravo *et al.*, 2021).** Recent investigations utilizing in-vitro models have discovered peptides derived from Ocimum basilicum seeds that possess antioxidant properties, as well as the capacity to inhibit a-glucosidase and a-amylase enzymes **(Chaudhary *et al.*, 2016).**

Concerning basil oil, **Zeweil *et al.*, (2017)** concluded that the blend of peppermint and basil essential oils could be used as a feed additive for developing rabbits because it shows beneficial results on FI without having any detrimental effects on growth performance, carcass or digestibility. The predominant compounds in Essential oil (EO) extracted from fresh basil leaves and flowers were linalool, δ-cadinene, germacrene, γ-cadinene, methyl cinnamate, methyl chavicol (estragole), β-selinene, methyl eugenol, eugenol, and α-bergamotene as well as camphor, thymol, p-cymen, geraniol and α-terpineol **(Özcan and Chalchat, 2002)**. Additionally, the therapeutic agents of basil essential oils, including their antioxidant, analgesic, antimicrobial, anti-inflammatory, antibacterial, and antifungal properties, have been demonstrated in multiple investigations **(Saggiorato *et al.*, 2012; Avetisyan *et al.*, 2017 and Kavoosi and Amirghofran, 2017). .**

Figures and Tables

Table (1): The protective effect of basil seed and oil on body weight gain (BWG) and relative organs weight in adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

groups	B.W.G (%)	Liver weight(g)	Kidney weight (g)
(-ve) control	26.5 ± 1.32^{a}	3.75 ± 0.19^{a}	0.84 ± 0.14^{a}
(+ve) control	13.33 ± 1.49^{d}	2.82 ± 0.25^{c}	0.54 ± 0.05^{c}
5% basil seed + ZnONPs	15.52 ± 0.50^{c}	3.26 ± 0.05^{b}	0.61 ± 0.06^{b}
5% basil oil + ZnONPs	17.69 ± 1.03^{b}	3.34 ± 0.04^{b}	0.64 ± 0.05^{b}

Every value indicates the mean ±SD. At $p \leq 0.05$, the means in the same column containing various superscript letters were significant.

Hematological investigations

The findings reported in Table 2 showed the protective impact of basil seed and oil on blood count (arthrogram) in adult rats with renal injury induced by zinc oxide nanoparticles.

Results recorded for hemoglobin (M± SD) that in the case of control (-ve) was (12.38 ± 0.82 g/dl), while for control (+ve) was (11.09 ± 0.18 g/dl). Results for MCV demonstrated a significant reduction in the (+ve) control group (71.52 ± 2.49 fl) as compared to the (-ve) control group (90.49 ± 1.24 fl). MCH of the control (+ve) group significantly reduced (22.53 ± 0.52 pg) while in control (-ve) was (33.04 ± 0.95 pg). MCHC, RBCs, and hematocrits also significantly reduced ($P<0.05$) in the control (+) group. Rats medicated with basil seed (5%) and basil oil (5%) demonstrated significant elevations ($P<0.05$) in hematological parameters when compared with the control positive. Anemia in patients with kidney dysfunction can be triggered via numerous processes, involving deficiencies in iron, folate, vitamin B12, erythropoietin, and abnormally low reactivity to erythropoietin. The levels of zinc found in the examined organs and tissues were particularly noteworthy in the bone, kidney, liver, and blood. These outcomes confirm earlier findings (**Llobet *et al.*, 1988)**. Exposure to elevated levels of zinc can have harmful effects on the hematopoietic system. Research indicates that ZnNPs can lead to the enlargement and dilation of veins and blood sinusoids, potentially affecting the permeability of cell membranes in the endothelial lining of blood vessels. Additionally, ZnNPs can cause changes in blood viscosity **(Ma et al., 2009; Guan et al., 2012)**. Previous studies conducted on rats have demonstrated that high concentrations of zinc can exhibit cytogenetic toxicity. Specifically, zinc has been found to induce the formation of micronuclei in polychromatic erythrocytes within the rats' bone marrow, resulting in a reduced lifespan of red blood cells. Furthermore, zinc can lead to deficiencies in copper and iron, leading to impaired growth and the development of anemia. **(Piao *et al.*, 2003).** The amelioration in the basil seed group is in line with **Chaudhary *et al.*, (2016)**, who observed that the administration of AEOBS extract improved the levels of red blood cells (RBCs) and related indices. This suggests that AEOBS contains certain phytoconstituents that have the potential to reverse the erythropoietin deficiency

in kidney dysfunction. *O. basilicum* is a medicinal plant with high flavonoids, tannins, alkaloid, saponins, naphthoquinone, and triterpenes (anti-inflammatory and antioxidant compounds), so they can diminish the hemolytic anemia's rate **(Zangeneh *et al.*, 2019)**. In addition, Basil seeds are a natural anti-oxidant traditionally believed to be utilized as medicine to enhance blood circulation, decrease inflammation and elevate immune function **(Afifah *et al.*, 2016).**

Table (2): The protective impact of basil seed and oil on blood count (erythrogram) in adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

Groups	Hemoglobin (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RBCs (x10^6/cmm)	Haematocrit %
(-ve) control	12.38 ± 0.82^{a}	90.49 ± 1.24^{a}	33.04 ± 0.95^{a}	30.62 ± 0.72^{a}	4.26 ± 0.17^{a}	37.65 ± 0.57^{a}
(+ve) control	11.09 ± 0.18^{c}	71.52 ± 2.49^{d}	22.53 ± 0.52^{d}	24.57 ± 0.45^{d}	3.10 ± 0.15^{d}	30.64 ± 0.53^{d}
5% basil seeds + ZnONPs	11.75 ± 0.31^{b}	78.15 ± 0.96^{c}	24.62 ± 0.42^{c}	26.08 ± 0.31^{c}	3.43 ± 0.23^{c}	32.84 ± 0.29^{c}
5% basil oil + ZnONPs	12.06 ± 0.10^{ab}	80.56 ± 1.12^{b}	26.24 ± 1.06^{b}	27.45 ± 0.55^{b}	3.84 ± 0.13^{b}	34.36 ± 0.51^{b}

The mean ±SD is indicated by each value. Significant at $p \leq 0.05$ were the means in the same column with different superscript letters

Results in Table 3 demonstrated the protective impact of basil seed and oil on blood count (leukogram) in adult rats with renal injury induced by zinc oxide nanoparticles.

Mean values of WBCs, Lymphocytes, and Monocytes indicated a significant decline ($P < 0.05$) in the control (+ve) group (4.63 ± 0.30, 84.62 ± 0.29, and 1.15 ± 0.03) as compared with a negative control group (9.76 ± 0.59, 85.95 ± 0.21 and 1.39 ± 0.16) respectively. On the other hand, basil seed (5%) and oil (5%) groups demonstrated significant elevations ($P<0.05$) when compared with the control positive. The reduction in white blood cell count was likely brought on by either the stress induced by the spleen's cell manufacturing activity or the toxic action of zinc on WBC. Furthermore, as corticosteroid and cortisol hormones are crucial for both preventing and treating inflammation, the drop in WBC count may also be linked to an increase in their secretion. The considerable reduction in zinc-induced lymphocytes can be attributed to the cortisol hormone's suppressive influence on lymphocyte development, which ultimately results in immunosuppression. These consequences could be linked to metal damage to the immune system's cells **(Çelik *et al.*, 2013)**.

The positive impacts noted in the group that got basil seeds align with the findings of **Chaudhary et al. (2016)**. In their investigation, it was revealed that AEOBS' administration at doses of 250 mg/kg and 500 mg/kg resulted in an increase in various white blood cell (WBC) components, such as lymphocytes, monocytes, neutrophils, basophils, and eosinophils, bringing them closer to normal levels. These findings were attributed to the immunomodulatory properties of AEOBS in the context of kidney dysfunction. *Ocimum basilicum*, commonly known as basil,

is a significant medicinal plant and culinary herb that is known to contain numerous antioxidant compounds. It has also been reported to have notable effects at the cellular level, including its ability to inhibit platelet aggregation **(Tomar et al., 2010)**.

Table (3): The protective effect of basil seed and oil on blood count (leukogram) in adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

groups	WBCs (x10^3/cmm)	Lymphocytes (%)	Monocytes (%)
(-ve) control	9.76 ± 0.59^{a}	85.95 ± 0.21^{a}	1.39 ± 0.16^{a}
(+ve) control	4.63 ± 0.30^{d}	84.62 ± 0.29^{c}	1.15 ± 0.03^{c}
5% basil seeds + ZnONPs	5.55 ± 0.41^{c}	85.10 ± 0.18^{b}	1.25 ± 0.03^{b}
5% basil oil + ZnONPs	6.52 ± 0.38^{b}	85.31 ± 0.21^{b}	1.28 ± 0.04^{b}

Every value indicates the mean ±SD. At $p \leq 0.05$, the means in the same column containing various superscript letters were significant

Kidney functions

The findings presented in Table (4) indicated the protective impact of basil seed and oil on renal functions, including (Creatinine, Urea, Creatinine Clearance, and Uric acid) in adult rats with renal injury induced by zinc oxide nanoparticles.

The mean value of creatinine in the control (+ve) group significantly elevated compared to the control (-ve) group; this recommends a great defect in renal function. Supplementing the diets with 5% basil seed and 5% basil oil led to notable reductions ($P<0.05$) in the Creatinine's levels in rats with renal injury. The values recorded were 0.69 ± 0.02 mg/dL and 0.67 ± 0.01 mg/dL, consecutively, compared to the control group (+ve). This indicates that both basil seed and basil oil improved Creatinine levels in the rats, with the most significant improvement observed in the group supplemented with 5% basil oil.

In terms of Uric acid, the control group (+ve) exhibited higher values (2.62 ± 0.51 mg/dL) in comparison to the control group (-ve) (1.50 ± 0.01 mg/dL). However, the groups treated with 5% basil seed and 5% basil oil demonstrated significant reductions ($P<0.05$) in Uric acid levels in comparison to the control group (+ve). Therefore, both basil seed and basil oil had a positive impact on reducing Uric acid levels, with the most effective being the 5% basil oil group. Similar trends were observed for Urea. The control group (+ve) showed a significant elevation in Urea levels compared to the control group (-ve), but the supplemented diets with 5% basil seed and 5% basil oil induced significant decreases in Urea levels compared to the control group (+ve). The most favorable outcome was observed in the rats fed with 5% basil oil.

In contrast, the mean value of Creatinine Clearance in the control (+ve) group significantly declined as compared to the control (-ve) group (1.12 ± 0.05 and 1.80 ± 0.01, respectively). In

contrast, supplemented diets with 5% basil seed and 5% basil oil induced a significant increase compared to the control (+ve) group. The ideal outcome was noted in rats fed on 5% basil oil. Zinc can cause acute and chronic toxicities on some target organs such as kidneys, liver, and spleen after excessive exposure, inducing oxidative stress, necrosis, apoptosis, and other histological changes **(Piao *et al.*, 2003).** The kidneys exhibited the most significant histological abnormalities, with notable lesions observed in the glomerular Bowman's capsule characterized by flattened epithelial cells, as well as in the proximal convoluted tubules where there was tubular epithelial cells' desquamation and the presence of pyknotic nuclei. These structural changes indicate damage to the nephrons and impaired kidney function. Consequently, elevated levels of Creatinine and Urea were noted in the group exposed to zinc oxide nanoparticles (ZnONPs). The nephrotoxic effects induced by ZnONPs can be attributed to the oxidative stress triggered by the generation of reactive oxygen species **(Yan *et al.*, 2012).**

On the other hand, *Ocimum basilicum*, also known as basil, contains numerous antioxidant compounds, including flavonoids, tannins, saponins, and alkaloids. These compounds function as scavengers, protecting against ROS and oxygen-derived free radicals that are proven to contribute to aging and various disease processes. Saponins, for instance, have been shown to have beneficial effects on blood cholesterol levels, possess anti-cancer properties, and aid in maintaining overall health while stimulating the immune system. In contrast, tannins are recognized for their antiviral, antitumor, anti-inflammatory, and wound healing properties, particularly in relation to the kidneys and other bodily systems. Thus, *Ocimum basilicum* extract, which contains saponins and tannins, could be useful for medicinal purposes **(Daniel *et al.*, 2011).** In addition, (**Saber and Wael, 2012)** concluded that aqueous basil extract has a helpful influence on nephrotoxicity in albino rats via its antioxidant impact.

The existing literature supports the notion that basil seeds possess significant antioxidant potential owing to the exitance of various phenolic compounds involving orientin, vicenin, and rosmarinic acids, as well as other phytochemicals including tannins, terpenoids, saponins, flavonoids, alkaloids, and steroids. Phenolic compounds are known to perform diverse physiological functions in plants, and their consumption has been associated with protection against serious illnesses such as cancer, renal diseases, and cardiovascular disorders **(Calderón Bravo et al., 2021)**.

Furthermore, basil essential oils, which contain components such as methyl eugenol, methyl cinnamate, methyl chavicol, geraniol, linalool, citral, limonene, and various terpenes, have been reported to have antioxidant, antimicrobial, anti-inflammatory, and radical scavenging properties. Among these components, basil essential oils exhibit the greatest capacity to neutralize free radicals **(Avetisyan et al., 2017).**

Table (4): The protective impact of basil seed and oil on Kidney functions in adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

Groups	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)	CR.CL (ML/min/1.73 m2)
(-ve) control	0.53 ±0.01^{c}	1.50 ± 0.01^{c}	34.57 ± 0.45^{d}	1.80 ± 0.01^{a}
(+ve) control	0.95 ± 0.03^{a}	2.62 ± 0.51^{a}	74.91 ± 1.52^{a}	1.12 ± 0.05^{d}
5% basil seeds + ZnONPs	0.69 ± 0.02^{b}	2.41 ± 0.08^{ab}	50.10 ± 1.02^{b}	1.52 ± 0.04^{c}
5% basil oil + ZnONPs	0.67 ± 0.01^{b}	2.27 ± 0.03^{b}	47.01 ± 1.56^{c}	1.60 ± 0.1^{b}

Every value indicates the mean ±SD. At p≤0.05, the means in the same column containing various superscript letters were significant.

Serum electrolytes

Data presented in Table (5) demonstrated the protective impact of basil seed and oil on serum levels of some electrolytes (sodium, potassium, Calcium, and Magnesium) in adult rats with renal injury induced by zinc oxide nanoparticles.

Sodium, potassium, Calcium, and Magnesium values indicated a significant reduction ($P < 0.05$) in the control (+ve) group (138.81 ± 0.57, 4.23 ± 0.02, 8.46 ± 0.17, and 1.36 ± 0.01) as compared with a negative control group (150.62 ± 0.59, 5.40 ± 0.02, 11.53 ± 0.02 and 2.27 ± 0.01) consecutively. Rats who received a diet with basil seed (5%) and basil oil (5%) indicated significant elevates ($P<0.05$) in Serum electrolytes when compared with control positive. The greatest outcome was found in rats fed on 5% basil oil. Electrolyte imbalance is a characteristic associated with impaired kidney function. It has been noted that ZnONPs reduce the glomerular filtration rate, which can increase electrolyte excretion in urine and thus decrease its rate in serum. Also, disrupt sodium and water reabsorption as a sign of nephrotoxicity with resultant loss of electrolytes and body fluids **(Noori *et al.*, 2014).** Furthermore, ZnONPs promote cytotoxicity by generating ROS, which can cause damage and depolarization of cell membranes, resulting in electrolyte imbalance **(Ng *et al.*, 2017).** In addition, **Najafzadeh *et al.*, (2013)** reported that excessive Zn might reduce the absorption of calcium or phosphorus. The significant increase observed in the basil seed group aligns with the findings of **Chaudhary et al. (2016)**, who demonstrated that the administration of AEOBS at doses of 250 mg/kg and 500 mg/kg effectively corrected electrolyte imbalances. Additionally, Daniel et al. (2011) reported that Ocimum basilicum has a high potassium content, along with appreciable levels of calcium, sodium, and magnesium. Furthermore, according to Calderón Bravo et al. (2021), basil seeds contain significant amounts of minerals such as calcium, potassium, and magnesium. These mineral contents may explain the observed increase in these electrolytes in the groups treated with basil seeds, indicating their potential role in maintaining electrolyte balance and contributing to the observed protective effects.

Table (5): The protective effect of basil seed and oil on serum levels of some electrolytes (sodium, potassium, Calcium and Magnesium) in adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

groups	sodium (mmol/l)	Potassium (mmol/l)	Calcium (mg/dL)	Magnesium (mg/dL)
(-ve) control	150.62 ± 0.59[a]	5.40 ± 0.02[a]	11.53 ± 0.02[a]	2.27 ± 0.01[a]
(+ve) control	138.81 ± 0.57[c]	4.23 ± 0.02[c]	8.46 ± 0.17[c]	1.36 ± 0.01[c]
5% basil seeds + ZnONPs	143.66 ± 3.14[b]	4.59 ± 0.06[b]	9.55 ± 0.51[b]	1.93 ± 0.31[b]
5% basil oil + ZnONPs	143.83 ± 1.44[b]	4.61 ± 0.17[b]	9.64 ± 0.07[b]	2.13 ± 0.04[a]

Every value indicates the mean ±SD. At $p \leq 0.05$, the means in the same column containing various superscript letters were significant.

Zink in kidney tissue and serum

Table (6) demonstrates the protective effect of basil seed and oil on Zink levels in kidney tissue and serum in adult rats with renal injury induced by zinc oxide nanoparticles. The mean value of Zink in kidney tissue in the control (+ve) group (174.28 ± 0.99 µg/dl) was a significant elevation as compared to the control (-ve) group (40.36 ± 1.32 µg/dl). The control group (+ve) exhibited a significant increase in serum zinc levels (96.58 ± 1.18 µg/dL) compared to the negative control group (12.18 ± 0.37 µg/dL). However, the rats that received 5% basil seed and 5% basil oil demonstrated a significant reduction ($P<0.05$) in both kidney tissue zinc levels and serum zinc levels compared to the control group (+ve). The most favorable outcome was noted in the rats that received 5% basil oil. It is worth noting that normal zinc and zinc compounds have been reported to have toxic effects, including digestive or respiratory system irritation, dental damage, skin ulceration, and symptoms such as chills, nausea, fever, muscular aches, vomiting, and weakness when exposed to zinc fumes **(Piao et al., 2003)**.

ZnONPs have been suggested to induce the ROS formation and cause oxidative stress in the kidneys. The oxidative stress and lipid peroxidation elicited by ZnONPs can disrupt cell membranes, induce cytotoxicity, and promote apoptosis. Studies by **Xia et al. (2008) and Yan et al. (2012)** reported that ZnONPs disrupt cellular zinc homeostasis and interact with components of renal cells, leading to mitochondrial damage and cell death.

Table (6): The protective impact of basil seed and oil on Zink levels in kidney tissue and serum in adult rats with renal injury induced by zinc oxide nanoparticles(n= 6)

groups	Zink (µg/dl) tissue	Zink (µg/dl) serum
(-ve) control	40.36 ± 1.32[d]	12.18 ± 0.37[c]
(+ve) control	174.28 ± 0.99[a]	96.58 ± 1.18[a]
5% basil seeds + ZnONPs	130.52 ± 3.13[b]	48.50 ± 5.81[b]
5% basil oil + ZnONPs	123.57 ± 0.63[c]	44.67 ± 4.03[b]

Every value indicates the mean ±SD. At p≤0.05, the means in the same column containing various superscript letters were significant.

Liver enzymes

Table 7 demonstrates the protective effect of basil seed and oil on liver enzymes, specifically ALT, AST, and ALP, in adult rats with renal injury induced by ZnONPs. The control group (+ve) exhibited significantly higher mean values of liver enzymes (132.51 ± 3.48 U/L for AST, 114.35 ± 1.68 U/L for ALT, and 183.91 ± 2.63 U/L for ALP) compared to the control group (-ve) (93.94 ± 10.10 U/L for AST, 53.42 ± 0.59 U/L for ALT, and 135.47 ± 0.79 U/L for ALP). However, rats that consumed the treatment diets showed a significant decrease (P<0.05) in these liver enzymes compared to the control group (+ve), with the optimal outcome observed in the group fed a diet supplemented with 5% basil oil.

The rise levels of AST, ALT, and ALP indicate liver cell damage since these enzymes are released into the bloodstream from hepatocytes when liver cells are compromised **(Aragon and Younossi, 2010)**. **Ding et al. (1998)** stated that great zinc levels triggered liver toxicity in mice and inhibited AST activity in liver homogenates, suggesting that zinc might directly or indirectly impact AST synthesis. The toxic impacts of zinc and other heavy metals on AST levels may involve different mechanisms. ZnONPs have the ability to interact with enzymes and proteins in the interstitial tissue of the liver, upsetting the antioxidant defense system and producing reactive oxygen species, which trigger an inflammatory response. ZnONPs also exhibit necrotic effects on liver tissue, characterized by the lobular structure's destruction, congestion of RBC, hepatocyte vacuolization (fat deposits), and leukocyte infiltration. Additionally, apoptosis has been observed in the liver tissue of animals treated with ZnONPs **(Johar et al., 2004)**. **Park et al. (2007)** discovered that ZnONPs were the most harmful to A549 cells out of the six distinct nanoparticles, as demonstrated by experiments on DNA fragmentation and apoptosis.

Ocimum basilicum leaf extracts have been shown to safeguard the liver from heavy metals **(Sharma et al., 2002)**. Linalool and methyl chavicol are major constituents of O. basilicum essential oil **(Avetisyan et al., 2017)**. Linalool acts on inflammation by inhibiting the secretion of pro-inflammatory factors and reducing lipopolysaccharide (LPS)/D-galactosamine (GalN)-induced liver injury in mice. This action is achieved through the inhibition of caspase-3 and

caspase-8 expression, suppression of the inflammatory response via NF-kB suppression, and elimination of inflammatory response **(De Andrade et al., 2017)**.

Table (7): The protective effect of basil seed and oil on liver enzymes in adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

groups	AST (U/L)	ALT (U/L)	ALP (U/L)
(-ve) control	93.94 ± 10.10^{c}	53.42 ± 0.59^{c}	135.47 ± 0.79^{d}
(+ve) control	132.51 ± 3.48^{a}	114.35 ± 1.68^{a}	183.91 ± 2.63^{a}
5% basil seeds + ZnONPs	108.32 ± 2.57^{b}	80.79 ± 6.24^{b}	151.53 ± 2.70^{c}
5% basil oil + ZnONPs	113.20 ± 4.06^{b}	83.74 ± 2.21^{b}	157.74 ± 5.82^{b}

Every value indicates the mean ±SD. At $p \leq 0.05$, the means in the same column containing various superscript letters were significant.

Protein fractions

Table (8) shows the protective effect of basil seed and oil on protein fractions (total albumin, protein, and globulin) in adult rats with renal injury induced by zinc oxide nanoparticles. Our results revealed that the mean values of Total protein were significantly lower in the (+ve) control group (5.01 ± 0.07 g/dl) compared to (-ve) control rats which were (6.30 ± 0.06 g/dl). The similar table shows the mean values of albumin. The control (+ve) group demonstrated a significant reduction as compared to the (control -ve) group (2.45 ± 0.22 and 3.22 ± 0.15 g/dl, consecutively). Also, in the same table control (+ve) group decreased in Globulin values as compared to the (-ve) control group (2.55 ± 0.16 and 3.08 ± 0.09 g/dl), consecutively. In contrast, all of the total protein, albumin, and globulin demonstrated a significant elevation when feeding on basil seed (5%) and Basil oil (5%) diets as compared to the control (+ve) group. Hypoalbuminemia is a characteristic of proteinuria, renal dysfunction, and glomerular lesions. Hypoalbuminemia is related with elevated lipid peroxidation and can contribute to oxidative stress in chronic renal disorders **(De Castro *et al.*, 2014; Zhang *et al.*, 2019)**. **Sallam *et al.*, (2023)** noted that basil essential oil nanoemulsion enhanced total protein and albumin in albino rats medicated with titanium nanoparticles.

Table (8): The protective impact of basil seed and oil on protein fractions in adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

groups	T.P (g/dl)	ALb (g/dl)	G (g/dl)	A/G
(-ve) control	6.30 ± 0.06^{a}	3.22 ± 0.15^{a}	3.08 ± 0.09^{a}	1.05 ± 0.08^{a}
(+ve) control	5.01 ± 0.07^{d}	2.45 ± 0.22^{c}	2.55 ± 0.16^{b}	0.97 ± 0.15^{a}
5% basil seeds + ZnONPs	5.23 ± 0.04^{c}	2.65 ± 0.09^{b}	2.59 ± 0.14^{b}	1.02 ± 0.09^{a}
5% basil oil + ZnONPs	5.38 ± 0.03^{b}	2.68 ± 0.07^{b}	2.70 ± 0.04^{b}	0.99 ± 0.04^{a}

Every value indicates the mean ±SD. At $p \leq 0.05$, the means in the same column containing various superscript letters were significant.

Antioxidant enzymes and lipid peroxide (MDA)

Data presented in Table 9 demonstrated the protective impact of basil seed and oil on antioxidant enzymes (SOD, CAT, and GSH) and malondialdehyde (MDA) in kidney tissue of adult rats with renal injury induced by zinc oxide nanoparticles. These results denote that the mean value of antioxidant enzymes (SOD, GSH, and CAT) in the control (+ve) group was extremely lower than the control (-ve) group. Also, results revealed that the levels of lipid peroxidation marker (MDA), which was low in (control -ve) rats increased in the case of the control (+ve) group, which demonstrated values of (55.30 ± 2.61 and 123.34 ± 2.53 nmol/mg respectively). Supplemented diets with 5% basil seed and 5% basil oil significantly increased antioxidant levels compared with the control (+ve) group. Meanwhile, there was a marked reduction in MDA levels The lipid peroxidation and oxidative stress response's exitance was induced by ZNP exposure, as evidenced by the higher MDA levels and decreased SOD and GPx activity in the kidney. In rat kidney tissue, ZNP has been shown to cause cytotoxicity, apoptosis, oxidative stress-mediated DNA damage, and cell membrane defects **(Yan *et al.*, 2012; Ng *et al.*, 2017) (Heidai-Moghadam *et al.*, 2018).**

Although **Singh *et al*. (2007)** suggested that the high particle surface area is connected to the oxidative stress' generation following NPs therapy, the exact mechanism is yet unknown. Ocimum basilicum essential oil (OBO) possesses cytotoxic, antibacterial, and radical scavenging properties. As a result, it can be utilized as a secure antibacterial additive as well as a reliable and efficient supply of natural antioxidants to enhance oxidative stability. They may also be considered as a contender for the development of anticancer drugs. OBO's capacity to scavenge radicals may be associated with geraniol, then methyl chavicol. Superoxide dismutase activity, an antioxidant enzyme, increased by 45% and glutathione concentration by 120% when geraniol was added to cells. Furthermore, geraniol was found to be able to scavenge radicals, significantly lessen lipid peroxidation, and stop nitric oxide (NO) and reactive oxygen species (ROS) from being released in pretreatment cells as opposed to stressed cells. By scavenging ROS and RNS and triggering antioxidant defense mechanisms including glutathione and superoxide dismutase, these results showed the antioxidant activity of geraniol and also

suggested that the product may be useful in reducing damage to biological tissues **(Shirazi *et al.*, 2014).**

Table (9): The protective impact of basil seed and oil on antioxidant enzymes and malondialdehyde (MDA) in kidney tissue of adult rats with renal injury induced by zinc oxide nanoparticles (n= 6)

groups	SOD (U/mg)	CAT (ng/mg)	GSH (ng/mg)	MDA (nmol/mg)
(-ve) control	32.89 ± 1.86[a]	56.89 ± 5.22[a]	9.86 ± 1.09[a]	55.30 ± 2.61[d]
(+ve) control	12.08 ± 0.49[d]	26.88 ± 0.42[d]	2.01 ± 0.06[d]	123.34 ± 2.53[a]
5% basil seed + ZnONPs	21.00 ± 1.55[c]	40.66 ± 1.86[c]	4.67 ± 0.52[c]	82.66 ± 2.25[b]
5% basil oil + ZnONPs	25.66 ± 1.03[b]	46.00 ± 1.55[b]	6.00 ± 0.89[b]	77.33 ± 2.25[c]

Every value indicates the mean ±SD. At $p \leq 0.05$, the means in the same column containing various superscript letters were significant.

Histological Results:

Histopathological examination of kidney tissue

Under a microscope, the kidneys of the rats in group (1) Control (-ve) exhibit normal renal sections with normal tubules, glomeruli, and interstitial tissue stained in group (A). A kidney section from group (2) Control (+ve) (B) displays the following: diffuse interstitial edema; tubular lumen dilation; tubular cell necrosis (red arrow); foci of denuded basement membrane; intraluminal casts (short black arrow); and interstitial inflammatory cell infiltrates. kidney section from group (3) basil seeds (5%) treated group(C) shows moderate diffuse tubular hydropic degeneration (black arrows). kidney section from group (3) basil seeds oil (5%) treated group(D) shows show decreased tubular hydropic degeneration (black arrows) and few dilated tubules with mild cast formation. Zinc oxide nanoparticles caused renal corpuscle distortion and tubule degeneration in the form of cytoplasmic vacuolation in a different study. Additionally, it was noted that some vascular glomeruli were enlarged and closely filled the Bowman's capsule, lacking the capsular gaps **(Yousef *et al.*, 2019).** These results are in line with **Sallam *et al.*, (2023)** who found that rats medicated rats with TiO2- NPs plus basil oil demonstrating some enhancement noted in minimal fibrous tissue and little diffusion of inflammatory cell infiltration.

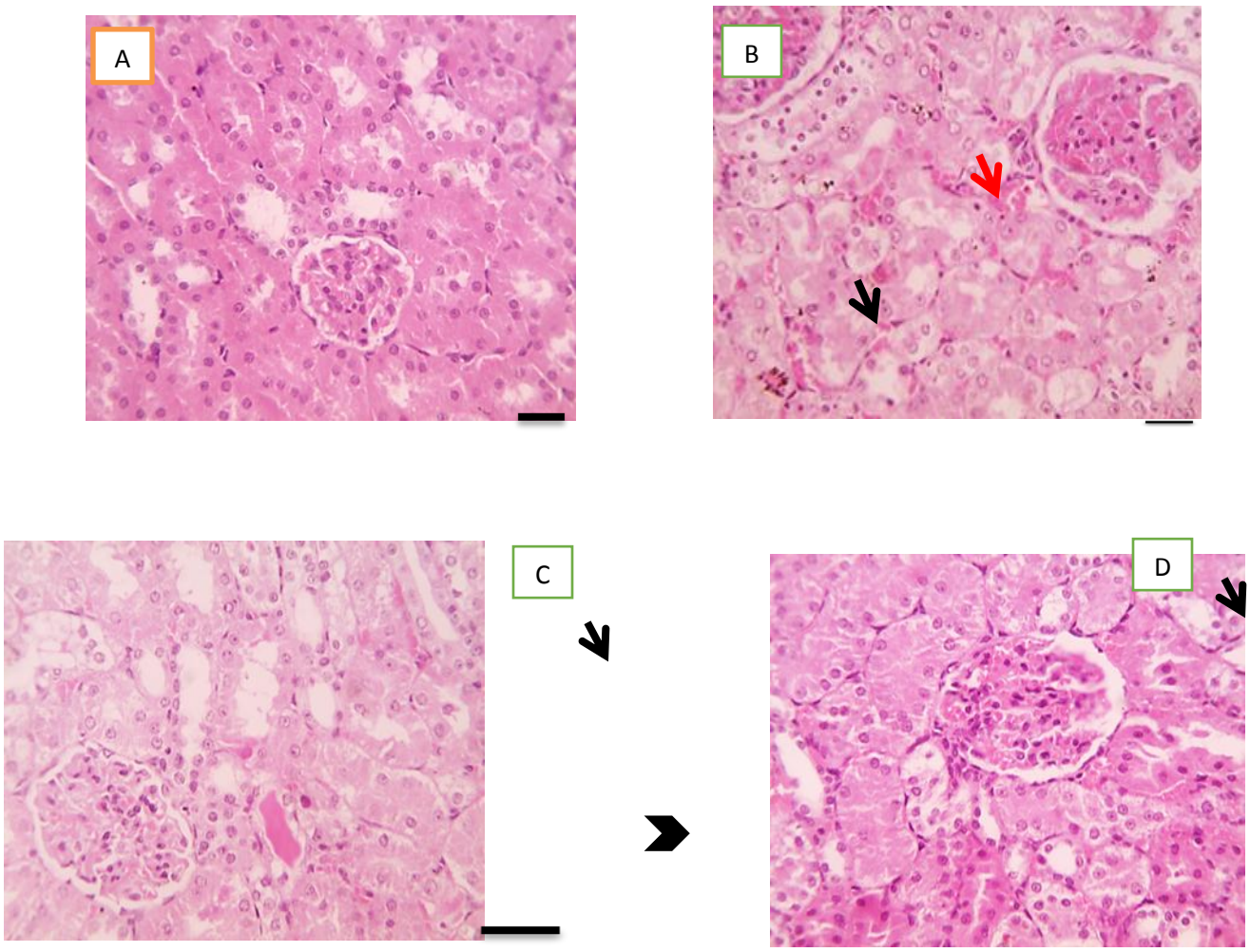

Fig. 1. Representative images of Haematoxylin and Eosin stained kidney sections (x400) from A Normal Control group, B ZnO nanoparticles (positive control group), C basil seeds (5%) treated group, D oil seeds (5%) treate group.

CONCLUSION

Basil seeds and oil improved kidney functions and renal antioxidant enzymes. Therefore, they can be a potential preventive agent against ZnONPs-induced nephrotoxicity.

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