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Abstract:

Nanoparticles are compounds with special qualities that are very small in size (less than 100 nm). As a result, nanoparticles pose many different health concerns. Therefore, this study aimed to estimate basil leaf extract's protective effect on nephrotoxicity tempted by zinc oxide nanoparticles (ZnONPs) in male albino rats. Twenty-four male *Sprague Dawley* rats were divided into four groups. Group 1 rats were given a basal diet for 4 weeks and served as normal control. Group 2 rats were fed on a basal diet+ 500mg/ kg b.w. from leaves extract by gavage) for 4 weeks. Group 3 rats have a single dose of ZnONPs (600 mg/kg i.p.) on the 29th day. Group 4 rats received a single dose of ZnONPs (600 mg/kg i.p.) on the 29th day+ 500 mg/ kg b.w. leaf extract via gavage for 4 weeks. At the end of the experiment, blood samples were taken, internal organs were collected, weighted. A complete blood count was performed. Serum was separated to biochemical examination. Basil leaves extract recorded the highest catechein, benzoic, P-OH-benzoic, ellagic and gallic acids, respectively. Serum creatinine, uric acid, urea, zinc, Calcium, magnesium, sodium, potassium and the renal levels of malondialdehyde increased after injection of ZnONPs, reduced hemoglobin, creatinine clearance, zinc's renal activity and antioxidant enzymes. On the other hand, the administration of basil leaves extract improved kidney functions and renal antioxidant enzymes. Therefore, basil leaves extract can be used as a potential preventive agent against ZnONPs induced nephrotoxicity.

Keywords: Basil leaves; liver functions; ZnO nanoparticles; nephrotoxicity; oxidative stress.

Introduction

The kidney is an important excretory organ. It has important functions in the metabolism and elimination of medications, xenobiotics, and environmental pollutants. It is exposed to a higher part of medications or poisons than other organs (**Hosohata,2016)**.

Many different nanoparticles are used in businesses and consumer items due to the rapid growth of nanoengineering. Because of their catalytic activity, ultraviolet, and visible absorption qualities. (ZnONPs) are becoming more popular. They've been employed in semiconductors, catalysts, paints, and a variety of other applications. **(Osmond and McCall 2010; Ludi and Niederberger 2013; Ma *et al.*, 2013).** Because of its digestibility, it can be used in the construction of food packaging or

pharmaceuticals. **(John *et al.*, 2010; Tankhiwale and Bajpai 2012).** Toxicity depends on the physicochemical parameters of NPs, such as particle size and shape and the amount of free space on their surfaces **(Pasupuleti *et al.*,2012).** On the other hand, nanomaterials have many drawbacks, including toxicity and negative environmental effects. A large number of research have found harmful effects in various experimental models of ZnONPs due to the widespread use of ZnONPs in numerous consumer products **(Adamcakova-Dodd *et al.*, 2014).** NZnO alters rat renal tissue energy metabolism and causes a cell membrane abnormality **(Yan *et al.*, 2012**). In embryonic kidney cells, NZnO caused oxidative stress (**Guan *et al.*, 2012).** It's also been found that 14 days of oral ZnO NP delivery damages the mitochondria and cell membranes in rats' kidneys **(Yan *et al.*, 2012).** It has been shown that ZnONPs have toxic impacts on numerous tissues such as the kidney **(Xiao *et al.* 2016),** Liver **(Almansour *et al.* 2017). Al-Al Zerjawe and Al-Bairuty (2020**) found that ZnONPs lead to kidney damage, including the growth of inflammatory cells near blood vessels, sloughing, and renal tubule lining epithelium, nucleus hypertrophy, necrosis, congestion of blood vessel, glomerulus loss and Calcium deposition within the tubules.

Culinary herbs have been stated to own antioxidant activities and might have potential human health benefits **(Yanishieva *et al.*, 2006)**. Basil, often known as sweet basil (*Ocimum basilicum)*, is a plant that belongs to the Labiatae family and has been proved to be effective in avoiding disease in many nations. Basil leaf extract has been found to have prevailing antioxidant, anti-cancer, anti-aging, antiviral, and antibacterial activities in several investigations **(Akujobi *et al.*, 2010).** Basil is recognized as one of the most important sources of medicine and pharmaceuticals due to many phytochemical active compounds such as terpenoids, saponins, anthraquinone, flavonoids, steroids, cardiac glycosides, alkaloids, and tannins **(Daniel et al.,2011).** Basil had antioxidant, nephroprotective, and anti-inflammatory properties and effectively reduced renal inflammation and glomerular filtration rate **(Abdulwahab *et al.*, 2018).** Basil displayed substantial antioxidant and nephroprotective action, as well as a protective effect against biochemical and histological alterations in tacrolimus-treated mice, according to **Oyounia *et al.*, (2018)**. So, the current study was intended to investigate the protecting effect of basil leaves extract against zinc oxide nanoparticle-induced nephrotoxicity in rats

Materials and methods

Materials:

1- Basil leaves powder was obtained from Sourour market in Tanta, Egypt.

2- Soybean oil and starch were purchased from the local market. Casein, cellulose, vitamins, minerals, dextrin, L-cysteine, and choline chloride were obtained from the Cairo Company for Chemical Trading, Cairo, Egypt.
3- ZnO nanoparticles with a particle size less than 100 nm and a ZnO composition greater than 99.9 percent were acquired from Sigma-Aldrich, USA.
4-Twenty-four male albino rats (*Sprague Dawley* strain) were be attained from the Laboratory Animal Colony, Helwan, Cairo – Egypt, and their average weight ranged from 150±10g.
5- Kits were purchased from Egyptian American Company for Laboratory Service and Supplied by Alkan Company.

Methods:

Preparation of the raw materials

Basil leaves were harvested in the Tanta region, certified by a botanist, and used in an alcoholic extraction. 6kg of fresh basil leaves were collected, rinsed, and dried in the shade before being crushed in a grinder. Additionally, the dried leaves were steeped in 90% ethyl alcohol for 15 minutes in a tightly closed container before being analyzed. After that, it was placed in a percolator and macerated at room temperature for 24 hours before being slowly percolated. The procedure was repeated until no further extraction was possible, and the resulting residue was placed in a vacuum desiccator.

Determination of phenolic compounds

HPLC was used to separate phenolic acid from basil leaves extract, dissolved in a mobile phase. We used retention time and peak area to figure out how many phenolic chemicals were in each sample, according to **(Tarola *et al.*, 2013).**

Experimental design

Twenty-four adult male albino rats of the *Sprague Dawley* strain weighing (150± 10g) were housed in well-aerated cages and fed a basal diet according to Reeves et al., (1993) for one week for adaptation; then they were randomized into four groups. Each group contained sex animals. Group 1 rats were given a basal diet and served as normal control. Group 2 rats were fed on a basal diet+ 500mg/ kg b.w. from leaves extract by gavage. Group 3 rats have a single dose of ZnONPs (600 mg/kg i.p.) on the 29th day, according to **(Ahmad *et al.*, 2012)**. Group 4 rats received a single dose of ZnONPs (600 mg/kg i.p.) on the 29th day+ 500 mg/ kg b.w. leaf extract via gavage .The experiment lasted for (4weeks). The body weight gain (BWG) was determined according to **Champman *et al.*, (1959).** In addition, the rat's weight is recorded weekly**.** After 24 h of the last treatment of ZnONPs, the rats were fasted

overnight before being sacrificed. Each rat's blood sample was centrifuged for 10 minutes at 3000 rpm to separate the serum. The serum was carefully separated and stored at -20oC for analysis. kidneys and Liver were removed from each rat by careful dissection, cleaned from the adhesive matter by a saline solution (0.9%), dried by filter paper, weighed. The kidney was excised immediately for biochemical investigation. One kidney was placed in a dry clean aluminum foil and kept frozen till estimated antioxidant enzymes levels, and the other part was reserved in formalin solution (10%) for histopathological investigation.

Hematological investigations

According to the manufacturer's instructions, a complete blood count was performed on anticoagulant-treated blood samples using a haematological analyzer (Exigo Eos Vet, Sweden) **(Jain, 1986).**

Biochemical analysis of serum and urine

Alkaline phosphatase (ALP) activity was estimated according to **Kind and King, (1954).** Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated according to **Bergmeyer *et al.*, (1986).** Albumin **(Drupt, 1974)** and total protein were determined according to **(Sonnenwirth and Jaret, 1980).** Uric acid, urea nitrogen and creatinine were calculated in serum according to **Patton *et al.*,(1977), Faulkner and King, (1976) and Fossati *et al.*, (1980)**, respectively. Creatinine clearance (Cr Cl) was calculated from S Cr and U Cr levels and 24-hour urinary volumes. Sodium and potassium were determined according to **Sonnen wirth and Jaret (1980)**. Calcium, magnesium, and zinc were determined according to **Milner, Whiteside (1984).**

-Assessment of Oxidant/Antioxidant Activity in kidney tissue

After the kidney was removed, homogenized, and centrifuged at 10,000 rpm at 4°C for 20 minutes, the supernatant was used to estimate different antioxidant levels by calorimetric method with a spectrophotometer Elisa (microplate reader Ryto2100 C) at (520 and 535 nm)(Manoldehyde (MDA) by thiobarbituric acid-reactive substances (TBARS) methods **(Uchiyama and Mihara 1978),** Superoxide dismutase (SOD) by a technique developed by **Misra and Fridovich, (1972)** and Catalase (CAT) by colorimetric assay **(Sinha,1972)**. **Sedlak and Lindsay,(1968)** method estimated glutathione reductase (GSH).

-Statistical analysis

The results are presented as mean standard deviation (SD). Differences in means between groups were tested for significance using a one-way ANOVA followed by Duncan's test, and a P-value of 0.05 or less was considered significant according to **(Snedecor, 1969**) using SPSS (vertion20).

Results and Discussion

Determination of Phenolic compounds of basil leaves extract

Data in Table (1) showed the Phenolic content in basil leaves. The results revealed that basil leaves recorded the highest content of catechein, benzoic, p-OH-benzoic, and ellagic acids, which also contains gallic, caffeic, vanillic, ferulic and, p-coumaric acids. **Tarchoune *et al*., (2012)** found that basil leaves contain p-OH-benzoic, gallic, coumaric, vanillic, and ferulic acids. Also agree with **Złotek *et al*., (2016),** who found that basil is a rich supplier of phenolic compounds, especially phenolic acids such as (benzoic acid, vanillic acid, p-coumaric acid, caffeic acid, ferulic acid, and protocatechuic acid).

Table (1): Phenolic compounds of basil leaves extract b(ppm.)

Phenolic compounds Ppm.	basil leaves
Gallic	16.79
Pyrogalloi	113.04
4-Aminobenzoic	19.89
Protocatchuic	30.31
Catechein	341.50
Chlorogenic	59.50
Catechol	28.05
Caffeine	67.10
P-OH-benzoic	150.10
Caffeic	22.40
Vanillic	15.04
P-coumaric	12.11
Ferulic	13.15
Iso- Ferulic	92.13
Ellagic	128.91
Alpha –coumaric	4.34
Benzoic	184.36
Salycillic	115.62
3,4,5-methoxy-cinnamic	58.75
Coumarin	29.31
Cinnamic	3.76

Biological evaluations

The consequences presented in Table (2) showed the influence of basil on body weight gain (BWG) and organs weight in adult rats with renal injury induced by zinc oxide nanoparticles (ZnONPs). Our findings revealed that the mean value of body weight gain was significantly reduced in the positive (+ve) control group (1.24 ± 0.11g) compared to the negative (-ve) control group (2.38 ± 0.94g). But when compared to the (+ve) control group, the basil-treated groups showed significant increases($P<0.05$). Liver and kidney weights recorded substantial decreases in the (+ve) control group compared to the (-ve) control group. In contrast, basil-treated groups showed significant increases compared to (+ve) control group. Administration of basil leaves extract showed significant increases when compared to(+ve) control group. These results agree with **Yousef *et al*., (2019),** who found that body weights in groups treated with ZnONPs were significantly lower than control rats. Also, the liver and kidney weights showed a significant decrease compared to the negative control group. ZnONPs may exert their toxicity through the formation of free radicals, lipid peroxidation, oxidative stress, DNA degradation, systemic inflammation, and anemia, as well as alterations in the expression of hepatic genes governing mitochondrial biogenesis. When consumed, nanoparticles can travel to various regions and organs of the body due to their small size. They can enter the bloodstream via the small intestine, the brain, the lung, the heart, the kidney, the liver, the spleen, the stomach, and the large intestine. Following intestinal absorption, biodistribution investigations demonstrated that the kidneys, liver, and spleen are the primary target organs for nanoparticles. Toxicological symptoms such as diarrhea, Liver and kidney cytotoxicity, membrane injury, DNA damage, inflammatory response, and apoptosis were also observed in ZnONPs treated rats. ZnONPs also disturb energy metabolism and cause cell membrane defects in the renal tissue of rats **(Yan *et al*., 2012).**

Instead, basil leaf extract administration enhances body weight gain, liver and kidney weight. Basil is a diuretic and a nephroprotective in treating drug-induced renal impairment, nephrocarcinoma, kidney, and urethral stones. It is reported that basil has anti-inflammatory, antiplatelet, antiulcer, antiviral, antibacterial, antifungal, and anticancer properties. In addition, flavonoids, polyphenols, tannins, steroids, phytosterols, saponins, polysaccharides, and fatty acids are found in basil leaves. These compounds are natural antioxidants and free radical scavengers that have been shown to protect against ZnONPs -induced oxidative damages **(Zaveri *et al*., 2011 and Alkaladi *et al*., 2015).**

Table (2): The protective effect of basil leaves on (BWG) and organs weight against zinc oxide nanoparticle-induced nephrotoxicity in rats

Groups	B.W.G (g/day)	Liver weight(g)	Kidney weight(g)
(-ve) control	2.38 ± 0.94^{a}	7.70 ± 0.23^{a}	2.23 ± 0.22^{a}
Basil leaves	2.43 ± 0.33^{a}	7.73 ± 0.13^{a}	2.30 ± 0.23^{a}
(+ve) control	1.24 ± 0.11^{b}	7.03 ± 0.41^{c}	2.10 ± 0.23^{b}
basil leaves + ZnONPs	1.40 ± 0.21^{b}	7.30 ± 0.78^{b}	2.15 ± 0.18^{b}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$

Hematological investigations

Data presented in Table (3) showed basil leaf extract's effect on blood count (erythrogram) in adult rats with renal injury induced by zinc oxide nanoparticles.

Results for hemoglobin recorded a significant decrease in the mean value of the (+ve) control group (11.08 ± 0.13 g/dl) compared to (-ve) control group (11.39 ± 0.66 g/dl). Basil groups showed a marked increase when compared to the (+ve) control group. Results for MCV and MCH recorded a significant decrease in the mean value of the (+ve) control group compared to the (-ve) control group. Basil groups showed a marked increase compared to the (+ve) control group. These data agree with **Alkaladi *et al*., (2015),** who found a significant decrease in hemoglobin and MCV concentrations in ZnONPs groups. Anemia is associated with a reduction in erythrocyte indices. This normocytic normochromic anemia could be caused by a reduction in RBC life span or suppression of bone marrow stem cell activity. It could also be related to hemodilution or hemoconcentration caused by Zn's osmotic alteration of blood viscosity **(Oti and Avoaja, 2005).** The main toxic mechanism of ZnO nanoparticles was possibly by cumulative cellular oxidative stress **(Hao *et al*., 2013).** ZnONPs are also cytotoxic and genotoxic to erythrocytes. These erythrocyte abnormalities could be caused by the release of reactive oxygen species (ROS), damaging the cell membrane by causing lipid peroxidation of unsaturated fatty acids **(Ahmad *et al*., 2006).** When nanoparticles enter the bloodstream, they confront an immune cell and plasma protein web. Immune cells recognizing nanoparticles as foreign particles may produce reactive oxygen and nitrogen species (ROS/RNS) and modify cytokine levels **(Hou *et al*., 2010)**. The amelioration in basil groups can be attributed to that basil is a rich source of tannins, steroids, polyphenols, including (phenolic acids, simple or complex flavonoids, and colored anthocyanins). In terms of pharmacological activity, they can decrease the level of ROS and thus the oxidative stress caused by

ZnONPs toxicity. Basil also has a high Iron content, which improves hemoglobin level **(Alkaladi *et al*., 2015 and Filip, 2017).**

Table (3): The protective effect of basil leaves on blood count (erythrogram) against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	Hemoglobin (g/dl)	MCV (fl)	MCH (pg)
(-ve) control	11.39 ± 0.66^{b}	92.43 ± 2.95^{a}	29.21 ± 0.42^{b}
Basil leaves	12.44 ± 0.32^{a}	92.44 ± 2.96^{a}	30.85 ± 0.18^{a}
(+ve) control	11.08 ± 0.13^{b}	88.49 ± 3.35^{b}	27.62 ± 0.50^{c}
Basil leaves+ ZnONPs	11.09 ± 0.14^{b}	90.47 ± 4.12^{ab}	27.64 ± 0.49^{c}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$

Results for WBCs recorded a significant decrease in the mean value of (+ve) control group (4.93 ± 0.14 x10^3/cmm) when compared to (-ve) control group (7.90 ± 0.27 x10^3/cmm). Results for lymphocytes recorded a significant decrease in the mean value of(+ve) control group (85.21 ± 0.67 %) when compared to (-ve) control group (85.73 ± 0.56 %). The monocyte results recorded a significant decrease in the mean value of the (+ve) control group (1.14 ± 0.07 %) compared to the (-ve) control group (1.30 ± 0.05 %). While basil groups showed a marked elevation when compared to (+ve) control group (Table4). **Alkaladi *et al.*, (2015)** discovered stress-related leukopenia, heteropenia, lymphopenia, monocytopenia, and eosinopenia in all treated groups. The decrease in the number of white blood cells (leukopenia) may be due to the negative impact of ZnONPs on bone marrow and lymphoid tissues as a result of Zn bioaccumulation in various tissues, which causes toxicity and affects splenic cell production **(Celik *et al.*, 2013 and Faiz *et al.*, 2015**). The improvement in basil groups may be due to the basil content of phenolic acids such as (Benzoic, caffeic, and p-coumaric). The primary function of phenolic acids is as antioxidants. The redox characteristics of phenolic acids are essential in neutralizing free radicals, quenching singlet and triplet oxygen, and degrading peroxide. Hence, they have other benefits, such as being protective against cardiovascular disease, acting as antiplatelet and anti-inflammatory agents, as well as its content of flavonoids such as (quercetin, apigenin, luteolin, and kaempferol, mostly as O-glycosides) which have a variety of biological activities such as antioxidant, anti-inflammatory, antimicrobial, antiviral, antiallergenic and vasodilating properties. The antioxidant activity of flavonoids has sparked the most interest, owing to their ability to reduce free radicals.

Additionally, it contains a range of vital elements, including vitamin A, vitamin C, calcium, and phosphorus. Further, it includes a high concentration of carotenoids, such as β- carotene, which the body converts to vitamin A.β- carotene is more beneficial than vitamin A alone. It is also a potent antioxidant **(Filip, 2017).** According to **Batra and Gupta, (2006)**, supplementation with basil leaf reduced the severity of hepatitis, hydropericardium, myocarditis accompanied by hemorrhages, pulmonary edema, and lymphocytic depletion in lymphoid organs focal interstitial nephritis.

Table (4): The protective effect of basil leaves on blood count (leukogram) against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	WBCs (x10^3/cmm)	lymphocytes (%)	Monocytes (%)
(-ve) control	7.90 ± 0.27^{b}	85.73 ± 0.56^{b}	1.30 ± 0.05^{b}
Basil leaves	10.18 ± 0.94^{a}	87.21 ± 0.85^{a}	2.13 ± 0.39^{a}
(+ve) control	4.93 ± 0.14^{c}	85.21 ± 0.67^{b}	1.14 ± 0.07^{b}
Basil leaves + ZnONPs	4.95 ± 0.15^{c}	85.25 ± 0.71^{b}	1.29 ± 0.05^{b}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$

Kidney functions

Results for creatinine recorded a significant increase in the mean value of (+ve) control group (0.92 ± 0.02 mg/dL) comparing to (-ve) control group (0.82 ± 0.02 mg/dL). Basil groups showed a marked improvement when compared with (+ve) control group. The mean value of urea in (+ve) control group (71.95 ± 7.89 mg/dL) showed a significant increase when compared with the (-ve) control group (36.60 ± 2.86 mg/dL). Administration of basil extract showed a marked amelioration when compared with (+ve) control group. control (+ve) group showed a significant increase in Uric acid values (1.97 ± 0.10 mg/dL) when compared with control (-ve) group (1.52 ± 0.14 mg/dL). basil groups showed a marked improvement when compared with the control (+ve) group. While the mean value of CR.CL in control (+ve) group showed a significant decrease (1.14 ± 0.11 ML/min/1.73 m^2) as compared with negative control group (1.82 ± 0.08 ML/min/1.73 m^2). basil groups showed a marked improvement when compared with (+ve) control group (Table 5). These findings are consistent with those of **Faddah *et al.*, (2012),** who discovered that ZnONP-induced nephrotoxicity was associated with an increase in serum inflammatory markers such as TNF-, IL-6, and C-reactive protein (CRP). The observed increase in creatinine could be attributed to a decrease in the glomerular filtration rate or that nanoparticles significantly impact liver and kidney function. Nanoparticles can cause cytotoxicity through various mechanisms,

including increased production of reactive oxygen species (ROS) accompanied by oxidative stress, lipid peroxidation, genotoxicity, and induction of inflammatory pathways. It has been reported that ZnONPs interact with proteins and enzymes within mammalian cells and can interfere with the antioxidant defense mechanism, resulting in ROS generation, the initiation of an inflammatory response, an increase in the tumor suppressor p53, and mitochondrial perturbation and destruction, causing apoptosis or necrosis in liver and kidney tissues **(Schrand *et al.*, 2010 and Babele, 2019)**. ZnONPs can cause toxicity by interacting directly with cell organelles, forming chemical compounds with DNA, RNA, proteins, and other molecules, and accumulating in cells, tissues, and organs, causing oxidative damage to organs **(Jiang *et al.*, 2009)**. ZnONPs produced histopathological alterations in the kidney, including inflammatory cell buildup in glomerular capillaries and tubule degradation in the proximal and distal tubules. It appears that ZnO NPs have a short-term influence on renal function. With the gradual elimination of nanoparticle absorption into the kidney, these effects vanish after a month **(Noori *et al.*, 2014).** Swelling and fusing of podocytes may enhance the enlargement of the glomerular filtration slits, allowing proteins to pass through the glomerular barrier (**Almansour *et al.*, 2019)**.

On the other hand, basil treatment for four weeks improved Kidney function indicators. Our findings are consistent with those of **Oyouni *et al.*, (2018),** who found that basil pretreatment dramatically decreased alterations in biochemical indicators of nephrotoxicity such as blood urea nitrogen and creatinine, showing a protective effect on renal glomerular filtration ability. This is because extracts of basil leaves display dominant anti-oxidant action in a variety of test settings. It contains antioxidants such as linalool, eugenol, methyl chavicol, methyl cinnamate, ferulate, methyl eugenol, triterpenoids, and steroidal glycosides. As a result, the extracts could be employed as a therapeutic to inhibit the activity of environmental toxins, drug-induced disturbances, or toxicity.

Table (5): The protective effect of basil leaves on kidney functions against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	CR.CL (ML/min/1.73 m^2)
(-ve) control	0.82 ± 0.02^{b}	36.60 ± 2.86^{c}	1.52 ± 0.14^{c}	1.82 ± 0.08^{a}
Basil leaves	0.81 ± 0.01^{b}	34.97 ± 1.13^{c}	1.49 ± 0.09^{c}	1.79 ± 0.03^{a}
(+ve) control	0.92 ± 0.02^{a}	71.95 ± 7.89^{a}	1.97 ± 0.10^{a}	1.14 ± 0.11^{c}
Basil leaves + ZnONPs	0.85 ± 0.02^{c}	51.65 ± 2.06^{b}	1.70 ± 0.13^{b}	1.57 ± 0.06^{b}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$.

Serum electrolytes

In Table 6, the mean value of calcium in (+ve) control group (8.60 ± 0.35 mg/dL) was significantly lower as compared to the negative control group (11.54 ± 0.77 mg/dL). Results for Magnesium in (+ve) control group (1.37 ± 0.09 mg/dL) showed significant reduction as compared to control (-ve) group (2.26 ± 0.07 mg/dL). Sodium for control (+ve) group (139.33 ± 1.86 mmol/l) was significantly lower when compared with control (-ve) group (150.00 ± 1.78 mmol/l). Likewise for Potassium in (+ve) control group (4.26 ± 0.09 mmol/l) showed significant decrease as compared to (-ve) control group (5.38 ± 0.09 mmol/l). Basil groups showed significant improvement for all electrolytes when compared with the positive control group. **Yousef *et al.* *(2019)*** demonstrated that ZnONPs caused focal degradation of renal epithelial tubules, vacuolation, distortion of renal corpuscles, capillary shrinkage in the glomerulus with capsular space cellular infiltration in both distal and proximal tubules with pyknotic nuclei. **Noori *et al.*, (2014)** also found that ZnONPs caused neutrophil and eosinophil accumulation in glomerular capillaries (due to capillary infiltration) and proximal and distal tubule degeneration walls. Reduced glomerular filtration rate can increase electrolyte excretion in urine and thus decrease its rate in serum. These changes in kidney tissue, such as glomerular fibrosis and inflammatory cell infiltration, indicate the cytotoxic properties of ZnONPs on distal and proximal tubules **(Najafzadeh *et al.*, 2013).** The swelling and detachment of glomerular endothelial cells as a sign of nephrotoxicity caused by ZnONPs may be responsible for disrupting sodium and water reabsorption **(Almansour *et al.*, 2019).** ZnONPs promote cytotoxicity by generating ROS, which can cause damage and depolarization of cell membranes, resulting in electrolyte imbalance. Changes in renal cell osmolarity cause zinc ions to enter the mitochondrial matrix, compromising the inner membrane's integrity **(Vandebriel and De Jong, 2012 and Ng *et al.*, 2017).** On the other side, **Sakr and Al-Amoudi, (2012)** reported that the aqueous extract of basil leaves had been shown to alleviate nephrotoxicity and oxidative stress in rats. This might be because basil contains a variety of bioactive compounds, including phenolic compounds, glycosides, proteins, amino acids, steroids, sterols, saponins, triterpenoids, tannins, flavones, and flavonoids, which are responsible for its antioxidant activity due to their ability to scavenge free radicals and decrease oxidative stress **(Raina *et al.*, 2016)**. In addition to these reports, **Masresha *et al.*, (2012); Kamyab and Eshraghian, (2013)** confirmed its traditional use as a potent anti-inflammatory agent. Basil also has a high magnesium and potassium content, one of the seven essential macrominerals, which

improve the cardiovascular system health and nerve impulses transmission **(Filip, 2017).**

Table (6): The protective effect of basil leaves on serum levels of some electrolytes against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	Calcium (mg/dL)	Magnesium (mg/dL)	sodium (mmol/l)	Potassium (mmol/l)
(-ve) control	11.54± 0.77^{a}	2.26 ± 0.07^{a}	150.00 ±1.78^{a}	5.38 ± 0.09^{a}
Basil leaves	11.50 ± 0.82^{a}	2.27 ± 0.07^{a}	151.33 ±1.36^{a}	5.42 ± 0.06^{a}
(+ve) control	8.60± 0.35^{c}	1.37 ± 0.09^{c}	139.33 ±1.86^{c}	4.26 ± 0.09^{c}
Basil leaves + ZnONPs	9.77± 0.36^{b}	1.94 ± 0.05^{b}	144.66 ±1.36^{b}	4.62 ± 0.17^{b}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at p≤0.05

Zinc in serum and kidney tissue

In Table 7, the mean value of serum zink in the positive control group (75.66 ± 7.28 µg/dl) showed a significant increase as compared to the negative control group (12.66 ± 3.14 µg/dl). Likewise, in tissue, zink in (+ve) control group (153.33 ± 10.93 µg/dl) showed a significant increase when compared to the negative control group (41.33± 5.46 µg/dl). While basil groups showed a significant decrease when compared with (+ve) control group. These findings agree with **Najafzadeh *et al.*, (2013)** who found that exposure to zinc nanoparticles caused an increase in zinc level in serum and liver and kidney tissues of lambs. Zinc is a component of many enzymes and transcription factors. The entrance of ZnONPs into the cell increases the cytosolic Zn^{+2} content, disrupting cellular zinc homeostasis (**Osmond and McCall, 2010 and Kao *et al.*, 2012)**. This indicates that the alterations caused by ZnONPs can increase intracellular zinc ions, causing interaction with renal cell components and oxidative stress **(Vandebriel and De Jong, 2012).** ZnONP exposure may produce renal ultrastructural alterations, resulting in nephron destruction and impaired kidney function. The oxidative dissolution of ZnONPs and the release of Zn^{2+} in the renal tissue may deplete the tissues' dissolved oxygen, resulting in ROS production **(Almansour *et al.*, 2019**). On the other hand, basil leaves possess good antioxidant and antistress properties **(Sethi, 2003).** In addition, **Batra and Gupta (2006)** found that supplementing with basil leaf reduced the severity of focal interstitial nephritis.

Table (7): The protective effect of basil leaves on Zink levels in serum and kidney tissue against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	Zink (µg/dl) serum	Zink (µg/dl) tissue
(-ve) control	12.66 ± 3.14^{c}	41.33± 5.46^{c}
Basil leaves	11.00 ± 0.89^{c}	38.66 ± 2.25^{d}
(+ve) control	75.66 ± 7.28^{a}	153.33 ±10.93^{a}

Basil leaves + ZnONPs	45.00 ± 5.86[b]	101.66 ± 4.22[b]

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at p≤0.05

Liver enzymes

The mean value of ALT in the positive control group (112.30 ± 11.71 U/L) showed a significant increase compared to the negative control group (54.04 ± 7.37 U/L). The mean value of AST in (+ve) control group (114.39 ± 5.41 U/L) showed a significant increase when compared to the negative control group (94.34 ± 10.05 U/L). Likewise, results for ALP showed a significant increase in (+ve) control group (181.28 ± 12.31 U/L) when compared to a negative control group (136.06 ± 5.69 U/L). Basil groups showed a significant decrease compared with the (+ve) control group (Table 8). These data agree with **Abdelbaky, (2013) and Yousef *et al.*, (2019),** who found a significant elevation in serum ALT, AST, and ALP in rats treated with ZnONPs. **Abdelbaky, (2013)** revealed that ZnONPs caused liver damage, as evidenced by a significant increase in serum ALT (a marker of liver tissue damage). These findings could result from cellular leakage and loss of functional integrity of liver cell membranes, indicating that the liver is one of the target organs of such NPs toxicity **(Chen *et al.*, 1992).** According to **Ding *et al.*, (1998),** excessive dietary zinc induced liver damage in mice and reduced AST activity in mouse liver homogenate. ZnONPs caused a substantial reduction in the expression of hepatic genes (PGC-1 and mTFA), which might imply reduce mitochondrial biogenesis and defective mtDNA replication and transcription, potentially leading to mitochondrial malfunction. Because alteration of mitochondrial membrane potential is a critical step in the apoptotic process, it has been established that NPs exposure affects rat liver mitochondrial function, mainly through changes in mitochondrial membrane permeability **(Li *et al.*, 2012)**. According to **Yousef *et al.*, (2019),** ZnONPs caused hepatocellular necrosis, edema, congestive dilation of central veins and degeneration in the hepatocytes. In addition, **Najafzadeh *et al.*, (2013)** reported that ZnONPs caused liver changes such as swelling and necrosis of hepatocytes. Elevation of the serum marker enzymes ALT, AST, and ALP results from liver damage that releases these enzymes into the blood circulation following hepatotoxin administration **(Kew, 2000).** On the other hand, **Yacout *et al.*, (2012)** demonstrated the protective effect of basil against liver fibrosis. According to **WenChuan and Wei-Lii (2006),** Basil can help alleviate liver hepatocytes' synthetic function. **Sharma *et al.*, (2002)** found that basil leaf extracts protect the Liver from heavy metals. Supplementation with basil leaves has also been shown to reduce the

severity of hepatitis by **Batra and Gupta (2006)**. Basil is high in flavonoids, which have been linked to various biological characteristics associated with antioxidant processes. As a result, **(Sakr *et al.*, 2011)** concluded that the antioxidant activity of *O. basilicum's* flavonoids might be responsible for its hepatoprotective effect.

Table (8): The protective effect of basil leaves on liver enzymes against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	ALT (U/L)	AST (U/L)	ALP (U/L)
(-ve) control	54.04 ± 7.37^{c}	94.34 ± 10.05^{c}	136.06 ± 5.69^{c}
Basil leaves	51.73 ± 2.87^{c}	93.23 ± 2.87^{c}	134.46 ± 3.98^{c}
(+ve) control	112.30 ± 11.71^{a}	114.39 ± 5.41^{a}	181.28 ± 12.31^{a}
Basil leaves+ ZnONPs	96.17 ± 5.98^{b}	104.83 ± 16.07^{b}	162.13 ± 11.17^{b}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$

Protein fractions

Results for the total protein in the control (+ve) group (5.07 ± 0.27 g/dl) showed a significant decrease as compared to the negative control group (6.24 ± 0.22 g/dl). The mean value of albumin in (+ve) control group (2.66 ± 0.15 g/dl) showed a significant decrease when compared to a negative control group (3.08 ± 0.17 g/dl). Also results for globulin showed significant decrease in (+ve) control group (2.40 ± 0.12 g/dl) when compared to control (-ve) group (3.16 ± 0.16 g/dl). Basil groups showed a significant increase compared with the (+ve) control group (Table 9). These findings align with **Yousef *et al.*, (2019)**, who found a marked decrease in total proteins in male rats treated with Zno nanoparticles. The accumulation of oxygen reactive species (ROS) induced by ZnONPs causes oxidative stress in cells. Serum levels fall in inflammation, oxidative stress, acute or chronic liver failure, and hepatorenal syndrome due to decreased hepatic albumin synthesis **(Arroyo *et al.*, 2014)**. Basil has antioxidant, anti-inflammatory, antitoxic, antimutagenic, antiarrhythmic, immunomodulatory, and hepatoprotective activities **(Khair-ul-Bariyah *et al.*, 2012).**

Table (9): The protective effect of basil leaves on protein fractions against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	T.P (g/dl)	ALb (g/dl)	G (g/dl)	A/G
(-ve) control	6.24 ± 0.22^{a}	3.08 ± 0.17^{b}	3.16 ± 0.16^{a}	0.97 ± 0.08^{b}
Basil leaves	6.36 ± 0.20^{a}	3.36 ± 0.10^{a}	3.00 ± 0.17^{a}	1.12 ± 0.06^{a}
(+ve) control	5.07 ± 0.27^{c}	2.66 ± 0.15^{c}	2.40 ± 0.12^{b}	1.10 ± 0.01^{a}
basil leaves + ZnONPs	5.75 ± 0.29^{b}	2.80 ± 0.08^{c}	2.95 ± 0.20^{a}	0.94 ± 0.02^{c}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$

Antioxidant enzymes and lipid peroxide (MDA)in kidney tissue

The mean value of GSH in the positive control group (1.95 ± 0.45 ng/mg) showed a significant decrease compared to the negative control group (8.58 ± 0.78 ng/mg). Mean values of SOD in (+ve) control group (12.66 ± 3.72 U/mg) showed a significant decrease when compared to a negative control group (31.33 ± 1.36 U/mg). Results for CAT in control (+ve) group (27.33 ± 3.72 ng/mg) showed significant decrease as compared to negative control group (57.00 ± 2.36 ng/mg). While results for MDA (lipid peroxidation marker) showed a significant increase in (+ve) control group (120.33 ± 6.28 nmol/mg) when compared to a negative control group (58.33 ± 2.73 nmol/mg). Basil groups showed significant improvement when compared with (+ve) control group. These data agree with **Yousef *et al*., (2019),** who found a marked decrease in levels of antioxidant enzymes in kidney tissue in male rats treated with zinc oxide nanoparticles. ZnO nanoparticles cause cytotoxicity in a dose- and time-dependent manner, with the mechanism involving oxidative stress, lipid peroxidation (LPO), cell membrane damage, and oxidative DNA damage **(Xia *et al*., 2008 and Lin *et al*., 2009).** Pretreatment with *O. basilicum* for four weeks reduced mitochondrial LPO, indicating a protective action against LPO. Polyphenolic compounds are the most abundant natural antioxidants in *O. basilicum*, and their radical scavenging abilities help reduce oxidative stress. *O. basilicum* extract has also been shown to have immunomodulatory properties. It has been demonstrated that it inhibits the production of serum inflammatory markers and tumor necrosis factors **(Tsai *et al*., 2011).**

Table (10): The protective effect of basil leaves on antioxidant enzymes and malondialdehyde (MDA) against zinc oxide nanoparticle-induced nephrotoxicity in rats

groups	GSH (ng/mg)	MDA (nmol/mg)	SOD (U/mg)	CAT (ng/mg)
(-ve) control	8.58 ± 0.78^{b}	58.33 ± 2.73^{c}	31.33 ± 1.36^{b}	57.00 ± 2.36^{b}
Basil leaves	11.00 ± 0.89^{a}	48.00 ± 2.36^{d}	41.00 ± 0.89^{a}	62.66 ± 2.25^{a}
(+ve) control	1.95 ± 0.45^{d}	120.33 ± 6.28^{a}	12.66 ± 3.72^{d}	27.33 ± 3.72^{d}
Basil leaves + ZnONPs	4.53 ± 1.03^{c}	81.66 ± 6.28^{b}	21.66 ± 2.25^{c}	38.66 ± 2.87^{c}

Each value represents the mean ±SD. Means in the same column with different superscript letters were significant at $p \leq 0.05$

Conclusion

Taken together, basil leaves extract pretreatment in male albino rats protected against zinc oxide nanoparticle induced nephrotoxicity, at least in part, by increasing free radical scavenging activity, which might be attributed to the extract's antioxidants and flavonoids. The protective effectiveness was augmented by a substantial reduction in oxidative stress parameters in mitochondria isolated from the kidney, as well as an improvement in renal function biochemistry.

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الملخص العربى

الجسيمات النانوية عبارة عن مركبات ذات صفات خاصة صغيرة الحجم جدًا (أقل من ١٠٠ نانومتر). نتيجة لذلك ، تشكل الجسيمات النانوية العديد من المخاوف الصحية المختلفة. لذلك ، تهدف هذه الدراسة إلى تقدير التأثير الوقائي لمستخلص أوراق الريحان على السمية الكلوية التي تسببها جزيئات أكسيد الزنك النانوية في ذكور الجرذان البيضاء. تم تقسيم أربعة وعشرين ذكور جرذان سبراج داولي إلى أربع مجموعات. تم إعطاء جرذان المجموعة الأولى نظامًا غذائيًا أساسيًا لمدةاربعةأسابيع كمجموعة طبيعية سالبة . تم تغذية فئران المجموعة الثانية بنظام غذائي أساسي + 500 ملجم / كجم من وزن الجسم من مستخلص اوراق الريحان الكحولى لمدة ٤ أسابيع. تم حقن جرذان المجموعة الثالثة فى اليوم التاسع والعشرون جرعة واحدة من نانو اكسيد الزنك فى البطن بمقدار ٦٠٠ ملجم / كجم من وزن. تلقت جرذان المجموعة الرابعة جرعة واحدة من نانو اكسيد الزنك فى البطن بجرعة ٦٠٠ملجم /كجم من وزن الفأرفي اليوم التاسع والعشرين + ٥٠٠ مجم / كجم من وزن الجسم من مستخلص أوراق الريحان الكحولى بالتزقيم لمدة ٤ أسابيع. في نهاية التجربة ، أخذت عينات الدم ، وتم جمع الأعضاء الداخلية ، ووزنها. تم إجراء تحليل الدم الكامل. تم فصل المصل للفحص البيوكيميائي. سجل مستخلص أوراق الريحان أعلى نسبة من الكاتشين والبنزويك والبنزويك والبنزويك والإيلاجيك والجاليك على التوالي. زادت نسبة الكرياتينين في الدم ، وحمض البوليك ، واليوريا ، والزنك ، والكالسيوم ، والمغنيسيوم ، والصوديوم ، والبوتاسيوم ، ومستويات مالوندي الديهيد فى نسيج الكلى فى مجوعة النانو زنك ، وانخفاض الهيموجلوبين ، والكرياتينين ، والزنك فى نسيج الكلى ، والإنزيمات المضادة للأكسدة. من ناحية أخرى ، تناول مستخلص أوراق الريحان ادى إلى تحسين وظائف الكلى و الانزيمات المضادة للأكسدة فى النسيج الكلوى .لذلك ، يمكن استخدام مستخلص أوراق الريحان كعامل وقائي محتمل ضد السمية الكلوية التي يسببهانانو اكسيد الزنك فى الجرذان .

الكلمات المفتاحية: السمية الكلوية – مستخلص اوراق الريحان – وظائف الكبد – نانو اكسيد الزنك –الاجهاد التأكسدى .