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Antioxidant and anti-gout effects of orally administered zinc oxide nanoparticles in gouty mice

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ABSTRACT

Background: Zinc is an essential trace element which is involved in controlling oxidative stress, growth and immune system by regulating inflammatory cytokines. Gouty arthritis is the inflammation of joints and tissues caused by the accumulation of monosodium urate crystals.
Method and objective: This study involved the oral administration of zinc oxide nanoparticles at a various concentration (5 ppm, 10 ppm, and 20 ppm) and study their antioxidant and anti-gout effects on Balb/C mice. Various parameters such as ROS, superoxide, peroxide, catalase, TBARS, RFTs, LFTs, lipid profile and blood count were studied.
Results: ZnO nanoparticles at the concentrations of 10 and 20 ppm were significant ($P < 0.001$) in reducing serum uric acid concentration thus treating gouty arthritis. Reactive oxygen species and thiobarbituric acid reactive substances were significantly increased in comparison to zinc oxide nanoparticles treated groups. Furthermore, blood count and LFTs also showed the effectiveness of zinc oxide in the reduction of hyperuricemia. Histopathological analysis showed no apparent changes in liver, kidney and muscles tissues.
Conclusion: Zinc oxide nanoparticles can be effective in reducing oxidative stress and the treatment of gouty arthritis.

1. Introduction

Gout or gouty arthritis is characterized by the accumulation of monosodium urate crystals (MSUCs) in the joints and tissues resulting in hyperuricemia (HU). MSU crystals cause inflammation [1] mainly within the synovial area by its interaction with synovial fibroblasts, local macrophages, infiltrating neutrophils and monocytes, secreting proinflammatory mediators like interleukin -1β (IL-1β), tumor necrosis factor- α (TNF-α) [2] and chemokines (IL-8) [3–5]. These MSU crystals induce a mast cell degranulation that initiates inflammation [1]. HU happens when purine metabolism elevates serum concentration of uric acid beyond normal range [6]. HU is a precursor for other chronic diseases includes type 2 diabetes mellitus [7], joint pain (edema), dyslipidemia, abdominal obesity, kidney failure [8], metabolic syndromes [9] and hypertension [10]. Epidemiological studies revealed its prevalence ranging from 21% to 25.8% in American and Asian countries among adults respectively [11,12].

Zinc is a well-known micronutrient involved in controlling oxidative stress, growth, immune system [13]. It also regulates inflammatory cytokines [14] and cognitive impairment [15]. A report revealed that in hemodialysis patients there is a negative relationship between serum uric acid and zinc concentration [16]. Another research demonstrated that oral zinc intake improves hyperuricemia in a patient with Wilson's disease [17]. Previous studies depicted that zinc-flavonol complex treatment in diabetic rats improves the serum uric acid level [18]. One of the cross-sectional studies on middle-aged Chinese indicates the inverse relationship between dietary zinc intake and HU [19]. Previously, it has been reported that zinc plays an important role in scavenging free radicals and prevent inflammation in different biological processes [20]. Antioxidant effect of zinc has been confirmed by the prevention of formation of OH^{-1} (hydroxide ion) in antagonist transition metal-catalyzed reaction (Fenton reaction) [21]. The production of any OH^{-1} causes severe chronic localized damage in cellular components [22]. Studies revealed that zinc can reduce postischemic injuries in the brain,

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Table 1
Groups details and consideration taken for each group.

Group	Consideration
Group I (normal control)	Normal mice
Group II (NPs Control)	Normal mice orally administered with 10 ppm of ZnO NPs
Group III (diseased control)	MSU induced hyperuricemia/gouty mice
Group IV	Gouty mice orally administered with 5 ppm of ZnO NPs
Group V	Gouty mice orally administered with 10 ppm of ZnO NPs
Group VI	Gouty mice orally administered with 20 ppm of ZnO NPs
Group VII (positive control)	Gouty mice treated with 50 mg/kg of body weight Allopurinol

kidney, retina, stomach by replacing iron and copper from binding sites of metallothioneins (MT). MT's are metal-binding proteins having an affinity for zinc, copper, iron and many others. They are also involved in the reduction of oxidative stress, cytoprotective activity and anti-inflammation [23,24]. As the field of nanotechnology continues to expand, the production of nanoparticles is increasing rapidly. Recent advances in this field lead to the exponential growth in the developmental of new biocidal agents, medicine, consumer products, clothes, electronics, and sporting goods. This technology is capable of providing nano-scale size particles (1–100 nm) conferring large surface area and enhanced physicochemical and biological properties. Decreasing the size to nano range can modify their mechanical, structural, chemical, morphological, magnetic and optical properties. Thus, the nanoparticles are the research hot spot material in basic and applied sciences as well as nanobiotechnology nowadays.

ZnO is widely used due to its abundance, nontoxic effect, and bond strength and semi conductance ability [25]. It has been reported that zinc oxide nanoparticles can be synthesized by a green method using various plants including *Aloebarbadensis mitler* [26], *Calotropis procera* [27], *Polygala tenuifolia* [28] and *Poncirus trifoliate* [29]. Other applications involve gas sensor, environmental pollutant detoxification [30,31], antimicrobial agent and antibacterial agent in various ointments and mouthwashes [32]. Allopurinol is a commonly used medication for the treatment of gouty arthritis and hyperuricemia. It is well-known as a Xanthine oxidase inhibitor (XOI) used to decrease the high levels of serum urate [33]. Long term usage of allopurinol has side effects which include jaundice, nausea, dark urine, weight loss and many more [34]. For the reduction of long term side effects, zinc oxide nanoparticles (ZnO-NPs) were prepared and it was hypothesized that they might be effective for the treatment of gouty arthritis and hyperuricemia. ZnO-NPs have received increasing interest in recent years. It has been reported that ZnO-NPs are antibacterial agent [32]; they inhibit the growth of bacteria in the bacterial medium. Nano-sized ZnO exhibit different morphologies and over a large number of bacterial species have been explored with it. Due to its size, it enters into the bacterial cell and damages its membrane leading to its death by interacting with many intracellular components [35]. ZnO-NPs inhibits the synthesis of mRNA expression of inflammatory cytokines (IL-1β, IL-6, TNF-α). It has been revealed from the previous studies that ZnO-NPs are therapeutic for the treatment of mast cell-mediated allergies [36]. Zinc oxide nanowires were used as a biosensor of uric acid [37].

In our present study, we have prepared zinc oxide nanoparticles (ZnO-NPs) to investigate the anti-gout and anti-oxidant effects in case of gouty mice. No known studies have examined the relationships between zinc oxide nanoparticles intake and hyperuricemia. Therefore, this study aimed to examine the in vivo effect of metallic zinc nanoparticles on the treatment of gout and hyperuricemia.

2. Materials & method

2.1. Synthesis of ZnO nanoparticles

For the synthesis of zinc oxide nanoparticles, two solutions were prepared in de-ionized water. 0.5 M of NaOH solution was prepared in 50 ml of de-ionized water and another solution of 0.5 M $ZnCl_2$ was prepared in 50 ml of de-ionized water. NaOH solution (0.5 M) was added slowly in $ZnCl_2$ solution (0.5 M) with continuous stirring on a magnetic stirrer. During the whole reaction, the pH of the mixture was maintained up to 11 and air was used as a reaction medium. Precipitates were obtained and filtered with the help of a filter paper. Precipitates were heated at 300 °C for 5 h after washing with de-ionized water. After drying, precipitates were ground in mortar and pestle. The fine powder obtained was ZnO nanoparticles [38].

2.2. Characterization

To find out the structure and composition of ZnO-NPs XRD analysis was performed using AXS D8 Advance, while the surface morphology of ZnO-NPs was observed by SEM and elemental analysis by EDS (Tescan Vega 3).

2.3. Animals

The whole experimental study was performed on Balb/C mice of 25 ± 10 g weight of either sex. Adult mice were obtained from the National Institute of Health (NIH) Islamabad, Pakistan and were treated according to the guidelines of NIH. Mice were acclimatized for a week in a temperature-controlled room with a 12:12 -h dark-light cycle, provided with feed and water *ad-libitum*. Mice were given diet according to previously studied standards, which contained 10 mg/kg of zinc nutrient [39]. The departmental ethics committee of IIUI approved the experimental protocol (Letter No. IIU (BI&BT)/FBAS-IBBC-2015-4). Mice were divided into 7 groups, in which each group had 20 mice details of each group are given in Table 1.

2.4. Synthesis of drugs and test solutions for in vivo experiment

2.4.1. Preparation of mono sodium urate (MSU) crystals

For the preparation of Mono Sodium Urate (MSU) crystals, 0.001 M sodium hydroxide (NaOH) and 1.68 g uric acid were used. Uric acid was added in NaOH solution by heating it at 70 °C. For the maintenance of pH from 7.1 to 7.2 NaOH or hydrochloric acid (HCl) solution was added. The solution was left for 24 h at room temperature until the crystals were obtained. After discarding supernatant the crystals were washed with distilled water many times. Needle shaped MSU crystals were obtained when observed under a microscope for further experimentation [40,41].

2.4.2. Preparation of allopurinol solution

Powdered allopurinol tablet was dissolved in deionized water for the preparation of its suspension. Allopurinol solution was prepared according to the medium weight of each group and was given with the help of oral gavage to hyperuricemic mice (group 6).

2.5. Experimental

In order to evaluate the anti-hyperuricemic activity of zinc oxide nanoparticles, the diseased experimental mice were prepared by using

MSU crystals. Animals were divided into six groups and each group was administered with MSU crystals except control. Initially, MSU was administered by intra-articular injection on the left ankle of mice by the interval of one day for 3 weeks. For the next 2 weeks, MSU was injected intra-peritoneally. Blood samples were collected to measure the concentration of uric acid in mice regularly. Inflammation of ankles was measured by using Vernier caliper. After which mice were orally administered with zinc oxide nanoparticles at different concentrations for the 3 weeks, details are given in Table 1. At the end of the experiment, mice were euthanized and their muscle, liver and kidney samples were collected and stored in paraffin blocks for histopathology and tissues zinc level analysis. The tissue sections (3–5 μm) were prepared using microtome and were microscopically evaluated. Atomic absorption spectroscopy was utilized to analyze the zinc concentration in liver, kidney and muscle tissues.

2.6. Biochemical analysis

Renal function test and liver function test were analyzed using commercially available kits of AMP diagnostics AMEDA Labordiagnostik GmbH, Austria. All the procedures were carried out according to the manufacturer's instructions. Antioxidant enzymes level was estimated in liver tissue of control and treated animals. Superoxide dismutase (SOD) was assessed by the protocol adopted by [42]. Catalase (CAT), Guaiacol peroxidase (POD) levels were analyzed as described in [43] with some modifications. ROS value was determined using the protocol used by [44] and Thiobarbituric reactive acid substances (TBARS) levels were measured using [45] method on Smart Spec TM plus spectrophotometer. Lipid profile includes: Total cholesterol (TC), Triglycerides (TG), High-density lipoprotein (HDL-C), low-density lipoprotein cholesterol (LDL-C) were analyzed using commercially available kits of AMP diagnostics AMEDA Labordiagnostik GmbH, Austria. All the procedures were carried out using the manufacturer's instructions. Complete blood picture was analyzed using commercially available kits of AMP diagnostics AMEDA Labordiagnostik GmbH, Austria. Serum zinc level of mice was measured using atomic absorption spectroscopy.

2.7. Statistical analysis

All the data were expressed as mean ± SEM. One way analysis of variance (ANOVA) followed by Dunnett test was used to compare control with different experimental groups using Graph pad PRISM 5 software (San Diego, CA, USA). A Probability value of less than 0.05 was considered statistically significant.

3. Results

3.1. Characterization of ZnO-NPs

The XRD analysis of ZnO-NPs showed (Fig. 1) highest peak at 36.18° 2θ at x-axis corresponding to the intensity indicated the presences of zinc oxide nanoparticles [38]. Debye-Scherrer equation was used to calculate the size of these particles. The mean particle size was around 37 nm.

$$\text{Debye-Scherrer equation} \tau = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

Scanning electron microscopy (SEM) analysis of zinc oxide nanoparticles (Fig. 2) indicated the size and morphology. It was depicted that these chemically synthesized particles were amorphous and were in the nano range. The average diameter of the particles was 49.11 nm.

Energy-dispersive X-ray spectroscopy (EDS) of zinc oxide nanoparticles revealed the number of different elements which constitute the ZnO. EDS analysis can be better understood by observing Fig. 2.

3.2. Effect of ZnO-NPs on renal function test

To assess the extent of renal functionality the urea concentration of serum was measured by using the AMP diagnostic kit in control and treated mice. The MSU crystal-induced mice had a significant increase ($P < 0.001$) in urea level while, in ZnO-NPs ($P < 0.001$) and allopurinol ($P < 0.05$) treated mice had a significant decrease in urea level as compared to control as shown in Fig. 3.

The serum concentration of uric acid in experimental mice was decreased significantly when compared with the diseased control. On the other hand, ZnO-NPs and allopurinol treated group showed a significant decrease in uric acid concentration as compared to control. The Creatinine concentration of control was compared with all treated groups. MSU- induced gouty mice showed a significant increase ($P < 0.05$) in Creatinine values.

3.3. Effect of ZnO-NPs on liver function test

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values showed a significant increase ($P < 0.001$) in monosodium urate crystals when compared with control. ZnO-NPs (5 ppm) treated group showed significant decrease in ALT ($P < 0.05$) and AST ($P < 0.01$) values. ZnO-NPs (10 ppm) also showed significant decrease in ALT ($P < 0.01$) and AST ($P < 0.001$) values. The high dose treated group of ZnO-NPs (20 ppm) depicts a significant decrease in ALT and AST values ($P < 0.001$). Evaluation of total bilirubin revealed that its values increase significantly in MSU treated group, while other groups did not show any significant change.

Alkaline Phosphatase (ALP) of all the treated groups except the mice treated with ZnO-NPs (5 ppm) showed no significant changes in the values in comparison to the control groups. There was a significant ($P < 0.001$) decrease in the ALP values of mice treated with ZnO-NPs (5 ppm) (Table 2).

3.4. Effect of ZnO-NPs on antioxidant profile

The MSU- induced gouty group showed significant ($P < 0.001$) decrease in SOD, POD and CAT values. The SOD values of ZnO-NPs (5 ppm) showed a significant increase while other treated groups have a non-significant increase in SOD values in comparison with control. The POD values of ZnO-NPs (5 ppm) showed a significant ($P < 0.001$) decrease and other treated group groups showed a non-significant increase as compared to control. ZnO-NPs (5 ppm) showed a significant ($P < 0.001$) decrease in CAT values while other treated groups showed a non-significant increase when compared with control (Fig. 4).

ROS and TBARS values of MSU treated group was significantly ($P < 0.001$) increased in comparison with control. ZnO-NPs and allopurinol treated groups showed a significant decrease when compared with control.

3.5. Effect of ZnO-NPs on lipid profile

Lipid profile included evaluation of cholesterol, HDL, LDL, and triglycerides. The result showed that cholesterol, LDL and triglyceride significant ($P < 0.001$) increased in the diseased group as compared to control, yet remained within the normal range. All treated groups showed significant ($P < 0.001$) decrease in cholesterol, LDL and triglyceride in comparison with control. The HDL level significantly ($P < 0.001$) decreased in MSU treated group while, it showed significant ($P < 0.01$), ($P < 0.05$) increase in ZnO-NPs (20 ppm) and allopurinol treated groups respectively (Table 3).

3.6. Effect of ZnO-NPs on blood count test

The blood count test was performed to evaluate the effects of gout and ZnO-NPs on mice, the amount of white blood cells (WBCs)

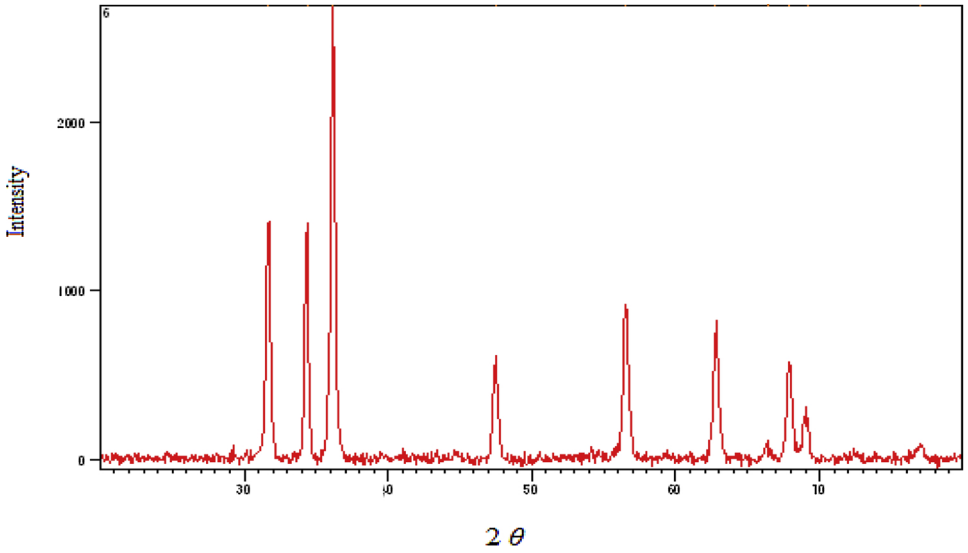

Fig. 1. X-ray diffraction pattern of synthesized zinc oxide nanoparticles.

increases with the inflammation of joints as gout progresses. All parameters were significantly high in MSU treated group in comparison with the normal control group. The number of white blood cells (WBCs) significantly ($P < 0.001$) increased to $7.15 \pm 0.20 \times 10^3/mm^3$ (Table 4).

3.7. Zinc level in serum and tissues

The average serum zinc level of mice treated with zinc oxide nanoparticles was significantly raised in comparison to the normal and diseased mice. Mice treated with 20 ppm of ZnO NPs showed the highest level of serum zinc level as shown in Fig. 5 (a). The average zinc level in liver tissues was higher in the ZnO NPs treated mice in comparison to normal and diseased mice all of the results can be visualized in Fig. 5(b).

3.8. Histopathology

Histopathology of diseased control group kidney tissue revealed mild mononuclear infiltrate in the interstitium as well as few interstitial

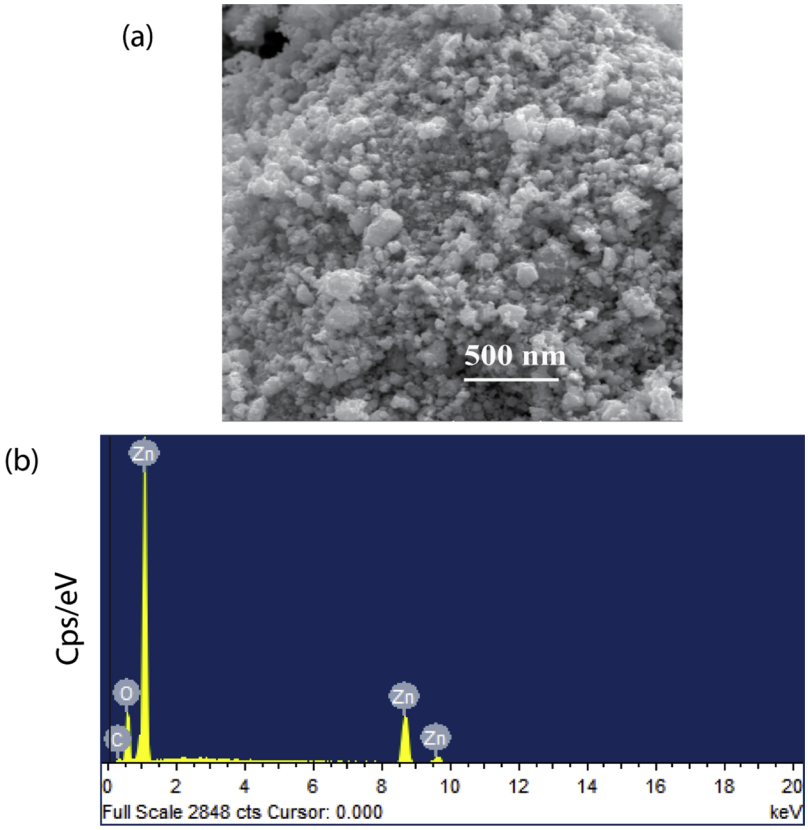

Fig. 2. (a) Scanning Electron Microscope (SEM) image of synthesized zinc nanoparticles (b) Energy dispersive X-ray spectroscopy (EDS) spectrum of synthesized zinc nanoparticles.

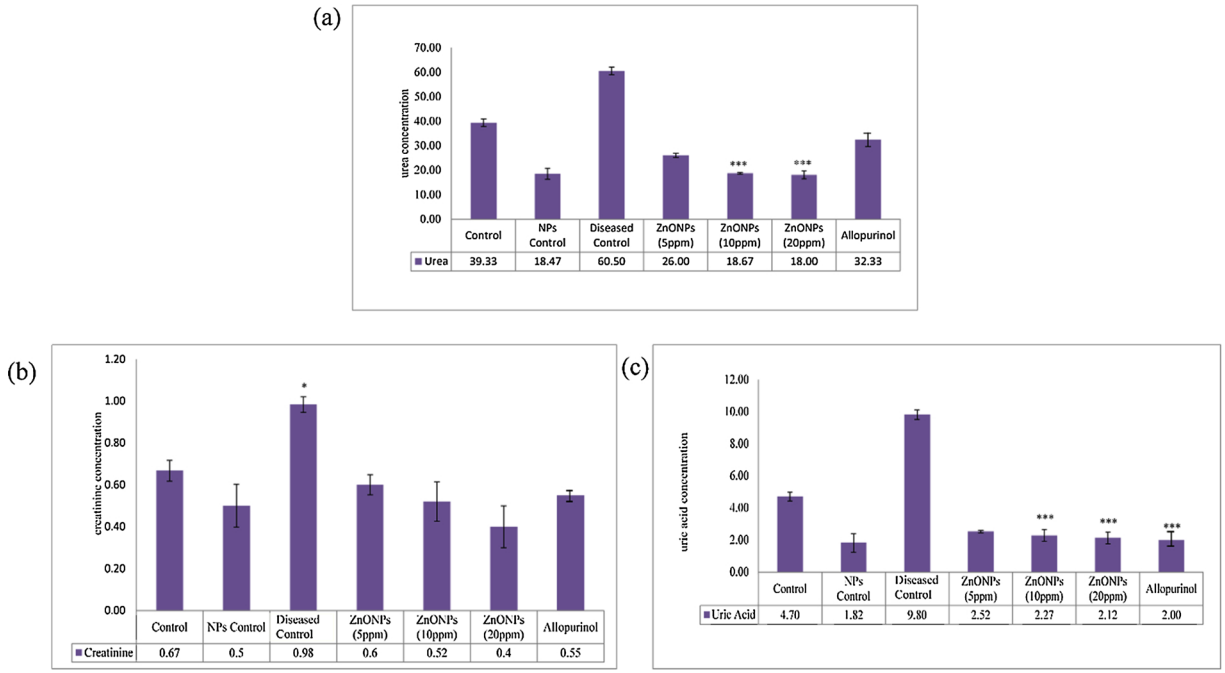

Fig. 3. Effect of ZnO NPs on Renal function test (RFTs) after three weeks of treatment. In which (a) is Urea Concentration (b) is creatinine concentration and (c) is serum Uric Acid. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

hemorrhages. There was also some loss of epithelial cells in proximal and distal convoluted tubules. However, treatment with ZnO NPs at 5, 10, and 20 ppm reduced the inflammatory infiltrate and decreased the cell loss of renal epithelial cells. Histopathology of liver sections of MSU induced gouty as well as treated mice showed no changes in histology i.e. no fatty change, no inflammation, and no fibrosis. Histopathology of muscle tissue sections showed no change in the histology of the MSU induced gouty mice group as compared with the ZnO NP Treated groups. Histopathological slides of all the groups can be visualized in Fig. 6.

4. Discussion

ZnO-NPs have been widely used in biomedical applications, as UV blockers cosmetic lotions [38], and food products [46]. Many studies have been reported on ZnO-NPs and its several activities. However, there are no reports on anti-gout activities of ZnO-NPs. Metal oxide nanoparticles are believed to be safe for applications because they are more stable and with salient properties [47]. Previous studies showed that oral and intraperitoneal administration of ZnO-NPs distributed the NPs in the mice liver, spleen, kidneys and adipose tissue [48]. Its systemic absorption increases the zinc concentration in the liver, adipose tissue and pancreas and produce anti-diabetic effect [49]. Studies [50,51] have shown that there is an inverse association between dietary zinc uptake and hyperuricemia in both men and women. Furthermore, in a previous study the effect of zinc oxide nanoparticles on renal function tests was studied in which it was observed that the serum uric acid was reduced significantly in comparison to the control group [52].

The present study aimed to evaluate the anti-gout and antioxidant activity of orally administered ZnO-NPs. Gout was induced by using MSU crystals, the symptoms appeared later were a significant increase ($P < 0.001$) in urea, creatinine and uric acid that was an actual sign of induction of gout [53]. Post-treatment of gout induced mice with ZnO-NPs significantly reduces ($P < 0.001$) the concentration of urea, creatinine and uric acid. It has been reported from the previous studies that zinc is responsible for inhibition of XOD as it suppresses the uric acid formation effectively [54]. Similarly, a previous study conducted the administration of copper nanoparticles to the mice having hyperuricemia, significantly ($P < 0.001$) reduces the serum uric acid levels. The serum uric acid levels were reduced to (2.167 ± 0.08 mg/dL) [55].

In our current study, it was observed that after the administration of

Table 2
Effect of ZnO NPs on liver function test (LFTs) after three weeks of treatment.

Groups	Parameters			
	ALT (SGPT)	AST (SGOT)	Total bilirubin	Alkaline Phosphatase
Unit	**U/L**	**U/L**	**mg/dl**	**U/L**
Control	46.33 ± 7.9	48.04 ± 4.3***	0.50 ± 0.20	174.00 ± 28.1*
MSU	71.37 ± 10.3***	65.83 ± 8.01**	0.82 ± 0.11**	164.83 ± 58.7***
NPs Control	34.66 ± 4.22	35.36 ± 1.2**	0.52 ± 0.12	154.33 ± 4.11*
ZnO NPs (5 ppm)	37.67 ± 4.8*	38.00 ± 1.4***	0.56 ± 0.17	136.00 ± 6.4***
ZnO NPs (10 ppm)	35.83 ± 5.06**	34.00 ± 1.4***	0.50 ± 0.14	147.00 ± 2.8
ZnO NPs (20 ppm)	32.50 ± 1.4***	29.50 ± 1.8	0.30 ± 0.08	180.00 ± 1.4***
Allopurinol	41.84 ± 2.8	40.00 ± 4.9	0.50 ± 0.17	145.33 ± 27.9

Values are expressed as Mean ± SD.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

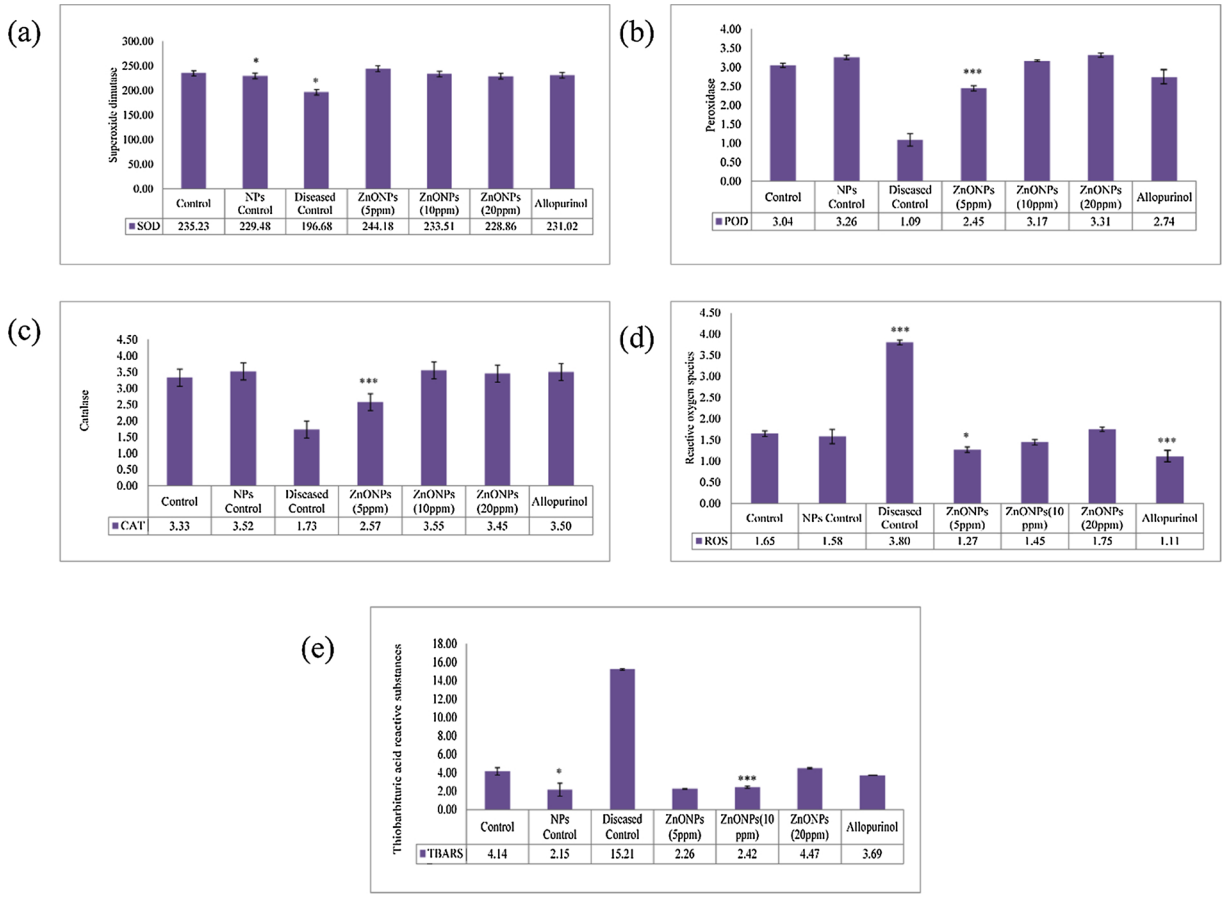

Fig. 4. Effect of ZnO NPs on antioxidant activity after three weeks of treatment. In which (a) is Superoxide dimustase (b) is Peroxidase (c) is Catalase (d) is Reactive oxygen species and (e) is Thiobarbituric acid reactive substances. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Table 3
Effect of ZnO NPs on lipid profile after three weeks of treatment.

Groups	Parameters			
	Cholesterol	HDL	LDL	Triglycerides
Unit	**mg/dl**	**mg/dl**	**mg/dl**	**mg/dl**
Control	167.83 ± 43.50	54.67 ± 17.6	5.11 ± 10.5	304.17 ± 27.87
MSU	250.83 ± 36.06***	24.33 ± 3.0***	5.50 ± 5.51***	389.17 ± 51.96***
NPs Control	98.44 ± 4.26**	52.62 ± 1.8	1.33 ± 1.2*	189.12 ± 22.44**
ZnO-NPs (5 ppm)	101.17 ± 2.63***	51.67 ± 3.8	1.56 ± 2.0***	238.00 ± 1.41***
ZnO-NPs (10 ppm)	100.17 ± 2.06***	55.83 ± 1.5	1.33 ± 1.4***	200.67 ± 1.43***
ZnO-NPs (20 ppm)	78.33 ± 2.041***	67.67 ± 2.0**	1.00 ± 2.0***	148.00 ± 3.44***
Allopurinol	117.67 ± 17.56***	45.33 ± 2.3*	3.00 ± 4.0***	138.50 ± 15.04***

Values are expressed as Mean ± SD.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.00'$.

ZnO-NPs the ALT, AST and total bilirubin levels of all the treatment groups were significantly reduced. Furthermore, the groups administered with commercially available drugs also had elevated levels of hepatic enzymes. In a study conducted by Fagugli et al [56], the adverse effects of allopurinol were discussed, which were linked with the acute renal and hepatic failure during prolonged usage. Furthermore, histopathological studies showed no apparent changes in the liver, kidney and muscle tissues as evident by a previous similar study in which antidiabetic effects of ZnO NPs was studied [57].

Furthermore, cholesterol, LDL and triglycerides levels in all MSU groups showed significant ($P < 0.001$) increase while in ZnO-NPs significantly decreased ($P < 0.001$) which indicated the effectiveness of ZnO-NPs in increasing HDL concentration similar to a previous study [55]. The normal biological function of the cells gets disturbed due to oxidative stress. A Significant increase ($P < 0.001$) was observed in SOD, POD and CAT values of ZnO-NPs treated group while a significant decrease was observed in the ROS and TBARS values. This decrease revealed the free radicals scavenging activity of ZnO-NPs. Furthermore,

Table 4
Effect of ZnO NPs on Blood count after three weeks of treatment.

	WBCs Count	RBCs Count	Haemoglobin	Hematocrit	MCV	Platelet Count	RDW CV
Unit	10^3/ mm^3	mL/mm^3	g/dl	%	F1	$10^3/mm^3$	%
Control Group	5.52 ± 1.1	7.13 ± 0.2	11.78 ± 1.1	38.50 ± 2.6	38.50 ± 2.46	230.16 ± 94.6	19.33 ± 2.33
MSU	7.15 ± 4.5***	8.88 ± 1.3***	8.83 ± 2.184***	32.50 ± 3.1	32.50 ± 4.44***	199.67 ± 46.9	21.83 ± 2.43
NPs Control	5.32 ± 1.2**	7.55 ± 0.8*	14.21 ± 1.88	40.50 ± 2.8	40.50 ± 1.5	533 ± 10.8	19.2 ± 1.22*
ZnO NPs (5 ppm)	6.40 ± 0.1**	8.42 ± 0.02**	14.97 ± 0.17***	50.0 ± 2.0***	50.00 ± 4.66***	646.1 ± 14.4***	28 ± 2.2***
ZnO NPs (10 ppm)	5.05 ± 0.2	7.35 ± 0.026	14.31 ± 0.48**	43.50 ± 2.8*	43.50 ± 1.67***	595.8 ± 6.3***	18 ± 1.42
ZnO NPs (20 ppm)	4.37 ± 0.1***	7.24 ± 0.011	11.30 ± 0.88	38.00 ± 2.1	38.17 ± 2.11***	240.89 ± 6.3	7 ± 1.11***
Allopurinol	3.44 ± 0.5***	6.06 ± 0.09*	9.97 ± 0.87*	44.00 ± 2.7*	44.00 ± 2.71***	1184.83 ± 70.4	17.3 ± 1.53

Values are expressed as Mean ± SD.
* P < 0.05.
** P < 0.01.
*** P < 0.001.

past studies also depicted the antioxidant activity of ZnO-NPs that was confirmed by using DPPH method [58].

5. Conclusion

From this experiment, it has been concluded that the mice having hyperuricemia and gouty arthritis when treated with ZnO-NPs showed a significant reduction in serum uric acid levels. Furthermore, lipid profile tests also showed that the nanoparticles treatment was highly effective in reducing the cholesterol and LDL levels in comparison to control. However, hepatic enzymes (ALT, AST, and ALP) were increased in nanoparticles as well as allopurinol treated groups. Histopathological analysis of liver, kidney and muscles tissues showed no apparent changes. ZnO-NPs efficacy in decreasing the serum uric acid levels, cholesterol and LDL from oral administration can be concluded from this research.

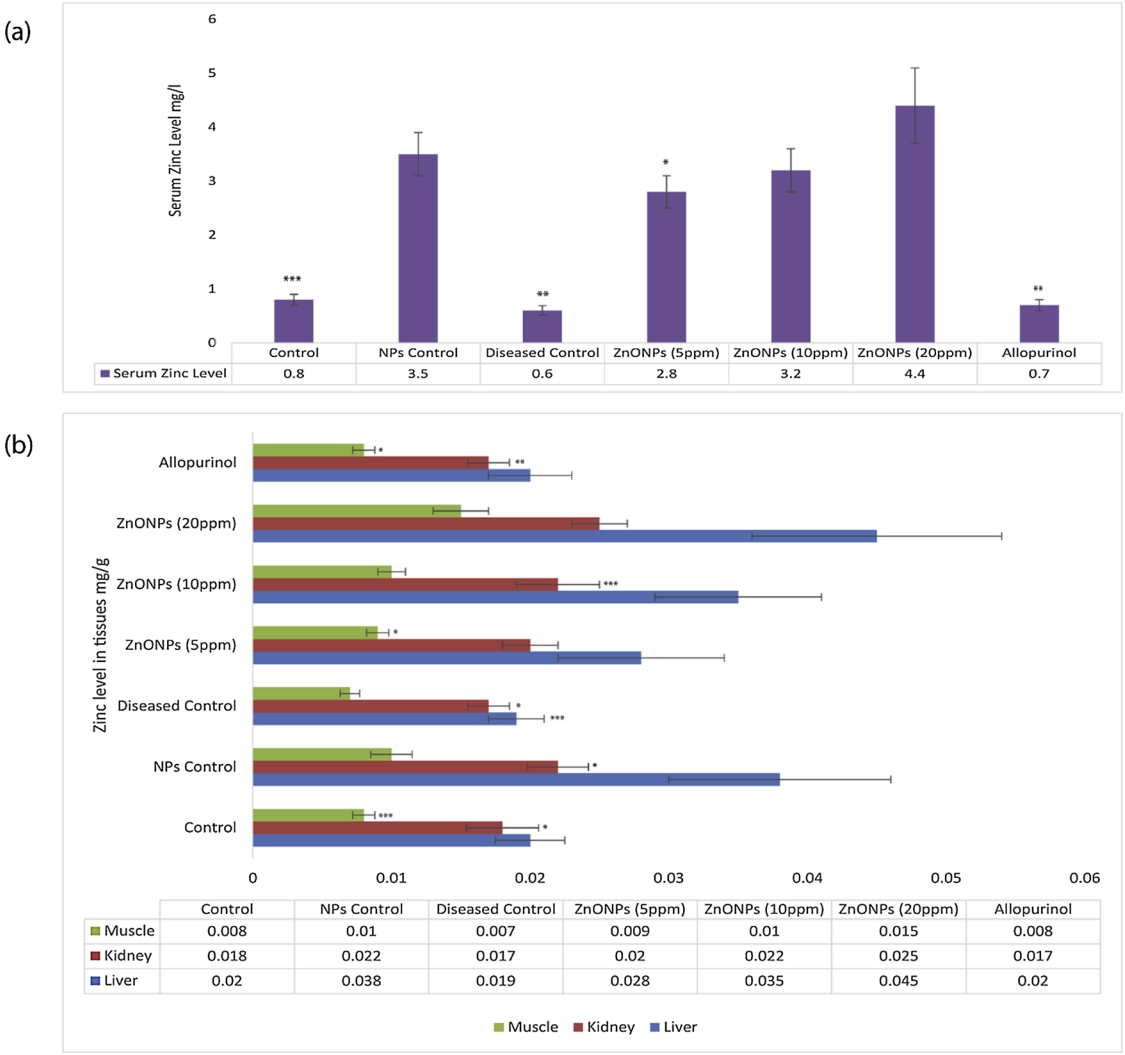

Fig. 5. (a) Average serum zinc level of mice treated with ZnO NPs for 3 weeks (b) Average zinc level in liver kidney and muscles tissues of mice treated with ZnO NPs for 3 weeks. (* P < 0.05, ** P < 0.01, *** P < 0.001).

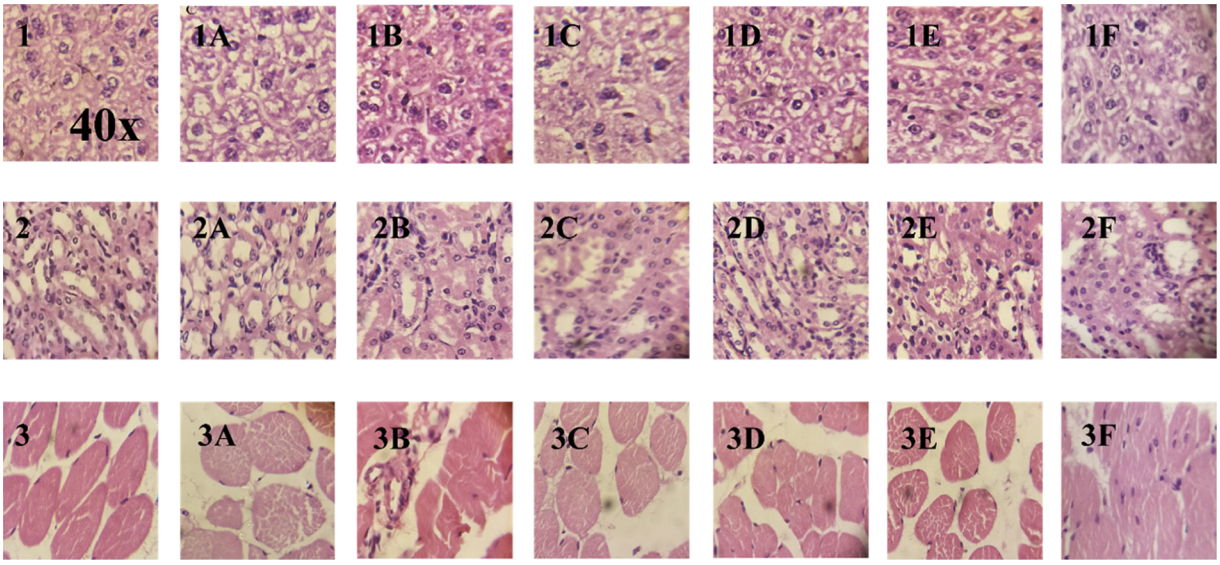

Fig. 6. Histopathological slides of all the groups in which Series 1 represents Liver, where 1 is the normal control, (1A) is the NPs control, (1B) is the diseased control, (1C) is the treatment with 5 ppm ZnO NPs, (1D) is the treatment with 10 ppm ZnO NPs, (1E)) is the treatment with 20 ppm ZnO NPs and (1 F) is the treatment with allopurinol; Series 2 represents kidney, where 2 is the normal control, (2A) is the NPs control, (2B) is the diseased control, (2C) is the treatment with 5 ppm ZnO NPs, (2D) is the treatment with 10 ppm ZnO NPs, (2E)) is the treatment with 20 ppm ZnO NPs and (2 F) is the treatment with allopurinol; and Series3 represents muscle, where 3 is the normal control, (3A) is the NPs control, (3B) is the diseased control, (3C) is the treatment with 5 ppm ZnO NPs, (3D) is the treatment with 10 ppm ZnO NPs, (3E)) is the treatment with 20 ppm ZnO NPs and (3 F) is the treatment with allopurinol.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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