



# Nephroprotective effects of curcumin loaded chitosan nanoparticles in cypermethrin induced renal toxicity in rabbits

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## Abstract

Cypermethrin, a pyrethroid insecticide, may cause several adverse effects including nephrotoxicity. Curcumin is a nutraceutical with many pharmacological effects including nephroprotective effects. But its effective clinical use is limited due to poor bioavailability, physicochemical instability, low bioactive absorption, quick metabolization, less penetration, and targeting efficacy. To resolve these issues, curcumin is incorporated in chitosan nanoparticles. The focus of the study was to prepare and characterize curcumin loaded chitosan nanoparticles and evaluate their nephroprotective activity in a cypermethrin induced renal toxicity. The curcumin loaded chitosan nanoparticles were prepared by using solvent displacement method and characterized by particle size, zeta potential, polydispersity index, entrapment efficiency, and FTIR. The prepared formulation was stable and lies within nanometer range (264.8 nm), and possessed high drug loading capacity (84.64%). Cypermethrin (24 mg/kg body weight) and Curcumin loaded chitosan nanoparticles (15 mg/kg and 30 mg/kg body weight) were orally administered to 20 rabbits (4 groups) for 28 days. It was found that cypermethrin significantly increased the serum levels of creatinine, urea, and BUN and decreased glutathione S-transferase and superoxide dismutase. Co-administration of curcumin loaded chitosan nanoparticles provided pronounced beneficial effects against cypermethrin-induced biochemical alterations and oxidative damage in the kidneys of rabbits. 30 mg/kg body weight of curcumin loaded chitosan nanoparticles have better nephroprotective effects as compared to 15 mg/kg body weight.

**Keywords** Curcumin · Cypermethrin · Nanoparticles · Nephroprotective · Oxidative stress · Pesticide · Preventive therapy

## Introduction

The kidney is the main organ of the body possessing many biological roles. It plays a vital role in development, growth, health, and disease (Javaid et al. 2012). Human body is comprised of paired kidney that is less than 1% of the total body weight. The kidney has the ability to concentrate and extract toxic materials through highly specified cells and has high blood flow of approximately 21% of the cardiac output. The

toxic xenobiotics target the kidney and influence their functions (El Saied Azab et al. 2014).

Various drugs and chemicals like pesticides have the ability to cause nephrotoxicity. Nephrotoxicity is an inherent adverse effect of anticancer, antibiotics, and other synthetic molecules or produced by overdosing or toxic effects of few medicines and harmful materials (Azab et al. 2017). Pesticides are in use for about five decades. They are extremely used in the agricultural pest control worldwide (Sulak et al. 2005). The extensive usage of pesticides in agricultural and public health programs has instigated severe health hazards including human poisoning and environmental pollution (Mahjoubi-samet et al. 2008).

Cypermethrin pesticide can accumulate in the heart, blood, adrenal glands, skin, liver, lungs, kidneys, ovaries, and body fat in mammals. It is reported that exposure of human to cypermethrin is mostly occupational during application or by residues of pyrethroid like those detected in bread, cow's milk, vegetables, and fruits (Sankar et al. 2012). *In vitro* and *in vivo* studies showed that pyrethroids cause nephrotoxicity in the experimental animals. A single dose of cypermethrin

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increased urea, creatinine, total bilirubin, and blood urea nitrogen. Cypermethrin increased the lipid peroxidation (LPO) and oxidative stress in the kidneys, heart tissues, and liver in rats. Lipid peroxidation and oxidative stress contribute in the toxicology of pyrethroids (Sushma and Devasena 2010; Ahmad et al. 2018).

Medicinal plants are widely utilized as they offer health and medical benefits. Plant drugs are considered as nontoxic and lacking adverse effects. Here the focus will be on one of the most promising and extensively investigated nutraceutical i.e., curcumin. Curcumin is considered as safe dietary substance that naturally occurs and has been utilized since ancient times. It is a nutritional supplement with potential health benefits and utilized in traditional medicines to treat a variety of ailments like biliary diseases, rheumatism, cough, anorexia, sinusitis, diabetic wounds, hepatic, and renal disorders (Hashish and Elgaml 2015).

The main drawback related to the usage of curcumin is its poor stability and bioavailability. This is because of the poor absorption in the intestine, having higher metabolism in the liver and quick elimination in bile. Half-life of curcumin is relatively low due to its higher clearance rate from the body and serum, limiting its therapeutic potential (Venkatesan et al. 2000; Yadav et al. 2012). To avoid these drawbacks, several approaches like polymeric nanoparticles, lipid-based nanoparticles, encapsulation in liposomes, biodegradable microsphere, cyclodextrin, and hydrogels are developed (Akhtar et al. 2012). Nano-medicine that works in the field of medicine and health is used in early detection, better diagnosis, appropriate treatment, prevention, and follow-up of many diseases (Nikalje 2015). In medicine and biology, nanoparticle technology has gained much importance.

Chitin is a natural element and found in exoskeleton of crustaceans, some insects, and water plants and in some fungi. Chitosan is commonly utilized polymer in the drug delivery formulations. Chitosan is synthesized by N-deacetylation of chitin, and chitin is isolated from natural origins and therefore eradicates cellular toxicity. Chitosan is a pH-sensitive polymeric drug delivery carrier having a higher safety profile. It is nontoxic and has a high charge density; absorption promoting, mucoadhesive, wound healing properties; and low immunogenicity. It is biodegradable, nontoxic, and biocompatible. It is cationic and hydrophilic. Studies approved that chitosan has an adequate toxicological profile and endorsed by the FDA as an additive in nutrition. Chitosan nanoparticles have been reported as a new vehicle for enhanced delivery of many drugs (Yadav et al. 2012).

No study is available on the nephroprotective effects of curcumin loaded chitosan nanoparticles against cypermethrin induced renal damage in rabbits. Therefore, the objectives of this study were to prepare and characterize curcumin loaded chitosan nanoparticles, to evaluate the nephrotoxic effects of cypermethrin in albino rabbits, and to observe the

nephroprotective effects of curcumin loaded chitosan nanoparticles in cypermethrin-induced toxicity in albino rabbits.

## Materials and methods

### Experimental animal

Healthy New Zealand white adult female and male rabbits of the age (about 7–9 months) with body weight between 1 and 2 kg were acclimatized for 1 week before the study. Room humidity (45–70%), temperature (25–27 °C), and 12-h light-dark cycle were maintained throughout the study. The green fodder Berseem and Alfalfa were provided to rabbits in the morning and evening. The study protocol was approved from the Bioethics Committee of Institution vide letter number 2475, and the laboratory animal care guidelines were followed throughout the experimental period.

### Chemicals

Chitosan of high purity (MW 140,000–220,000), product number 740179, and curcumin, analytical standard, product number 08511, were purchased from Sigma-Aldrich, Germany. Cypermethrin (10% EC) was purchased from Sinopak Chemicals, Multan, Pakistan. Acetone, product number AC0352, was purchased from Merck, Darmstadt, Germany. All the reagents and chemicals were of analytical grade.

### Preparation of curcumin loaded chitosan nanoparticles

Curcumin loaded chitosan nanoparticles were prepared by following the solvent displacement method with little changes. 10 milligram of curcumin was added in 10 mL of acetone and mixed completely on vortex mixer. 20 milligram of chitosan was added in 10 mL of acidified water and dissolved completely on magnetic stirrer at room temperature (25 °C). Chitosan solution was placed on a magnetic stirrer under 120 rpm at 25 °C. Then, by using micropipette, curcumin solution was added in chitosan solution drop by drop gradually and steadily for the homogenous distribution of drug and polymer in the formulation. Thus, dispersion of nanoparticles was developed.

After freshly prepared dispersion of nanoparticles, it was kept on a magnetic stirrer for continuous stirring for 4 h at 25 °C so that the organic phase (acetone) was completely removed. The remaining dispersion was then ultracentrifuged at 25000 rpm for 30 min at 25 °C. The pallet of nanoparticles was settled down. The supernatant was separated. Then the settled down pallet was dispersed in distilled water on a vortex

mixer. The dispersion was sonicated for 10 min for complete homogenization (Tefas et al. 2015).

### Characterization of curcumin loaded chitosan nanoparticles

Zetasizer was used to measure the average particle size, zeta potential, and the polydispersity index (PDI) of curcumin loaded chitosan nanoparticles by light scattering method. Encapsulating efficiency was measured by ultracentrifugation method followed by UV-visible spectrophotometric analysis. Curcumin loaded chitosan nanoparticles were centrifuged at the speed of 25,000 rpm at 25 °C for 30 min. The resulting supernatant was analyzed at 402 nm by UV-visible spectrophotometer. The encapsulation efficiency was calculated by following the given formula (Bagad and Khan 2015).

%Encapsulation Efficiency

$$= \frac{\text{Curcumin weight in nanoparticles} \times 100}{\text{Initial weight of curcumin used}}$$

Compatibility of curcumin with chitosan was analyzed by using Fourier-transform infrared spectroscopy (FTIR). FTIR spectra of standard curcumin, pure chitosan, and curcumin loaded chitosan nanoparticles were recorded.

### Dose selection

. It was reported that the LD<sub>50</sub> of cypermethrin is 2400 mg/kg body weight when given orally to rabbits (Yousef et al. 2003). Cypermethrin was administered every other day to each animal following the method of Yousef et al. (2003) who administered cypermethrin at a dose of 24 mg/kg body weight of the rabbits for 28 days. In the current study, curcumin loaded chitosan nanoparticles (15 mg/kg and 30 mg/kg of body weight) were administered orally to each rabbit on alternate days (Yadav et al. 2012).

### Experimental design

The total number of rabbits ( $n = 20$ ) was divided into 4 groups ( $n = 5$ ), and following protocol was adopted for 0 to 28 days. Control rabbits (group 1) were given routine diet, while group 2 were given routine diet and Cypermethrin (24 mg/kg body weight, orally). Group 3 was administered routine diet, Cypermethrin (24 mg/kg body weight, orally) and Curcumin loaded chitosan nanoparticles (15 mg/kg body weight, orally) (Treatment 1). The rabbits of group 4 were administered routine diet, Cypermethrin (24 mg/kg body weight, orally) and Curcumin loaded chitosan nanoparticles (30 mg/kg body weight, orally) (Treatment 2). The body weights of rabbits were recorded at the start and at the end of the experiment.

### Blood sampling

For blood sampling, 3 mL of blood was collected from each rabbit in blood collection vials. The first blood sample was collected one day before drug administration. Then, blood samples were collected at 7th, 14th, 21st, and 28th day. Samples were allowed to clot for 15 min in vertical position and then centrifuged for 10 min at 4000 rpm. Separated serum was stored at -20 °C till analysis. During this study, all the rabbits were observed for morbidity and mortality. At the end of experimental period of 28 days, the rabbits were sacrificed, and the kidneys were collected for histopathological examination.

### Serum biochemical markers

Serum creatinine, urea, and blood urea nitrogen (BUN) are indicators of renal function. These levels were measured by using commercially available kits (Quimica Clinica Aplicada S.A. kit, Amposta, Spain).

### Assessment of antioxidative defense parameters

Antioxidative defense parameters glutathione S-transferase (GSH-ST) and superoxide dismutase (SOD) were measured in a serum. These levels were measured by using commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing Jiancheng Technology Co. Ltd).

### Histopathology

Histopathological studies were performed on kidney tissues to analyze the cellular changes. The rabbits were sacrificed after 28 days, and the kidneys were collected and preserved in 10% buffered formalin. Formalin-fixed kidney samples were processed in graded ethanolic concentrations and embedded in paraffin blocks. The 6 μm thick transverse tissue sections were cut and mounted on a glass slide. They were stained with Hematoxylin and Eosin (H & E). Slides were examined and photographed by binocular light microscope fitted with camera.

### Statistical analysis

GraphPad Prism (version 8.1.2) was used for statistical analysis. The results have been expressed as Mean ± SD and analyzed by two way analysis of variance (ANOVA). Statistical differences among different groups were measured by Tukey's multiple comparison test at 5% level of significance ( $P < 0.05$ ).

## Results

### Characterization of curcumin loaded chitosan nanoparticles

For the characterization of nanoparticles, the particle size, zeta potential, and polydispersity index (PDI) are important parameter. Particle size was measured at a count rate of 154.8 kcps. The prepared nanoparticles showed a particle size of 264.8d.nm which lies within the range of 10–1000 nm (Fig. 1a). Zeta potential was measured at a count rate of 362.5 kcps with an electric voltage of 148.9 V and the conductivity was 0.438 mS/cm. Zeta potential of curcumin loaded chitosan nanoparticles was  $+67.9 \pm 6.52$  mV as shown in Fig. 1b. Polydispersity index of curcumin loaded chitosan nanoparticles was 0.401 that shows nanoformulation was homogenous.

The encapsulating efficiency of the prepared formulation was 84.64%. It showed that curcumin was successfully loaded in chitosan nanoparticles. The FTIR spectra of standard curcumin (a), pure chitosan (b) and curcumin loaded chitosan nanoparticles (c) has been shown in Fig. 2. In standard curcumin spectra, characteristic peaks were obtained at  $3505\text{ cm}^{-1}$ ,  $1626\text{ cm}^{-1}$ ,  $1603\text{ cm}^{-1}$ ,  $1508\text{ cm}^{-1}$ ,  $1275\text{ cm}^{-1}$ ,  $1152\text{ cm}^{-1}$ ,  $1026\text{ cm}^{-1}$ ,  $962\text{ cm}^{-1}$  and  $808\text{ cm}^{-1}$ . In spectra of pure chitosan, characteristic peaks were obtained at  $3420\text{ cm}^{-1}$ ,  $1657\text{ cm}^{-1}$ ,  $1632\text{ cm}^{-1}$ ,  $1595\text{ cm}^{-1}$ ,  $1543\text{ cm}^{-1}$ ,  $1061\text{ cm}^{-1}$  and  $1026\text{ cm}^{-1}$ . In FTIR spectra of the curcumin loaded chitosan nanoparticles, characteristic peaks were obtained at  $3505\text{ cm}^{-1}$ ,  $3422\text{ cm}^{-1}$ ,  $1653\text{ cm}^{-1}$ ,  $1626\text{ cm}^{-1}$ ,  $1601\text{ cm}^{-1}$ ,  $1506\text{ cm}^{-1}$ ,  $1273\text{ cm}^{-1}$ ,  $1152\text{ cm}^{-1}$ ,  $1026\text{ cm}^{-1}$ ,  $962\text{ cm}^{-1}$  and  $808\text{ cm}^{-1}$ .

### Effects on serum biochemical markers

Mean  $\pm$  SD values of the serum creatinine (mg/dL), urea (mg/dL), and blood urea nitrogen (BUN) (mg/dL) has been shown in Fig. 3. Data shows that the mean  $\pm$  SD values of serum creatinine, urea, and BUN are significantly different in different treatment groups ( $P < 0.0001$ ). In the cypermethrin-treated group, time and dose dependent increasing trend was observed in creatinine, urea, and BUN concentrations. High

serum creatinine, urea, and BUN were recorded at week 3 and 4 in the cypermethrin-treated group. In treatment 1 (low dose of curcumin loaded chitosan nanoparticles) and treatment 2 (high dose of curcumin loaded chitosan nanoparticles) groups, creatinine, urea, and BUN levels decreased as compared to the cypermethrin-treated group. In treatment 2 group, creatinine, urea, and BUN level became normal as compared to treatment 1 group.

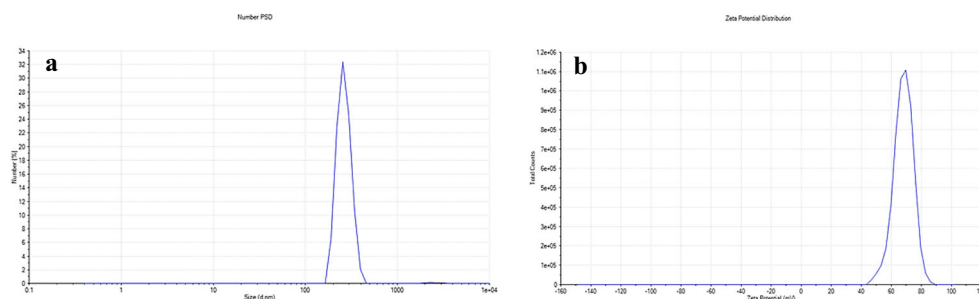
### Effects on antioxidant defense parameters

Mean  $\pm$  SD values of glutathione S-transferase (U/mL) and superoxide dismutase (U/mL) have been shown in Fig. 4. Data shows that the Mean  $\pm$  SD values of glutathione S-transferase are significantly different in different treatment groups ( $P < 0.0001$ ). In the cypermethrin-treated group, time and dose dependent decreasing trend was observed in glutathione S-transferase and superoxide dismutase concentration. The lowest glutathione S-transferase and superoxide dismutase level were recorded in week 3 and 4 in the cypermethrin-treated group. In treatment 1 (low dose of curcumin loaded chitosan nanoparticles) and treatment 2 (high dose of curcumin loaded chitosan nanoparticles) groups, the concentration of glutathione S-transferase and superoxide dismutase restored as compared with the cypermethrin-treated group.

### Histopathology

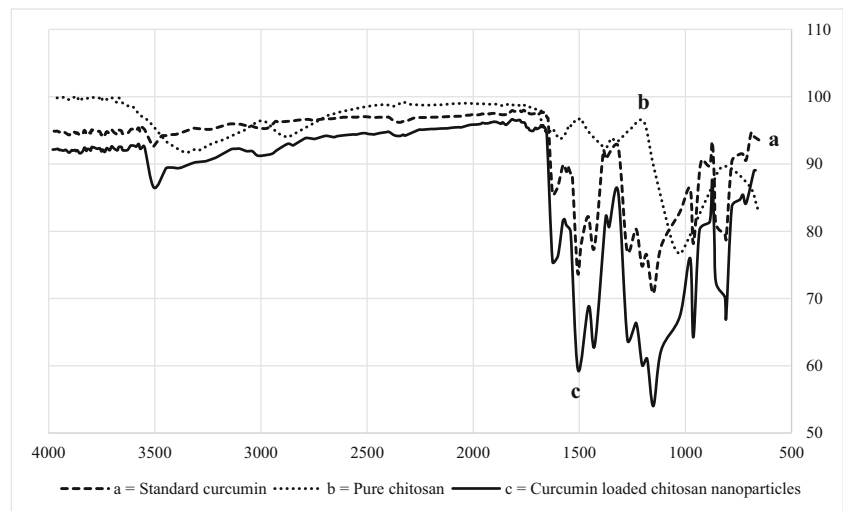
In the control group, nuclei, glomerulus, tubular epithelial cells, and Bowman's capsule were normal in appearance. Renal parenchyma and architecture of the kidney were normal as indicated in Fig. 5a. In CYP induced group, Bowman's capsule was dilated, and glomerular tufts were contracted. Severe congestion was present in renal parenchyma. Pyknotic nuclei were found in kidney tubules. Inflammatory cell infiltration and mild necrosis were present in tubular epithelial cells as indicated in Fig. 5b. In treatment 1 group, less dilation was found in Bowman's capsule, and glomerular tufts were less contracted as compared to the cypermethrin-induced group. Mild congestion was present in the renal parenchyma. The nuclei present in epithelial cell were normal at most of the places. Minute inflammatory cell infiltration and necrosis

**Fig. 1** Characterization of curcumin loaded chitosan nanoparticles. **a** Zeta size of Curcumin loaded chitosan nanoparticles. **b** Zeta Potential of curcumin loaded chitosan nanoparticles





**Fig. 2** FTIR spectrum of **a** standard curcumin **b** pure chitosan **c** curcumin loaded chitosan nanoparticles



were found. It indicated the ameliorated effects of low dose curcumin loaded chitosan nanoparticles (Fig. 5c). In treatment 2 group, minute congestion was present in the renal parenchyma. The nuclei present in epithelial cell were normal in appearance. The glomerulus was bounded by Bowman's capsule. No inflammatory cell infiltration and necrosis were found. It indicated the high dose of curcumin loaded chitosan nanoparticles showed nephroprotective effect (Fig. 5d).

## Discussion

Particle size is a critical determinant for the characterization of nanoparticle. Particle size determines the distribution of nanoparticles in the body. The particle size of the prepared curcumin loaded chitosan nanoparticles was 264.8d.nm. The size of nanoparticles usually lies between 1 to 100 nm, but some studies exhibit that the size of nanoparticles may increase up to 1000 nm (Mora-huertas et al. 2010). The size of the nanoparticle can affect the rate of release of drug as well as absorption of drug. If the size is too small, surface area will be increased and eventually the release of drug is increased. Curcumin loading into chitosan nanoparticles raised its particle size. The stirring speed may also affect the particle size (Nair et al. 2019). The particle size of our nanoformulation is comparable to Akhtar et al. (2012) who also synthesized curcumin loaded chitosan nanoparticles with a size greater than 200 nm.

Zeta potential analysis is a method to determine the surface charge of the nanoparticles in solution (colloids). The magnitude of the zeta potential determines the colloidal stability. High negative or positive zeta potential more than 30 mV leads to monodispersity. Lower values, less than 5 mV, lead to agglomeration. Dispersions with lower zeta potential values will ultimately aggregate because of the Van Der Waal interparticle attractions. Zeta potential is significant to understand

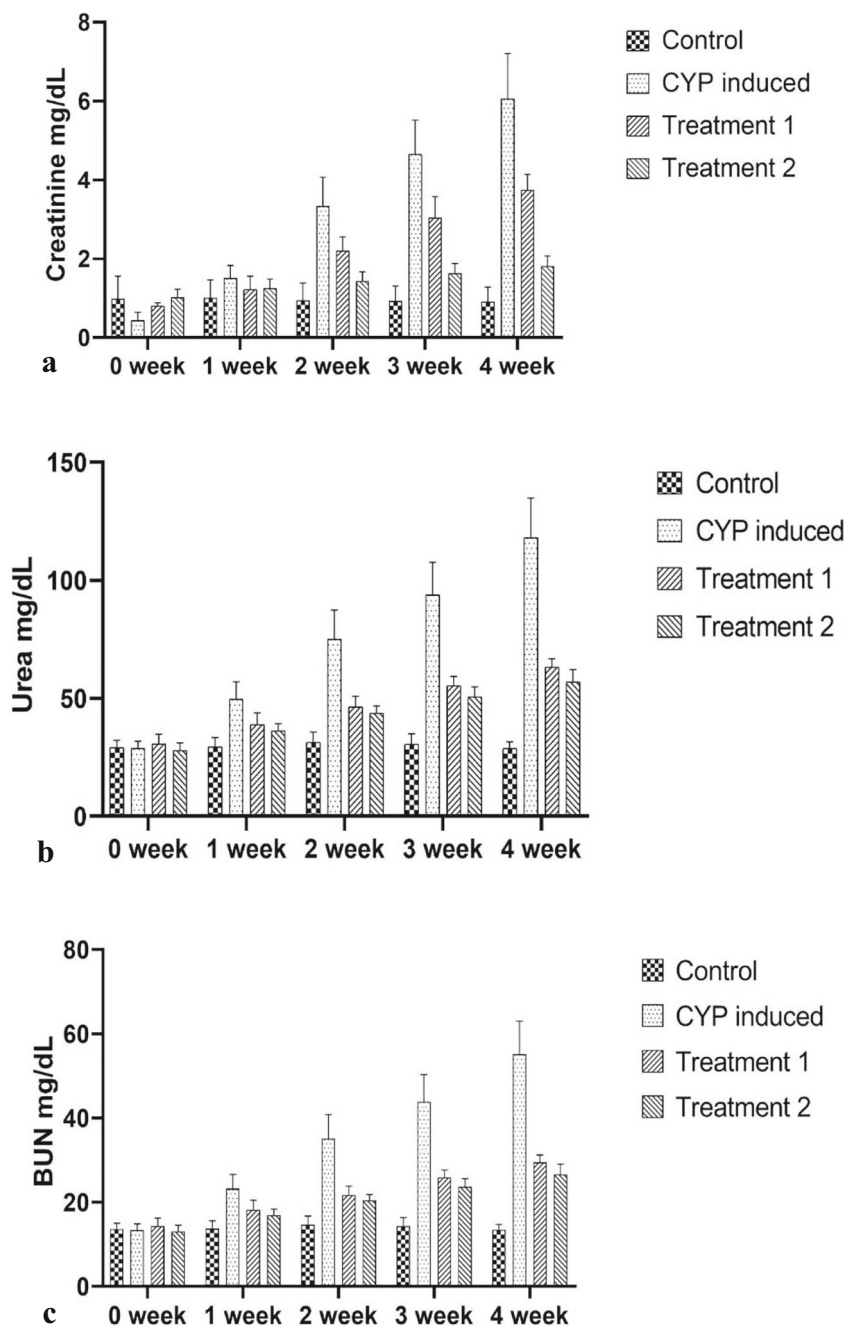
the state of the nanoparticle surface and to predict long term stability of nanoparticle (Gumustas et al. 2017). Chitosan is a cationic polymer and has positive zeta potential. The positive zeta potential is because of the amino group in chitosan. The higher positive charge is required to avoid aggregation. Studies reveal that nanoparticles with zeta potential values more than 30 mV have good stability and more than 60 mV have excellent stability (Honary and Zahir 2013; Nair et al. 2019). The zeta potential of curcumin loaded chitosan nanoparticles in the current study was + 67.9 mV indicating excellent stability of our nanoformulation.

Polydispersity index is the measurement of size distribution in a given sample. It ranges from 0 to 1. To achieve monodispersity, it is necessary that the dispersion possess the PDI values as low as possible. If a value of PDI is near to zero, it exhibits that the suspension is homogenous. Higher values of PDI > 0.5 usually exhibit wide size distribution. Therefore, it was necessary to keep PDI values as low as possible, and the observed value of curcumin loaded chitosan nanoparticle was 0.401 which is in agreement with Nair et al. (2019). It is evident from the results of size analysis that the particle size was within the range and dispersion was stable and homogenous.

Entrapment efficiency is the percentage of drug that is entrapped in the nanoparticles. Drug entrapment in nanoparticles can be important for improving the oral bioavailability of drug. In this study, the encapsulating efficiency of curcumin loaded chitosan nanoparticles was 84.64%. While in Jahromi et al. (2014) study, the entrapment efficiency was 75%. The encapsulation efficiency more than 80% was also reported in the study of Nair et al. (2019).

To characterize the chemical structure of curcumin loaded chitosan nanoparticles, FTIR studies of curcumin, chitosan, and curcumin loaded chitosan nanoparticles were performed. FTIR spectrum has been shown in Fig. 2. The values found were almost similar and showed that the drug

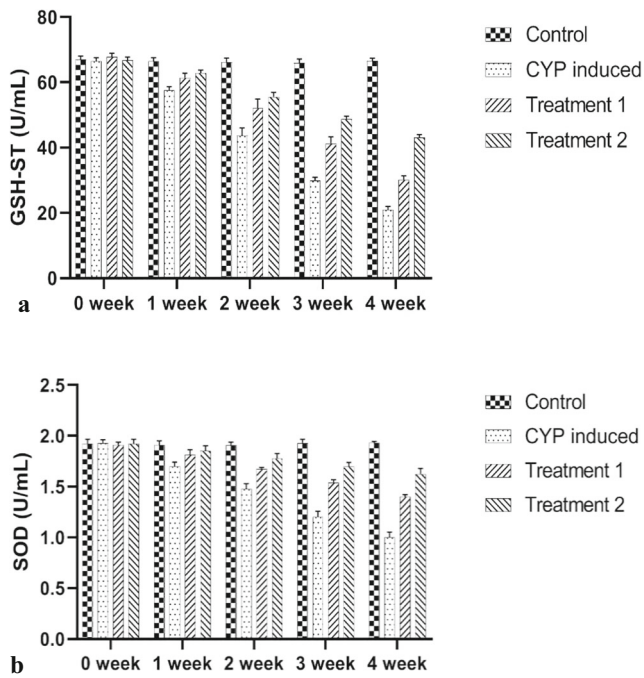
**Fig. 3** Mean  $\pm$  SD values for **a** creatinine **b** urea and **c** BUN level (mg/dL) with oral administration of cypermethrin and curcumin loaded chitosan nanoparticles in rabbits ( $n = 5$ /group) for 4 weeks



had good compatibility with the polymers used. A sharp peak at  $3505\text{ cm}^{-1}$  showed the presence of hydroxyl group (OH). Characteristic peaks at  $1626\text{ cm}^{-1}$ ,  $1603\text{ cm}^{-1}$ , and  $1508\text{ cm}^{-1}$  have predominantly mixed C=C and C=O characters. Enol C-O peak was achieved at  $1275\text{ cm}^{-1}$ . At  $1026\text{ cm}^{-1}$ , C-O-C peak was obtained. Benzoate trans-CH vibration was at  $962\text{ cm}^{-1}$ . The peaks obtained in our study were in agreement with the study of Mohan et al. (2012).

The band at  $3420\text{ cm}^{-1}$  is attributed to the combined peaks of the OH and  $\text{NH}_2$  group stretching vibration in chitosan. The

peak at  $1657\text{ cm}^{-1}$  corresponds to the  $\text{CONH}_2$  group. The bending vibration of  $\text{NH}_2$  at  $1595\text{ cm}^{-1}$  peak is sharper than the peak at  $1657\text{ cm}^{-1}$ . It exhibits high degree of deacetylation of chitosan. A shift from  $3420\text{ cm}^{-1}$  to  $3505\text{ cm}^{-1}$  has been shown in Fig. 2, and the peak is sharper in the curcumin loaded chitosan nanoparticles, which showed that hydrogen bonding is enhanced. Peaks  $1061\text{--}1026\text{ cm}^{-1}$  were due to stretching of C-O-C. The peaks found in our study were comparable with the study of Qi et al. (2004). This peak analysis indicates the successful loading of curcumin in chitosan and compatibility in nanoformulation.



**Fig. 4** Mean  $\pm$  SD values for **a** glutathione S-transferase (U/mL) and **b** superoxide dismutase (U/mL) with oral administration of cypermethrin and curcumin loaded chitosan nanoparticles in rabbits ( $n = 5$ /group) for 4 weeks

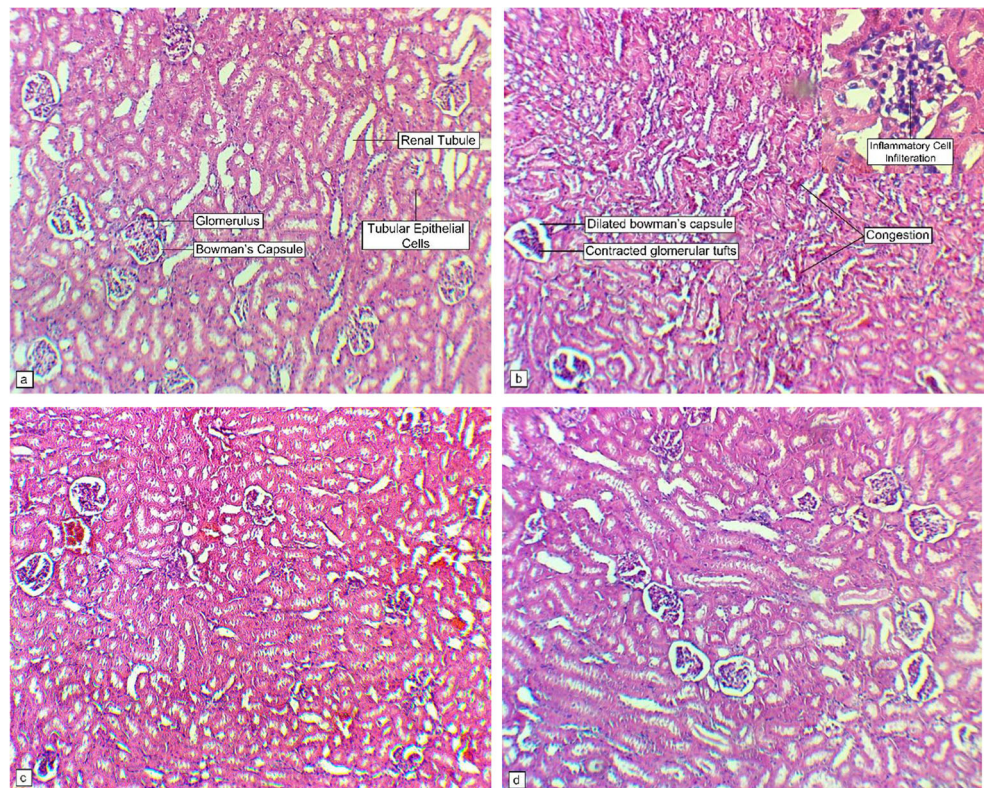
In this study, significant increase ( $P < 0.0001$ ) in the levels of serum creatinine, serum urea, and blood urea nitrogen was noticed. It was a classical sign that cypermethrin exposure

cause toxic effects in the kidney. Serum creatinine is more specific to the kidneys. Kidney damage is the only factor that increases serum creatinine in mammals (Garba et al. 2007). Urea is eliminated by the kidneys. The ability of the kidneys to eliminate urea from the blood diminished due to impaired renal function (Aslam et al. 2010). Serum urea may increase due to renal tissue damage, dehydration, quick urea production from ammonia, increased urea enzymes activity, low blood volume, troubled urea excretion, and decreased serum proteins (Garba et al. 2007). According to Yousef et al. (2003) and Ahmad et al. (2011), serum levels of creatinine, urea, and BUN were increased in cypermethrin treated animals.

The present study also indicated that low dose of curcumin loaded chitosan nanoparticles (15 mg/kg body weight) significantly decreased ( $P < 0.0001$ ) the levels of serum creatinine, urea, and BUN. High dose of Curcumin loaded chitosan nanoparticles (30 mg/kg body weight) significantly normalized ( $P < 0.0001$ ) the levels of serum creatinine, urea, and BUN. Similar changes in the levels of serum creatinine, urea, and BUN were reported in Sankar et al. (2012) and Nasri et al. (2016).

In the present study, glutathione S-transferase level was measured as a non-enzymatic antioxidant. Significant decrease ( $P < 0.0001$ ) in the level of glutathione S-transferase was noticed. Glutathione S-transferase is an important antioxidant that plays a significant role in defending cells against oxidative stress by scavenging reactive oxygen species. Cypermethrin cause decrease in the level of glutathione S-transferase in the kidneys. The decrease in glutathione S-

**Fig. 5** Kidney histopathology of **a** Control group: administered routine diet only **b** CYP induced: administered cypermethrin 24 mg/kg body weight per oral (Insert indicate inflammatory cell infiltration) **c** Treatment 1: administered cypermethrin 24 mg/kg body weight and low dose of curcumin loaded chitosan nanoparticles 15 mg/kg body weight per oral **d** Treatment 2: administered cypermethrin 24 mg/kg body weight and high dose of curcumin loaded chitosan nanoparticles 30 mg/kg body weight per oral (H & E, X 10)





transferase may be due to enhanced consumption of glutathione S-transferase for the detoxification of cypermethrin-induced free radicals (Raina et al. 2009).

In this investigation, superoxide dismutase enzyme activity was measured as enzymatic antioxidant. Superoxide dismutase (SOD) plays a vital role in balancing the anti-oxidation and oxidation in vivo and can eradicate the superoxide ions in biological system to avoid cell damage by the superoxide ions. Cypermethrin significantly decreased ( $P < 0.0001$ ) in the enzyme activity of SOD in the kidneys. Previous studies showed that the pyrethroid exposure produce oxidative stress. According to Raina et al. (2009) and Abdou et al. (2012), the level of GSH-ST enzyme and the enzyme activity of SOD significantly decreased in the cypermethrin-treated group. In this study, significant improvement was observed in biochemical parameters with curcumin loaded chitosan nanoparticles therapy in cypermethrin induced nephrotoxicity. Similar observations were also found in Buyuklu et al. (2014).

Curcumin is a potent antioxidant. The protective effect of curcumin loaded chitosan nanoparticles against cypermethrin induced nephrotoxicity was evaluated in this study. Curcumin loaded chitosan nanoparticles reveal the ability to reduce alterations associated with cypermethrin toxicity. The present study revealed that architecture of the kidney in the control group is normal in appearance. Cypermethrin treatment induced severe histopathological changes in the kidney. Dilation of Bowman's capsule and shrinkage of glomerular tufts lead to increased urinary space. Congestion in different areas of the kidney was observed in the same group. Epithelial cells of tubules in the kidney had pyknotic nuclei. Inflammatory cell infiltration and mild necrosis were also noted (Fig. 5). This is in agreement with many authors who reported the nephrotoxicity of cypermethrin (Ahmad et al. 2011; Abdou et al. 2012). Curcumin loaded chitosan nanoparticles treated animals ameliorated the histopathological changes in the kidney. This is in agreement with Sankar et al. (2012) and Hashish and Elgaml (2015).

## Conclusion

This study indicated that exposure of rabbits to cypermethrin induced adverse effects and profound alterations on the renal functions. The 30 mg/kg of curcumin loaded chitosan nanoparticles produced nephroprotective effects against cypermethrin induced biochemical alterations and oxidative damage in the kidneys of rabbits.

## Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest involved in this study. The authors alone are responsible for the content and writing of the paper.

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