Evaluated the Effect of Titanium Dioxide Nanoparticles on the Activity of Creatinine and Urea Levels in Dialysis Patients

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Abstract—Nanotechnology is an emerging field that has got a phenomenal interest in the last few decades. Medical application is considered one of the most important ambitions in this field. The objective was to evaluate the biological effects of (TiO_2-NPs) on urea and creatinine activity among kidney patients. The titanium dioxide (TiO_2) nanoparticles were created using the photocell technique, and for characterization used XRD and TEM. The research population consisted of 60 individuals, both male and female, with renal disease and older than 40 years old who were on dialysis without taking any drugs. Two groups were created out of them (30 control group and 30 dialysis patients group). The two groups' serum was collected using a straightforward sampling approach. Creatinine and urea levels were measured for the control group (0.6970±0.2432, 31.87±6.345), and for the dialysis group before the addition of TiO_2-NPs (p<0.0001); and also after the addition of TiO_2-NPs (p<0.0001, p<0.0002) respectively. Furthermore, creatinine and urea concentrations were determined using a spectrophotometric method. The XRD investigations showed that titanium dioxide nanoparticles are crystalline and the size ranged from 11-29 nm, according to photographs taken using TEM. The present investigation showed that (TiO_2-NPs) significantly lowered the levels of creatinine and urea in individuals with renal disease. Our findings demonstrated, for the first time, that TiO_2 nanoparticles considerably declined the levels of creatinine and urea among dialysis patients. Future research should be done to confirm this affects TiO_2-NPs.

Keywords— Nanotechnology, TiO_2-NPs, photocell, creatinine, urea.

1 Introduction

Nanotechnology is an enticing modern area of research, with various potential applications in industrial, domestic, and biological activities. Nanoparticles will have unique characteristics as a result of their surface area and little size. When these particles are introduced into a living creature, the biological activity that can be awoken by them can be both positive and desired (for instance, therapies with drug vectors, passing through cellular barriers for medication distribution in tissues, etc.) or negative and unwanted (e.g. cell dysfunction, oxidative stress, toxicity, and etc.), [1].

Nano medicine is a rapidly developing and expanding area. Nanoparticles (NPs) have been studied for a number of medicinal uses and are demonstrating promise as a delivery system for both therapeutic and diagnostic agents. Where traditional medicines fall short, it is thought that NPs may capable to obtain past some physical and biological obstacles [2].

The physical, chemical and remarkable compositions, forms, and architectures of the metal-oxide nanoparticles have all been put to the test [3]. Titanium is found in around 95% of its pure form as titanium dioxide, which is fully insoluble, non-flammable, and stable at steady temperatures. Titanium dioxide is used nowadays to make paintings, cancer photothermal therapy, water and wastewater treatment, cosmetics, optical-electronic devices, targeted medicine delivery, pigments, photocatalysts, ceramics, and many other things [4]. The use of TiO_2 nanoparticles is on the growth in a variety of biological and technological fields [5].

The kidney's intrinsic capacity to quickly remove particles smaller than 10 nm in diameter makes it a special organ for NP targeting [6]. The glomerular filtration unit, the interdigitating podocytes, and the basement membrane all contribute to this. A fenestrated endothelium in the renal corpuscle separates the mesangium from the extracellular matrix. As a result, 2 nm particles are easily removed by the kidney, but renal excretion is greatly reduced when the particle size is 6 nm and almost nonexistent when it is 11 nm [7].

Glomerular filtration capacity is reduced in chronic kidney disease (CKD), which results in a progressive decrease of kidney capacity over months to years. Toxins that the normal functioning kidney would normally eliminate might build up in the blood when there is CKD. Toxin buildup in the blood may occur before the substantial renal function is lost, which results in CKD diagnosis and therapy being delayed [8].

Creatinine levels in human blood and urine must be measured carefully since they are influenced by renal, muscular, and thyroid dysfunction [9]. Increased sera urea is the primary contributing factor to the steady reduction in renal function that results in CKD [10]. The advancement of chemistry and biology is greatly aided by urea. Being the primary nitrogen component of urine and the byproduct of protein breakdown, urea is extensively distributed in living organisms, making its measurement crucial for clinical chemistry, environmental monitoring, and the food business [11].

The research goal was investigating the impacts of titanium dioxide nanoparticles on the activity of creatinine and urea levels in renal patients (dialysis patients).

2 Methods and Materials

2.1 Synthesis of titanium dioxide nanoparticles

All chemical goods have been purchased untreated (BDH). The photocell is equipped with a 125W UV mercury lamp with a 365 nm wavelength and an ice-cooled pyrex tube to prevent a temperature increase brought on by UV radiation. The technique involved putting 15 ml of titanium isopropoxide ($C_{12}H_{28}O_4Ti$) gradually (drop by drop) into 30 ml of deionized water/ethanol (1:3). After that, 10 ml, 0.5 M of urea was gradually added to the mixture while stirring continuously for 10 minutes at 30 °C. The mixture was then exposed to radiation for 30 minutes. During the formation of precipitation. De-ionized water was used to clean and isolate a centrifuge. After drying for a day and calcining for three hours at 500 C° in an oven, a white precipitate of titanium dioxide nanoparticles was produced [12].

2.2 Specimens collection

The Kalar Dialysis Center/ Kalar General Hospital/ Iraq; provided a sample of 30 patients with kidney disease (dialysis), which equates to 30 controls in this study. All patients and controls had these samples drawn from them using plastic, disposable syringes, and they were given 15 minutes to clot at room temperature. Centrifugation was used to separate the components for 10 minutes at 700 g. To be studied later, the isolated serum samples were directly put in disposable tubes and maintained at -20 C°.

2.3 In vitro of TiO_2-NPs on creatinine and urea

In this investigation, the serum samples were mixed with TiO_2-NPs (2 µg /0.5 ml) in the range (1:2) after five minutes of mixing [13]. The concentrations of urea in serum determined by enzymatic colorimetric method using urea Berthelot diagnostic (liner kit) [14], and a creatinine Kinetic colorimetric technique fixed time (liner) kit used to determine creatinine in serum [15].

2.4 Statistical analysis

One-way ANOVA was performed to statistically analyze the data using GraphPad Prism 7. A statistically significant difference was recognized at a *p*-value of 0.0001. The information was presented as a mean ± standard deviation.

3 Result and Discussion

3.1 Considerations for structure and characteristics

Titanium dioxide nanoparticles were prepared using the photocell method the crystallinity of the portion is improved by calcination at 500 C°, and the TiO_2 phase crystal structure is created to verify the claim, Figure 1 shows the produced powder underwent XRD analysis.

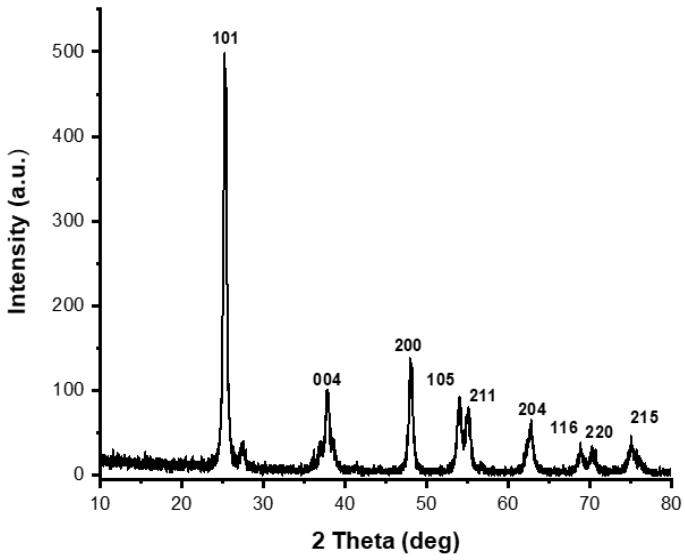

Fig. 1. XRD patterns of TiO_2-NP

The XRD results for TiO_2 nanoparticles were confirmed using Joint Committee on Powder Diffraction Standards (JCPDS file no. 73–1764). The diffraction peaks are 25.31°, 37.79°, and 48.04°, which correspond to the Müller values of 101, 004, and 200, respectively. The crystallographic planes of the anatase structure are seen. No other rutile phase peaks can be seen in the diffractograms of the TiO_2 as-produced. Hence, to ascertain the size of the crystallites in the particles, Scherer's equation was used [16]. TiO_2 powder has an average crystallite size of 15.72 nm.

Transmission electron microscopes (TEM) are used for analyzing the structure and composition of nanomaterial. The surface morphology of TiO_2 nanoparticles has been studied by using transmission electron microscopes (TEM). The outcome showed that the nanoparticle samples might have a restricted dispersion and minimal aggregation, as well as a consistent polygon shape and the size in the range of 11- 29 nm. The numbers from the XRD peak expansion measurement correspond to the average particle sizes obtained from TEM [12]. Where the result showed the average particle size as determined by TEM is 19.17 nm as seen in (Figure 2) A and B show the micrograms and particle size distribution, respectively. P. Anandgaonker et.al reported the TEM for the synthesis of TiO_2 by using the electrochemical method the result showing the tetragonal structure with a size of 25–30 nm [17].

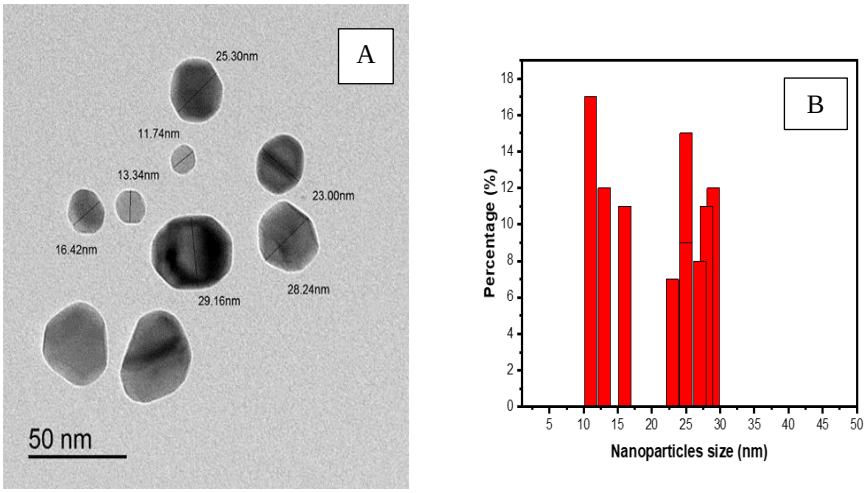

Fig. 2. (A) TEM image of TiO_2-NPs and (B) shows their distribution.

3.2 Effect of TiO_2-NPs on creatinine and urea

The effect of TiO_2 nanoparticles on patients with kidney disease, especially those with increase creatinine and concentrations are shown in Table 1 and Figure (3, 4).

Table 1. Comparisons of numerical variables for group 1, 2, and 3

Group	N	Creatinine (mg/dl) Means ±SD	P	P*	Urea (mg/dl) Means ±SD	P	P*
Control	30	0.6970±0.2432			31.87±6.345		
Before addition	30	6.088±2.206	S<0.0001		84.88±29.92	S<0.0001	
After addition	30	4.814±1.838	S<0.0001	S 0.0108	62.22±20.42	S<0.0001	S 0.0002

Regarding patients with high creatinine concentrations (6.088±2.206) before addition of TiO_2-NPs, the outcomes found a highly statistically significant increase (P<0.0001) in comparison with the control subjects (0.6970±0.2432). As well as, the creatinine results of kidney patients after the addition (4.814±1.838) were in a significant raise (P<0.0001) as compared with control group (0.6970±0.2432). Furthermore, after adding TiO_2-NPs; the outcomes revealed statistically significant reduction (P*=0.0108) in sera creatinine concentrations (4.814±1.838) compared to creatinine serum of patients before adding TiO_2 (6.088±2.206).

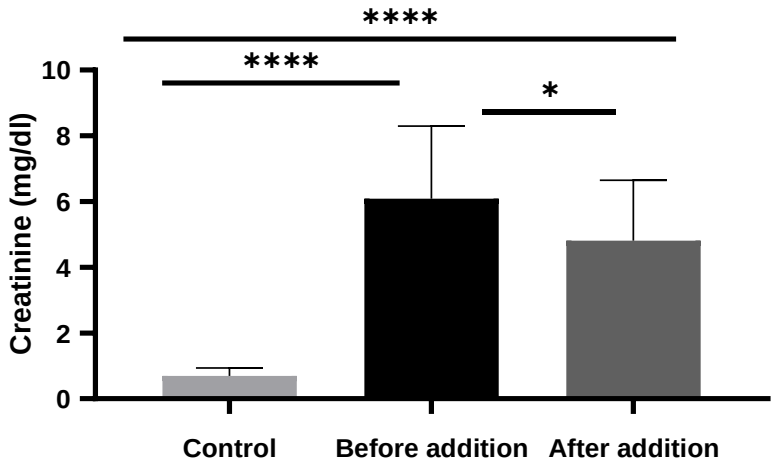

Fig. 3. Box plot of the results showed that sera creatinine was significantly higher in group 2 (patients before adding TiO_2-NPs) in compared to the group 3 (patients after adding TiO_2-NPs).

Moreover, the results showed in the case of urea sera (84.88±29.92) in kidney disease patients before adding TiO_2-NPs, a highly statistically significant increase (P<0.0001) in the sera levels of the urea in compared with that of control donors (31.87±6.345). Furthermore, the data illustrated that the sera levels of urea in group 1 (control subjects; 31.87±6.345) were doubleted significantly in comparison with urea concentrations of patients after adding TiO_2-NPs, (P<0.0001). In addition, the results also showed statistically significant drooping (P*=0.0002) in the sera levels of patients after adding TiO_2-NPs (62.22±20.42) compared to urea serum patients before adding TiO_2 (84.88±29.92).

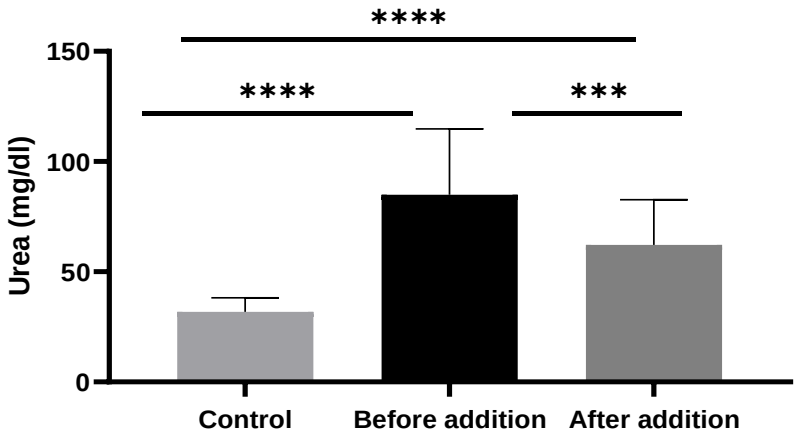

Fig. 4. Box plot of the results found that sera urea was significantly lower in group 3 (patients after adding TiO_2-NPs) in compared with the group 2 (patients before adding TiO_2-NPs).

Modern treatments may bring about and maintain illness cures, but they have substantial long-term side effects. By customizing the carrier features, nanoparticle drug carriers might lessen these negative impacts by more effectively delivering medications to the kidneys than free pharmaceuticals. Local pathophysiology, which may promote

increased nanoparticle deposition in certain tissues, is a significant extrinsic factor of nanoparticle biodistribution. The subject of studying nanoparticle systems for renal disorder implementations is expanding and has the potential to significantly alter the standard of care [18].

Nanomaterials are understood to have potential uses in the development of nanotechnology owing to their greater surface area to volume ratio, which boosts chemical reactivity and makes it simpler for them to enter cells. TiO_2-NPs among the various nanomaterials customarily are often thought of being chemically inert, nontoxic, and biocompatible substances. They have been extensively utilized in the paint industries as a coloring material, sunscreen sectors, and pharmaceutical [19]. Among the most well-liked nanoparticles today is TiO_2. It is a pigmented white additive with excellent coating and opacity qualities. It is often used for sterilization and industrial photolytic operations involving the decomposition of organic matter, pharmaceuticals, metallurgy, clothing, ceramics, car materials, paper, cosmetic, dye, food industries, rubber, and drug [20].

This study looked at how renal patients' levels of creatinine and urea were affected by TiO_2-NPs. In regard to patients with high creatinine and urea levels, prior and after to the addition of TiO_2-NPs, our data found that there were statistically powerful raise in both parameters when compared with control subjects. Moreover, the levels of creatinine and urea before adding of TiO_2-NPs were very high among patients with kidney disease (dialysis patients), but after adding TiO_2-NPs our outcomes observed a statistically remarkable downward trend of both creatinine and urea concentrations among those patients. Therefore, the main finding of this research demonstrated for the first time that TiO_2-NPs had an important effect on lowering the high levels of creatinine and urea among dialysis patients.

Our findings concur with the work that has been published by Amara et al. (2013); who mentioned that the biochemical assessment in plasma samples in the experimental group of rats treated by intraperitoneal injection of TiO_2-NPs showed significantly decreased creatinine levels compared with the control group [21].

Nevertheless, Shirdare et al. (2022); who showed that exposure to TiO_2 nanoparticles raised blood levels of creatinine and urea in rats, disagree with our findings [4]. The influences of TiO_2-NPs on the mouse kidney were examined in the research by Gui, et al. (2011). Creatinine increased and urea progressively reduced when the dosage of TiO_2-NPs was raised, indicating that mice with long-term low dose exposure to TiO_2-NPs had compromised nephric functions [19]. Another research discovered that the TiO_2 nanoparticles group's plasma concentrations of creatinine and urea in female rats were noticeably higher. On the other hand, as compared to the TiO_2 nanoparticles-intoxicated group, the plasma levels of creatinine and urea in the (quercetin + TiO_2-NPs) group were considerably lower [20]. Similarly, in another investigation on rats, quercetin was given to one group before the damage caused by TiO_2 was induced (preventive therapy), and to a different group after the injury was induced (therapeutic treatment). All groups' serum urea readings remained very consistent. No statistically significant deviations from the control groups were found. Nonetheless, there was a modest decline in the trend in the treated groups [1].

Our study did not agree with a study that was done by Hazelhoff et al. (2022); which claimed that subcutaneous treatment of TiO_2-NPs caused acute nephrotoxicity shown by histological and functional abnormalities in proximal tubule cells [22]. Furthermore, our findings, however, conflict with those of Feng et al., who studied rats exposed to silver nanoparticles (Ag NPs) at various time periods following exposure and discovered that the contents of creatinine and urea were noticeably increased in the rats' blood

[23]. Another study found that the plasma creatinine and urea concentrations in rats were markedly increased by zinc oxide nanoparticles treatment [24].

4 Conclusion

This study highlighted the areas of active clinical practice with NPs in the field of nephrology. In the medical field, nanoparticles have become a technology that is ideally suited for the detection and treatment of a number of disorders. This research looked at the impact of titanium dioxide nanoparticles (TiO_2-NPs) on renal patients' levels of creatinine and urea activity. Our results demonstrate for the first time that TiO_2 nanoparticles had a substantial influence on declining the levels of creatinine and urea among dialysis patients. These findings provide crucial physiological background for the potential novel, focused treatments for a renal disease that the nanoparticles may offer. In order to provide more accurate, trustworthy, and helpful findings for renal patients, more research is required to be done on these results using more dialysis patients, including different doses of TiO_2-NPs and putting some other criteria, and finally, try to examine it on rats in the future.

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