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Research article

Effect of Polyhydroxybutyrate Nano-Particles (PHB-NPs) in Mice Kidney Tissues: Histopathological Investigation

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ABSTRACT

Polyhydroxybutyrate (PHB) is a promising substance as it enters different applications such as medical, industrial, and food sectors. It is a safe material to prepare the PHB-nanoparticles (NPs) that are required to pass different chemo-physical reactions that may change its structure. That is why the PHB-NPs are required to assess their toxicity. The objective of the present investigation is to evaluate the cytotoxicity of PHB-NPs *in vivo* by examining the impact of PHB-NPs and other forms of this compound on kidney tissue. Within the confines of this study, PHB-NPs were synthesized in the laboratory through the application of ultrasound waves to PHB under varying pH conditions. The generation of PHB-NPs was estimated using a scanning electron microscope (SEM). Four cohorts of mice were intraperitoneally injected with 0.5 mg of PHB, PHB-NPs, PHB-cefotaxime (CTX), and CTX. The control group consisted of mice that were intraperitoneally injected with normal saline. The mice were subsequently dissected, and kidney slides from each group were prepared for histopathological examination. The method used in the current study is effective in producing PHB-NPs with diameters ranging from 20 to 35 nm. Furthermore, there was no discernible alteration in the kidney tissues of the mice in the four cohorts when compared to the kidney tissues of the control group. Thus, the present study concludes that there is no observed toxicity or any other adverse effects associated with the administration of PHB, PHB-NP, PHB-NP+CTX, and CTX on the histological characteristics of the experimental animals.

Keywords: Cefotaxime Kidney, Mice, Polyhydroxybutyrate, PHB-nanoparticles, Scanning electron microscope.

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1. INTRODUCTION

Polyhydroxybutyrate (PHB) is a polymer that finds application in the industry for the manufacture of biodegradable products which exhibits significant potential in addressing environmental concerns associated with plastic waste. PHB belongs to the polyhydroxyalkanoate (PHA) family, which are biopolymers naturally produced by various microorganisms. The biodegradebility, renewability, and versatile range of applications make PHB particularly noteworthy. In terms of production, PHB is synthesized by different microorganisms, including bacteria such as *Alcaligenes latus*. These bacteria accumulate PHB as granules within their cells [1]. It has found utility in the medical field, particularly for bioresorbable sutures and drug delivery systems, medical devices based on PHB can be utilized without the need for surgical removal after a specific period. Moreover,

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PHB can be employed in agricultural films for mulching and as a biodegradable substitute for conventional plastic covers [2]. In addition, PHB is employed in tissue engineering and regenerative medicine due to its biocompatibility and biodegradability. It can be integrated into scaffolds to facilitate cell growth and tissue regeneration [3].

The kidneys play a critical role in protecting the body against cytotoxicity, which refers to the detrimental effects of substances on cells. The kidneys also make a substantial contribution to this process. Specifically, the kidneys are responsible for the filtration of blood and the removal of waste products, toxins, and excess substances from the body through urine. To prevent potentially cytotoxic substances from circulating in the bloodstream and causing harm to cells throughout the body [4]. Furthermore, they help maintain the body's internal environment by regulating the balance of electrolytes, fluids, and pH. They prevent the accumulation of toxic substances by excreting them and maintaining the proper concentration of essential elements in the bloodstream [5]. Additionally, the kidneys can serve as sensitive indicators of systemic toxicity. When the body is exposed to cytotoxic materials, certain markers of kidney function, such as serum creatinine and blood urea nitrogen levels, may experience an increase [6]. Clinically, these markers are employed to assess kidney health and can also indicate exposure to substances that may be harmful to the kidneys or the body as a whole. These substances, including drugs and their metabolites, can be cytotoxic to cells if they accumulate in the body [7].

The kidneys aid in the elimination of these substances by filtering them out of the bloodstream, thereby preventing their buildup and potential cytotoxic effects. However, while the kidneys play a significant role in dealing with cytotoxic substances, they can also be susceptible to damage from certain toxic materials [8]. In particular, certain substances that are nephrotoxic (harmful to the kidneys) can impair kidney function or cause kidney damage, thereby reducing the organ's ability to effectively filter toxins.

Researchers frequently conduct investigations on the impact of various substances or materials on kidney cells *in vitro*, a context external to the human body, to comprehend their potential for cytotoxicity. Such studies play a crucial role in evaluating the safety of substances and materials, as well as aiding in the formulation of protocols or treatments to minimize any detrimental effects on kidney function. Previous investigations have examined the cytotoxic effects of nano-materials on both porcine kidney and human liver cells, utilizing two distinct metrics to assess cytotoxicity [9]. Another study delved into the hepatotoxic and nephrotoxic consequences of chloroacetonitrile (CAN) on the kidneys of rats, revealing structural damage to these organs [10]. Furthermore, an examination of the toxic effects of cobalt ferrite nanoparticles on kidney cells highlighted DNA damage at specific concentrations [11]. The toxicity of Chromium Copper Arsenate (CCA)-treated wood on the cell cycle of mouse kidney cells was also observed, suggesting potential hazards to the environment and public health [12]. Lastly, an investigation involving graphene oxide and gold nanoparticles found no significant toxic effects on kidney function parameters in mice [13]. However, there is no previous study showing the cytotoxic effect of either PHB or PHB-Nanoparticles (PHB-NPs) on the mice kidneys. Therefore, the aforementioned studies have convincingly underscored the significance of employing experimental animals to assess the toxicity of diverse materials. Hence, the present study aims to employ experimental animals to evaluate the toxicity of PHB, PHB-nanoparticles, and PHB-cefotaxime (CTX) compounds synthesized within our laboratory in terms of cytotoxic effect on the mice kidneys.

2. MATERIALS AND METHODS

2.1. Synthesis of PHB Nanoparticles

Five hundred microliter of Polyhydroxybutyrate (PHB) (Sigma-Aldrich, USA) was added to 25 ml of deionized double distilled water (pH 4 by HCl, 1N). The mixture was exposed to 4500 kh for 25 seconds of ultra-sonication (SONOREX SUPER RK 156 BH). Then, the pH was adjusted to 10 by NaOH (1N). After mixing for 120 min at 21 ºC, the mixture was stored at 21 ºC for 18 h. After the period of incubation, the pH was readjusted to 7.1 by HCl (1 N). The scanned electron microscopy (SEM, ZEISS Ultra Plus SEM, Germany) was used to check the production of PHB nanoparticles (PHB-NPs) [14].

2.2. Animals

BALB/c mice, aged between 8 and 9 weeks, and an average weight was 25 ± 2.1 gm. The animals were obtained from the central animal house of AL-Nahrain University in Baghdad, Iraq. The mice were housed in sanitary polypropylene cages and provided with a diet that was devoid of antibiotics and any medicines. Specifically, the mice employed in the present investigation were of the male gender.

2.3. Experiment

This methodology holds significant importance in ascertaining the toxicity of PHB-NP by assessing its impact on the histological characteristics of the mice kidneys. Within this experimental study, laboratory animals (mice) were employed as the test subjects. The experiment comprised four groups of mice, wherein each group comprised three individual mice. Group A, mice were injected intraperitoneal with 0.5 mg of PHB. Group B, mice were injected intraperitoneal with 0.5 mg of PHB nanoparticles. Group C, mice were injected intraperitoneal with 0.25 mg of PHB-NPs + 0.25 mg of CTX. Group D, mice were injected intraperitoneal with 0.5 mg of CTX, and group E, mice were injected intraperitoneal with sterile normal saline. The mice were sacrificed 72 hours post-administration of different materials intraperitoneally. All mice were dissected and kidneys were collected immediately after the sacrifice of animals. The kidneys were preserved in 10 % of formalin solution.

2.4. Histology

The methodology employed by Ibrahim et al. (2018) adhered to the standard protocol. To begin, the kidneys were impeded with 10% formalin (Sigma-Aldrich) and allowed to fix for a duration of 24 h. Subsequently, the kidneys were embedded in paraffin. A Leica microtome (Wetzlar, Germany) was employed to section the kidney blocks, with each section measuring a thickness of 5 µm. These sections were then affixed to slides. Following this, the sections of the mouse kidneys were subjected to staining using hematoxylin and eosin. To examine these stained sections, a compound light microscope (CH Series, Olympus LS, Japan) was employed. To ensure comprehensive evaluation, approximately five fields were examined within each section to assess any histological alterations [15].

3. RESULTS

3.1. PHB-NPs preparation

The method used in preparing PHB-NPs is considered one of the unique methods for preparing nanoparticles. The method was dependent on the pH gradient and exposure to sound waves. The size of the nanoparticles (PHB-NPs) prepared using

this technique ranges from 20 to 35 nanometers (nm), and this was confirmed using scanning electron microscope images.

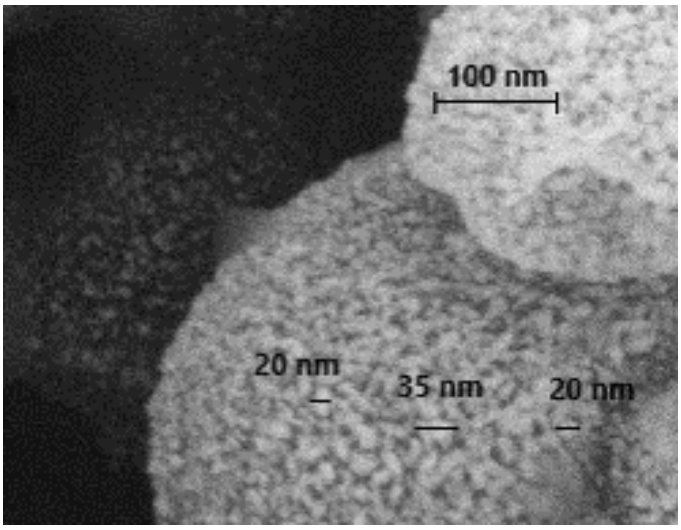

Fig. 1. Scanning electron microscope graph of PHB-NPs. The diameter of yielded particles ranged from 35 to 20 nm.

3.2. Effect of treatment dose on histological features of kidney

In the present study the instillation of 500 µg of PHB, PHB nanoparticles, PHB nanoparticles plus CTX complex and CTX intraperitoneal to study the cytotoxic effect of the above materials. The results were compared with control group of mice administrated with normal saline intraperitoneally. The results showed no significant changes in the histological structure of the cortex and medulla of mice kidneys, also no changes were observed in the glomerular capsule, tubules, and blood veins. Also, no changes were observed in all types of cells. In general, there is no histological changes were seen as compared with histological sections of the normal group. This finding proved that the substances given to mice have no toxic effect. That is why, this experiment proves the safety of using the substances mentioned above.

4. DISCUSSION

Many studies have highlighted the application of PHB in various fields [16,-19]. The extensive range of applications of PHB is due to its safety for both humans and animals [20, 21]. PHB-NPs, which are nanoparticles prepared from PHB, represent the new generation of PHB. The production of these nanoparticles requires exposure to physical and chemical stress, which may result in alterations in the chemical structure of PHB and render it unsafe. Consequently, this study aims to assess the toxicity of PHB-NPs by examining their impact on the kidneys of experimental animals. In this study, toxicity was evaluated by combining PHB-NPs with CTX and administering a dosage of 0.5 mg of various materials (PHB, PHB-NPs, PHB-NPs + CTX, and CTX) to the experimental mice. The results of this study revealed that the intraperitoneal administration of PHB, PHB-NPs, PHB-NP+CTX, and CTX did not produce any abnormal effects on the kidneys of the experimental animals, thereby confirming the safety of using these materials *in vivo*. This study is one of several conducted in our laboratory that focuses on evaluating the safety of these materials *in vivo*, which may pave the way for the utilization of PHB-NPs in various medical fields.

The effect of the injection of PHB on the kidney of an animal model is lake in the literature which is why we covered it in the current study. Several previous studies highlighted the use of changes in the kidneys of animal models as an indicator of the toxicity of several kinds of substances.

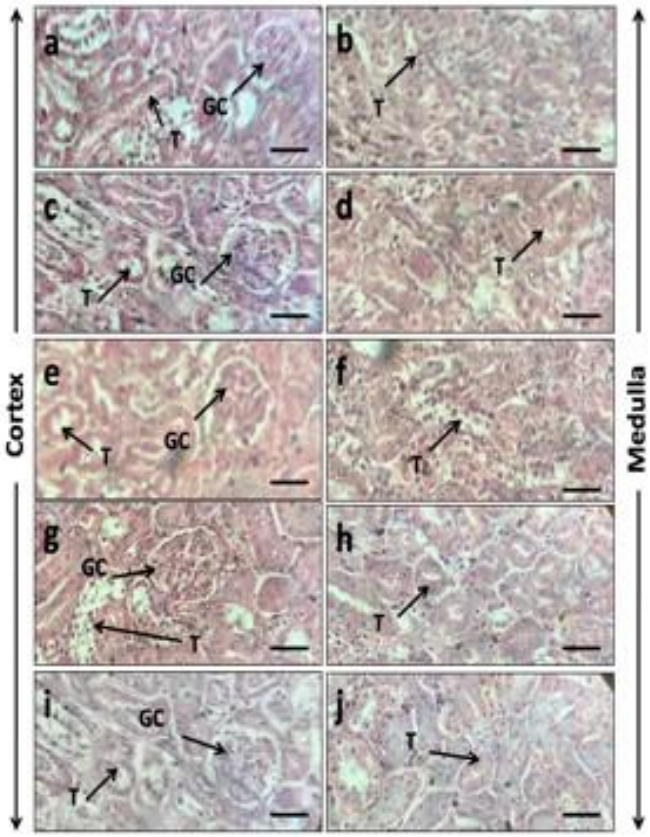

Fig. 2. Histopathological examination of mouse kidney (stained with hematoxylin & eosin) post-injected intraperitoneally with 500 µg of PHB (a, b), PHB nanoparticles (c, d), PHB nanoparticles plus cefotaxime complex (e, f) and cefotaxime (g, h). The results were compared with a control group of mice instilled with normal saline intraperitoneally. The results showed no histological changes in the sections of any mice groups as compared to the control. GC, glomerular capsule; T, tubules. All bars were 70 µm.

Chiou et al. (2020) used different methods to recruit the response of the kidney to different kinds of drugs to identify the toxicity of these materials [22]. Their findings help in the progress of the science of toxicology and push forward in this field and that will help in future work regarding measuring the toxicity of different substances. The present study matching with different studies proved the safety of using PHB *in vivo* [20, 21]. PHB has various applications in different fields. In the biomedical field, PHB is used for drug targeting and controlled drug release after nanoformulations. It is biocompatible and biodegradable, making it suitable for biomedical applications [16]. In the field of bioplastics, PHB is being researched as a substitute for non-degradable plastic wastes [17]. It is environmentally friendly and can be produced from bacteria, microalgae, actinomycetes, cyanobacteria, and plants [18]. PHB is also used in food packaging, agriculture, medicine, drug delivery, and tissue engineering [19]. According to the outcome of this study, we suggest that the PHB-NPs is a safe material but we also suggest doing more toxic evaluation experiments before judging the safety of this material.

5. CONCLUSION

The current investigation reached the definitive conclusion that the administration of PHB, PHB-NP, PHB-NP+CTX, and CTX to the mice at 0.5 mg did not exhibit any signs of toxicity or other adverse effects on the histological characteristics of the kidneys of the experimental subjects. This study proved the safety of PHB-NPs and their potential application.

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the Ministry of Health, Baghdad, Iraq (No 1205, 2022).

Author contributions

Jenan A. Ghafil. Conceptualization; Data curation; Investigation; Methodology; Project administration; Roles/Writing - original draft; Supervision; Validation.
Shamal A Al-Muffti. Roles/Writing - original draft; Visualization; Writing - review & editing.
Kadhim Hashim Alarajy. Formal analysis; Resources; Software.

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