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Research article

Histopathological Examination to Evaluate the Cytotoxicity of Polyhydroxybutyrate Nano-Particles (PHB-NPs) in Mice Livers

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ABSTRACT

Polyhydroxybutyrate (PHB) is considered a promising material in various medical, industrial, and food fields. The use of any substance in these areas must pass toxicity tests in vivo and in vitro. The current study aims to estimate the cytotoxicity of PHB nanoparticles (PHB-NPs) in vivo by checking the effect of PHB-NPs and other forms of this chemical on liver tissue. In this study, PHB-NPs were produced in the laboratory by exposing PHB to ultrasound waves in gradient pH. The formation of PHB-NPs was estimated using a scanning electron microscope (SEM). Four groups of mice were injected intraperitoneally with a treatment dose of PHB, PHB-NPs, PHB-cefotaxime (CTX), and CTX. The control group was the mice injected intraperitoneally with normal saline. The mice were dissected and liver slides of mice groups were prepared for histopathological examination. The results showed the efficiency of the method used in preparing the PHB-NP. The results of the experiments also showed that there was no change in the liver tissue of the mice of the four groups when compared with the liver tissue of the control group. The present study concluded that there is no toxicity or any other effect of administrating the mice with a treating dose of PHB, PHB-NP, PHB-NP+CTX, and CTX on the histologic features of the experimental animals. This study proves the safety of PHB-NPs and combines materials for use in in vivo study but after further cytotoxic experiments.

Keywords: Histopathological study, Liver, Mouse, Polyhydroxybutyrate Nano-Particles.

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1. INTRODUCTION

Polyhydroxybutyrate (PHB) is a biodegradable thermoplastic polymer that holds great promise in addressing environmental concerns related to plastic waste. It is a member of the polyhydroxyalkanoate (PHA) family, which are naturally occurring biopolymers produced by various microorganisms. PHB is of particular interest due to its biodegradability, renewability, and potential applications in a wide range of industries. PHB is produced by various microorganisms, including bacteria such as *Cupriavidus necator*, *Ralstonia eutropha*, and *Alcaligenes latus*. These bacteria store PHB as granules within their cells [1]. PHB serves as an intracellular carbon and energy storage compound, allowing these microorganisms to survive in nutrient-limited conditions. PHB is a linear, high-molecular-weight polyester composed of 3-hydroxybutyrate monomers. Its chemical structure is similar to that of synthetic petrochemical-based plastics, such as polypropylene. PHB is known for its physical properties, such as being a strong and stiff polymer with good oxygen barrier prop-

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erties. It is biodegradable in various environments, including soil, compost, and marine settings, making it an attractive alternative to traditional plastics. One of the key advantages of PHB is its biodegradability. When exposed to specific environmental conditions, microbial enzymes can break down PHB into its constituent monomers, which can then be used as an energy source by microorganisms. This natural degradation process helps reduce the environmental impact associated with plastic pollution [2].

PHB is used in the packaging industry for the production of biodegradable bags, films, and containers. Its biodegradability is a valuable feature for single-use plastic items, as it reduces plastic waste and its impact on the environment. PHB has applications in the medical field, particularly for bioresorbable sutures and drug delivery systems. Due to its biodegradability, PHB-based medical devices can be used without the need for surgical removal after a certain period. PHB can be used in agricultural films for mulching and as a biodegradable alternative to traditional plastic covers [3]. This reduces the environmental impact of agriculture. Some companies use PHB in cosmetic products like microbeads, which are biodegradable alternatives to plastic microbeads, often used in exfoliating products. PHB is used in tissue engineering and regenerative medicine due to its biocompatibility and biodegradability. It can be incorporated into scaffolds for cell growth and tissue regeneration [4].

The liver is a vital organ responsible for various critical functions in the body, including detoxification. It plays a central role in processing and eliminating toxic substances from the bloodstream. However, the liver can be severely affected by the presence of toxic materials, which can lead to a range of liver-related health issues. Toxic materials, including drugs, alcohol, environmental pollutants, and certain industrial chemicals, can cause liver toxicity [5]. When the liver processes these substances, it may convert them into metabolites that can be harmful to liver cells. Over time, the accumulation of these toxic metabolites can damage liver tissue and lead to liver dysfunction. Some medications and drugs can be toxic to the liver. Drug-induced liver injury can range from mild elevations in liver enzymes to severe liver damage. This condition may result from an adverse reaction to a specific drug, an overdose, or prolonged use of certain medications [6]. Exposure to toxic chemicals, such as solvents, heavy metals, and pesticides, can lead to liver damage. The liver's detoxification process may not be able to efficiently process and eliminate these toxic substances, leading to liver injury [7]. Chronic exposure to certain toxic materials, such as aflatoxins (produced by molds that grow on poorly stored grains and nuts), can increase the risk of developing liver cancer. Liver cancer often develops in the setting of underlying liver disease or cirrhosis [8].

It's important to note that the liver has remarkable regenerative abilities, and early-stage liver damage can often be reversed with appropriate intervention. Reducing or eliminating exposure to toxic materials, adopting a healthy lifestyle, and seeking medical treatment are essential steps in managing liver damage [6].

Different previous studies used the liver of animals as an indicator of the toxicity of the chemicals and different materials. van Dyk et al. (2007) checked the toxicity of the toxic effects, of two heavy metals, cadmium (Cd) and zinc (Zn), on the histology of the liver of the southern African freshwater fish *Oreochromis mossambicus*. They try to identify whether metal concentrations and exposure period influence the degree and nature of histological changes in the liver of exposed fish [9]. Padrilah et al. (2017) evaluated the toxicity of coopers in the environment by checking the histological changes in the liver of Clarias gariepinus [10]. Isoda et al. (2017) studied the effects and possible pharmacological interactions of nanoclays in vivo. When nanoclay particles were injected into mice through the tail vein, the liver was severely injured. Both liver and kidney damage was brought on by the co-administration of nanoclay with carbon tetrachloride, paraquat, or cisplatin. Thus, they checked the toxicity of nanoclay by administration of it in the mice intervene (i.v.) [7].

Thus the previous studies proved the importance of using the experimental mice in checking the toxicity of different materials that is why the present study aims to use the experimental animal to check the toxicity of the PHB, PHB-nanoparticles, and PHB-cefotaxime (CTX) prepared in our lab.

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 ^{o}C, the mixture was stored at 21 ^{o}C 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) [11].

2.2. Animals

BALB/c mice 6-8 weeks old, weighing 20-25 gm procured from central animal house, AL-Nahrain University, Baghdad, Iraq. Animals were kept in clean polypropylene cages and fed on the standard antibiotic-free diet. The mice that were used in the current study were male.

2.3. Experiment

This method is an important method that determines the toxicity of the PHB-NP by checking the effect of this material on the histologic features of the liver. In this experiment laboratory animals (mice) were used to do this method. Briefly, four groups of mice were used in this experiment. Each group consisted of three mice. Group A, mice injected under the skin with a treatment dose of PHB. Group B, mice injected under the skin with a treatment dose of PHB nanoparticles. Group C, mice injected under the skin with a treatment dose of PHB-NPs + CTX. Group D, mice injected under the skin with CTX, and group E, mice injected intraperitoneal with normal saline. The mice were sacrificed 73 hours post administration of treatment doses (0.5 mg/ ml) of the above materials under the skin. All mice were dissected. Livers were collected immediately after the sacrifice of animals. Livers were preserved in formalin solution.

2.4. Histology

The standard method of Ibrahim *et al.* (2018) was followed. The livers that were fixed by 10% formalin (Sigma-Aldrich) for 24 h were embedded in paraffin. Livers blocks were sectioned at a thickness of 5 µm using a Leica microtome (Wetzlar, Germany) and adhered to slides. The sections mouse were stained with hematoxylin and eosin and were examined by a compound light microscope (CH Series, Olympus LS, Japan). In each section, around five fields were examined to check different histological alterations [12].

3. RESULTS

3.1. Effect of treatment doses on histological features of Liver

Before starting the experiment the PHB-NPs were checked by examining the products under the SEM and the diameter of the PHB-NPs ranged from 30 nm to 22 nm. To identify the effect of administration intraperitoneally of 500 µg of PHB, PHB-NPs, PHB-NPs plus CTX complex and CTX on the liver of experimental mice was evaluated. The results were compared with the control group of mice administrated intraperitoneal injection of normal saline. The results showed no visible changes in the histological structure of mice livers and no changes in the liver cell level (Fig. 1). That 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. Thus it can be used for further experiments of treating the burn mouse model (the work is going on in our Lab).

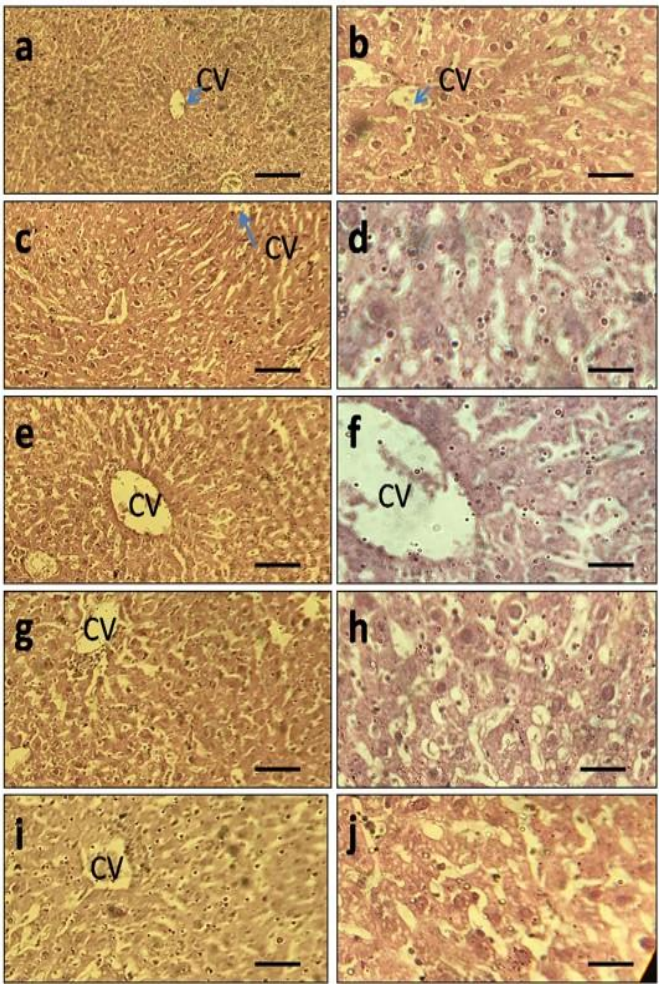

Fig. 1. Histopathology examination of mice livers post instillation with 500 µg of PHB (a, b), 500 µg PHB-NPs (c, d), 500 µg (1:1) of PHB-NPs plus CTX complex (e, f) and 500 µg of CTX (g, h). The results were compared with control group of mice instilled with normal saline intraperitoneal injection. The results showed no histological changes in any mice groups as compared to control. CV, central vessel. Bars were 125 µm for a; 60 µm for b; 75 µm for c & e; 50 µm for d, f, h & j; 65 µm for I & j. The slides were stained with hematoxylin and eosin.

4. DISCUSSION

There are many studies highlighted the application of PHB in different fields especially in medicine [5-8]. The wild range of application of PHB because the safety of this material to human and animals. The PHB-NPs is the new generation of PHB as prepared from PHB but in nanoparticle. To product the nanoparticle that required to expose to physical and chemical stress that may be make a change in the chemical structure of the PHB and that may make it non safe chemical. That is why, this study focused on evaluate the toxicity of PHB-NPs by evaluate the effect of this material on the liver of experimental animal. The present study evaluate the toxicity in the condition of mixing the PHB-NPs with CTX. The results of the present study showed that the administration of PHB, PHB-NPs, PHB-NP+CTX, and CTX intraperitoneally did not have any abnormal effect on the liver of experimental animals, which confirms the safety of the use of these materials *in vivo*. The present study is one of several studies in our lab focusing on the evaluation of the safety of these materials in vivo that will open the door to the possibility of using PHB-NPs in several lines of medical fields.
Several previous studies showed the changes in the liver as a good indication of the toxicity of substances [9-11, 13]. There is no literature that highlights the effect of PHB-NP on the livers of experimental animals. That is why the present study represents the pioneer study in this area of work. The toxicity of different solvents by evaluating the changes in the liver of experimental animals was checked by Malaguarnera *et al.* (2012) [14]. Calitz *et al* (2018) highlight the possibility of using an animal's liver as an indicator of toxicity of several substances [13]. Mosedale *et al.* (2020) gave an overview of the clinical profile, mechanisms, and risk factors underlying idiosyncratic adverse drug reactions as well as new approaches to study these reactions, focusing on idiosyncratic drug-induced liver injury [15]. 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 present study concluded that there is no toxicity or any other effect of administrating the mice with a treating dose of PHB, PHB-NP, PHB-NP+CTX, and CTX on the histologic features of the experimental animals. This study proves the safety of PHB-NPs and combines materials for use in in vivo study but after further cytotoxic experiments.

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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 Scientific Committee of the University of Baghdad, Baghdad, Iraq (No 134, 2020).

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