J Nanostruct 11(4): 647-653, Autumn 2021

RESEARCH PAPER

Effect of Free Prodigiosin Pigment and Mixed Prodigiosin With TiO_2 Nanoparticles in Some Viable Organs of White Mice

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ARTICLE INFO

Article History:
Received 29 June 2021
Accepted 09 September 2021
Published 01 October 2021

Keywords:
Free Prodigiosin
Mixed
TiO_2 nanoparticles
White mice

ABSTRACT

The study evaluated the effect of TiO_2 NPs mixed with pigment on liver and kidney weight. Liver weight in G1, which is administrated with low dose of prodigiosin decreased significantly compared to liver weight in control group. No significant differences were found in kidney weight between the study groups in comparison with the control. The results also, showed highly significant increase in the level of GOT enzyme in G3, G4, G5 and G. This may confirm the effect of TiO_2 NPs in restricting the prodigioisin effect and increasing its toxicity. All the study groups showed highly significant increase in urea level when compared to its level in the control group. G1, which is administrated with low dose of prodigiosin showed the highest urea levels among other groups. Same thing about GPT enzyme level, in G1 and G2 become less in a significant way than its level in the control group. The cytotoxic effects of TiO_2 NPs increased when the dose was raised, according to the results of this investigation. However, the presence of Prodigiosin inhibits the activity of nanoparticles and reduces their negative effects in essential organs (liver and kidney).

How to cite this article
Dehghankelishadi P, Dorkoosh FA. Effect of Free Prodigiosin Pigment and Mixed Prodigiosin With TiO_2 Nanoparticles in Some Viable Organs of White Mice. J Nanostruct, 2021; 11(4):647-653. DOI: 10.22052/JNS.2021.04.002

INTRODUCTION

Pigments are utilized as additives, color intensifiers, antioxidants, and other things in the food business [1]. Food coloring with pigments derived from natural sources has recently gained popularity and relevance around the world. Natural dyes are considered safe substitutes for synthetic colors, which are unpopular and dangerous in the food industry. As a result, it is critical to investigate various natural sources of food colorants and their potential. Because of their ease of manufacture and downstream processing, microbial colorants play an important role as food colorants. Because of the durability of the pigments generated and the ease of cultivation, microbial pigments are of great interest [2]. Natural compounds in the prodigiosin alkaloid family have a pyrrolylpyrromethene core and a rich, blood-red color. Microorganisms like Serratia marcescens create members of this family. Antimicrobial, antifungal, cytotoxic, and immunosuppressive properties of prodigiosins have been discovered.

Nanotechnologies are a collection of methodologies and techniques for treating matter with the goal of creating materials with new functions and better properties. Nanoparticles, among the numerous materials, serve a distinctive role in a wide range of applications, and there are many studies on titanium dioxide nanoparticles in particular (TiO_2 NPs) [3]. The properties of TiO_2 NPs and their usage in the degradation of compounds

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in aqueous solutions and the reduction of inorganic ions have been described in numerous research [4-5].

We investigated the effects of low and high dosages of prodigiosin on the function of two essential organs in human bodies, and to acquire a better understanding, we used a form of TiO_2 NPs that is known to interfere with the action of pigments. As a result, this research was started to see how TiO_2 NPs mixed with pigment affected the function of various essential organs (Liver and kidney). the aim of this study Effect of free Prodigiosin and mixed with TiO_2 nanoparticles in some viable organs of white mice.

MATERIALS AND METHODS

Solutions preparations

Nanoparticles solutions

• Sigma, USA provided titanium dioxide nanoparticles (TiO_2 NPs) solution (Conc. Wt. percent in water, size 50 nm).

• Distilled water was used to make nanoparticle solutions with a minimum concentration of 250 g/kg of TiO_2 nanoparticles, or 0.0075 g / mice.

Prodigiosin solution:

At ideal conditions, prodigiosin was extracted by solvent system from 250 ml volume flasks containing 50 ml of medium produced by selected isolates of S. Marcescens bacteria. S. marcescens G10 prodigiosin production culture after 42 hours. For the first 15 minutes, the culture media was centrifuged at 8000 rpm. The supernatant was discarded, and the extracted cell was agitated vigorously in a separating funnel at room temperature for at least 3 hours with an equal volume of solvent (chloroform). The organic phase (solvent phase) was collected and filtered through a 0.2 m filter unit before being dried at 45 degrees Celsius. The crustal pigment that resulted was quantified, and the crystal was kept in a safe place [6].

• A low dose of prodigiosin solution of 100 mg/kg body weight (0.05 mg/mouse) was made.

• A high dose of 250 mg/kg body weight of prodigiosin solution (0.125 mg/mouse) was made.

Phosphate buffer saline (PBS):

- (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4) Adjust pH to 7.4 with HCl after dissolving the following in 1L distilled H_2O.
- Design of the experiment: Seventy-two adult male mice were obtained from the National Center for Drug Control and Research's animal house and kept in the animal house of Al-Nahrain University's Biotechnology Research Center, where they were kept in separate cages at (25Co) room temperature and fed a suitable diet in addition to water.
- The animals in the experiment were divided into five groups at random:
- Group one (G1): A control group of 12 animals was given PBS orally for 30 days. This is the negative control group.
- Group two (G2): 12 mice were given a low dose of prodigiosin (100 mg / kg body weight) orally for 30 days.
- Group three (G3): 12 animals were given a high dose of prodigiosin (250 mg / kg body weight) orally for 30 days.
- Group four (G4): Four 30 days, 12 animals were given a low dose of prodigiosin (100 mg/kg body weight) combined with TiO_2 nanoparticles solution (0.25 m/kg) orally.
- Group five (G5): Four 30 days, 12 animals were given a high dose of prodigiosin (250 mg/kg body weight) combined with TiO_2 nanoparticles solution (0.25 mg/kg) orally.
- Group six (G6): 12 animals were given TiO_2 nanoparticles solution (0.25 m/kg) orally for 30 days. This is the positive control group.

At the conclusion of the test, the animals were weighed, and blood was taken by puncturing the heart, centrifuged for 10 minutes at 3000 r/m, and the animals were slaughtered. The liver and kidney were promptly removed and preserved in 10% formalin for histological study, while the serum was kept at -80 °C and utilized to determine the thalassemia level.

Laboratory investigations

1- Analysis of liver function tests: The serum levels of aspartate aminotransferase (AST) or glutamate oxaloacetate transaminase - GOT (U/L) and Alanine aminotransferase (ALT) or glutamate pyruvate transaminase - GPT (U/L) were determined using the ELIZA kit (Orgmetric/Germany). The biochemical tests were analyzed according to the manufacturer's instructions.

2-Kidney function tests: The serum concentrations of urea (mg/dl) and creatinine (g/dl) were determined using a kit (Randox kit/England). The biochemical tests were analyzed according to the manufacturer's instructions.

Statistical analysis

SAS (2010) was used to investigate the impact of various factors on the parameters under investigation. In this study, the least significant difference (LSD) test was utilized to compare means.

RESULTS AND DISCUSSION

Results of liver and kidney weight

Liver weight in G1, which is administrated with low dose of prodigiosin and in G2, which is administrated with high dose of prodigiosin decreased significantly compared to liver weight in control group. Liver weight in G3 which is administrated with low dose of prodigiosin + TiO_2 NPs and G4 which is administrated with high dose of prodigiosin + TiO_2 NPs is increased compared to liver weight in G1 and G2 but in a non-significant way, while its still less than liver weight in the control group.

This may confirm the effect of TiO_2 NPs in restricting the prodigiosin effect and increasing its toxicity. On the other hand, TiO_2 NPs alone results in decreasing liver weight significantly compared to control group. No significant differences were found in kidney weight between the study groups in comparison with the control.

Results of liver function tests

The level of GOT enzyme in G1 and G2. In G3 which is administrated with low dose of prodigiosin conjugated with TiO_2 NPs showed the highest levels of GPT enzyme compared to its level in the control group. No significant differences were found between GOT enzyme level in G5, G4 or G5.

Table 2 shows a highly significant increase in the level of GOT enzyme in G1, which was given a low dose of prodigiosin, and G2, which was given a large dose of prodigiosin. It was also noted that the enzyme level increased as the prodigiosin dose increased.

The G3 group, which received a low dose of prodigiosin coupled with TiO_2 NPs, had the greatest levels of GOT enzyme compared to the control group, with a very significant difference. In compared to the control group, GOT enzyme

Table 1. Organ weights (Liver and kidney) in the study groups expressed as (mean± sd)

Groups	Organ weights	
	Liver	Kidney
Control	4.731 ± 1.27 a†	0.284 ± 0.206
G1	2.967 ± 0.262 bc	0.272 ± 0.019
G2	1.938 ± 0.603 c	0.286 ± 0.074
G3	3.225± 0.652 b	0.496 ± 0.118
G4	2.914 ± 0.070 bc	0.463 ± 0.038
G5	2.026 ± 0.456 c	0.318 ± 0.002
P-Value	0.003**	0.069 N.S

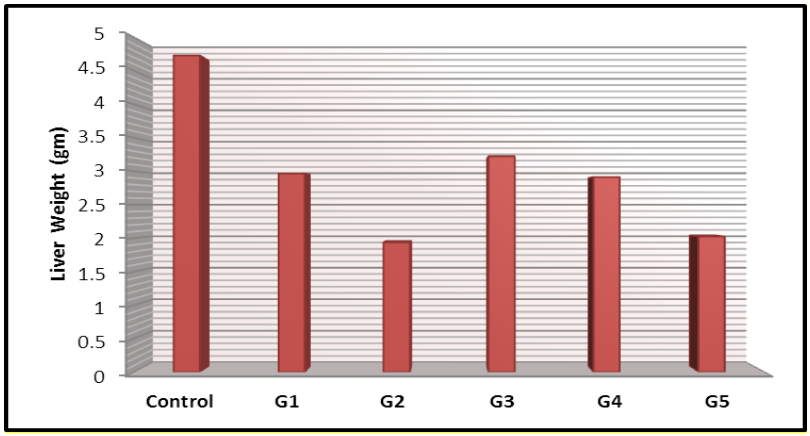

Fig. 1. Liver weight in study groups.

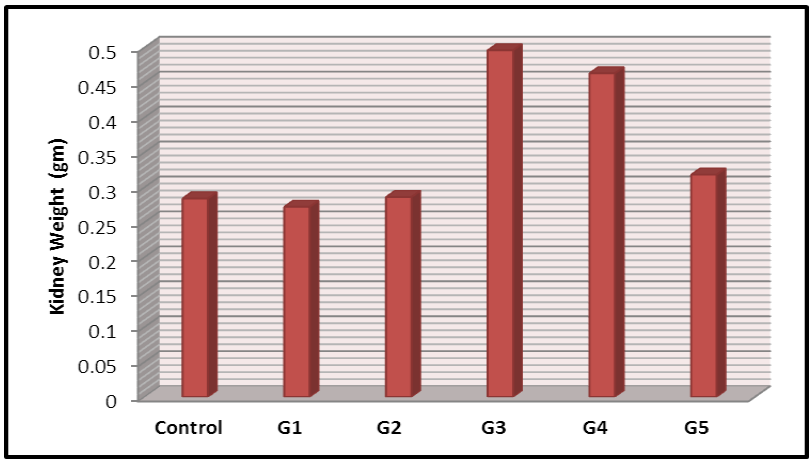

Fig. 2. Kidney weight in study groups

Table 2. Function tests for Liver in the study groups expressed as (mean± sd)

Groups	Liver Function			
	GOT		GPT	
Control	11.67 ± 0.58	d†	7.33 ± 1.52	c
G1	75.67 ± 6.51	bc	17.00 ± 2.00	b
G2	92.50 ± 23.44	b	15.25 ± 2.21	b
G3	120.00 ± 13.93	a	43.75 ± 4.85	a
G4	81.25 ± 5.68	bc	17.25 ± 1.50	b
G5	68.67 ± 3.51	c	14.33 ± 1.53	b
P-Value	0.000**		0.000**	

levels are lower in G4 when given a high dose of prodigiosin coupled with TiO_2 NPs, but the differences are still significant. When G5 was given TiO_2 NPs, the GOT enzyme level increased significantly compared to the control. GOT enzyme levels in G5 did not differ significantly from those

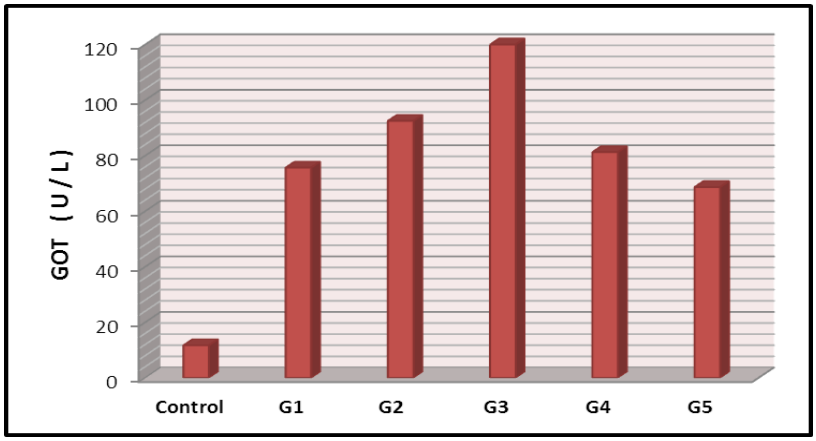

Fig. 3. Glutamate oxaloacetate transaminase - GOT concentration (U/L) in the study groups

in G4 and G1.

Similarly, the amount of GPT enzyme in G1 and G2 is much lower than in the control group. The G3 group, which received a low dose of prodigiosin conjugated with TiO_2 NPs, had the greatest levels of GPT enzyme when compared to the control group, and the differences were extremely significant. There were no significant variations in GPT enzyme level between G4 that was given a high dose of prodigiosin conjugated with TiO_2 NPs and G4 that was given a low dose of prodigiosin conjugated with TiO_2 NPs.

Results of Kidney function tests

When compared to the level in the control group, all of the study groups demonstrated a highly significant rise in urea. Among the other groups, G1 had the highest urea levels, although being given a low dose of prodigiosin. The same may be said for creatinine levels, which increased significantly in all research groups [7].

Organic pigments are generally extracted from fruits, vegetables, seeds, roots and microorganisms. They are sometimes called bio-colors because of their biological origin. The undesirable properties of natural pigments such as solubility and short term stability limit their application in the food industry. There is an ever growing interest in microbial pigments due to several reasons like their natural character and safety to use [8].

Prodigiosin has antifungal, antibacterial and antiprotozoal activities. The most significant goals of this experiment is to evaluate the safety of prodigiosins when its used as therapy and to discover its effect in the function of vital organs in

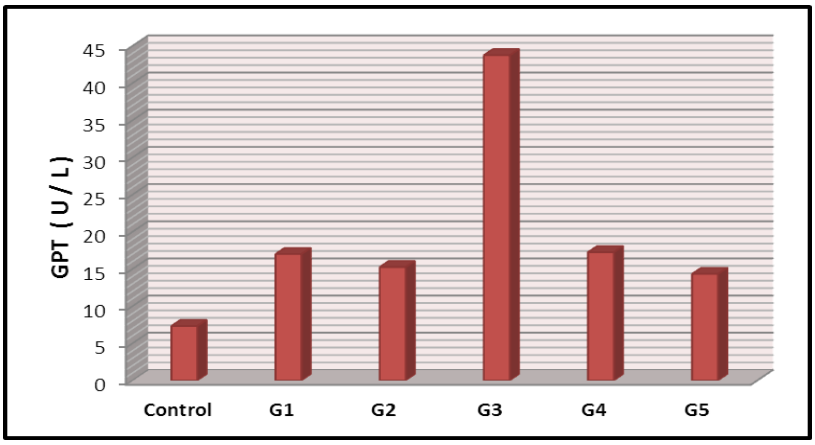

Fig. 4. Glutamate pyruvate transaminase - GPT concentration (U/L) in the study groups

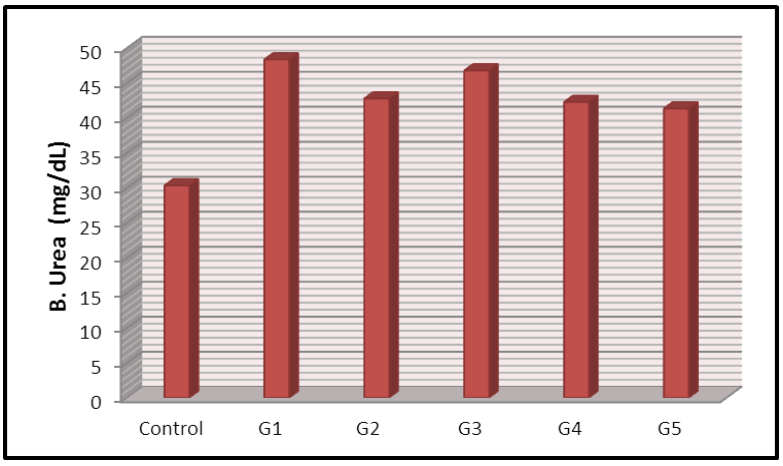

Fig. 5. Blood Urea concentration (mg/dl) in the study groups

Table 3. Function tests for kidney in the study groups expressed as (mean± sd)

Groups	Kidney Function	
	B. Urea	B. Creatinine
Control	30.33 ± 3.51 c	0.276 ± 0.065 c
G1	48.33 ± 5.68 a	0.746 ± 0.055 a
G2	42.75 ± 2.98 ab	0.472 ± 0.027 b
G3	46.75 ± 2.98 ab	0.530 ± 0.063 b
G4	42.25 ± 4.11 ab	0.472 ± 0.074 b
G5	41.33 ± 4.16 b	0.493 ± 0.030 b
P-Value	0.001**	0.000**

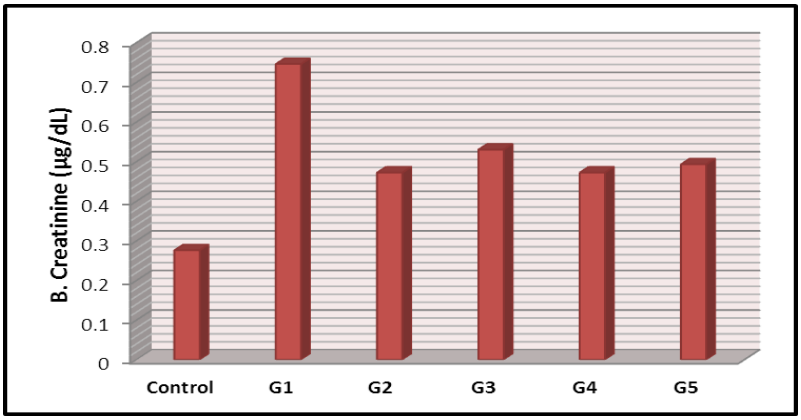

Fig. 6. Blood Creatinine concentration (µg/dl) in the study groups

human bodies. In this study we choose titanium dioxide nanoparticles (TiO_2 NPs) which have been shown to be effective in treating cancer cells from B-cell chronic lymphocytic leukemia patients. TiO_2 NPs are among the most commonly used metal oxide nanoparticles in industrial products, cosmetics, sunscreens, food products, paints and drugs. They have been reported to elicit various adverse cellular effects including oxidative stress and DNA damage. TiO_2 NPs induce histological and ultrastructural changes in the liver in the form of congestion and lymphocytic aggregation. The overproduction of ROS would break down the balance of the oxidative/anti-oxidative system in the tissues, resulting in the lipid peroxidation via ROS and MDA production. By passing time, the temporary disorders have been removed and the renal function has returned toward normal [9,10] In kidney tissue, the changes such as deposition of hyaline-like materials, the swelling, dilatation of Bowman's capsule and degenerations were seen. TiO_2 NPs caused damages in mitochondria and apoptosis of hepatocytes, generation of reactive oxygen species, and expression disorders of protective genes in the liver of mice. The study showed that titanium was distributed to and accumulated in the heart, brain, spleen, lung, and kidney of mice after intraperitoneal exposure.

CONCLUSION

In summry, in this work the effect of TiO_2 nanoparticles/pigment on liver and kidney weight was studied. Liver weight in G1, which is administrated with low concentration of prodigiosin reduced significantly compared to liver weight in control group. It was not shown any significant differences in kidney weight between the study groups in comparison with the control.

The findings revealedthe significant increase in the level of GOT enzyme in G3, G4, G5 and G. It relates to the TiO_2 nanoparticles which limits the prodigioisin effect and elevate its toxicity. All the study groups showed highly significant increase in urea level when compared to its level in the control group.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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