Effect of Nano Scale Titanium Dioxide in Quail Testes Tissue and Molecular Diagnosis in a RAPD_PCR Method

Zainab Al-Jomily[1], and Rayya G. Al-Sultan[2]
[1, 2]Department of Biology, College of Education for Girls, Mosul University, Mosul, Iraq
Email: zainab.20gep52@student.uomosul.edu.iq

Abstract
This study was conducted at the Central Laboratory/Faculty of Agriculture and Forestry/University of Mosul with the aim of detecting the genetic effects of nano titanium dioxide TIO2NPs in the quail's testes using the RAPD-PCR index, The birds were treated from 10 to 50 days old with this substance, where the dosage was from one day to the next and DNA was extracted from the adult birds' testicles after sacrifice, where the samples were subjected to the polymerization chain reaction. (PCR) The results of RAPD-PCR interactions showed a clear difference in the DNA testicular sample of the quail bird treated with nano titanium in the concentration of 35PPM compared with the control group, If this substance has the ability to indiscriminate genetic changes that occurred during the duration of the dosage and a mutation, deformation or alteration in the genetic composition of DNA occurs in a given region, this is an indicator of gene alteration shown by random designed RAPD-PCR and randomly associated in certain places. The results of the control testicle and the treatment testicle of nano titanium have been associated with the same area but with a lower or more sequence in terms of the number of nitrogens bases according to the marker division sequence ranging from 250Pb to 10000pb, suggesting that the DNA had mutations because of nano titanium and there was a clear difference between them.
Keywords: PCR, RAPD-PCR, TIO2NPs

1. Introduction

Humanity has undergone an enormous number of scientific revolutions and advances in all spheres of life, including nanotechnology (Albanese et al. 2012; Jiang et al. 2008), the term nanotechnology was first launched in 1974 by the world Taniguchi Noori (Bayda et al. 2020) The word nanoscale has a Greek origin and means something very small and the content of a meter is on one billion nanoscale parts (Wang, 2018), Nanotechnology is an important technique because it enters many fields and depends on the synthesis of objects with nanoscale dimensions. These objects have different qualities than the metals they formed, all properties of the materials will change such as melting point, optical properties, The electronic properties when their constituent molecules change into the nanoscale, the nanoscale technology is of outstanding importance as it can handle and control a single cell in terms of function and composition and is also capable of understanding or adapting living matter to its simple components of organic molecules that store DNA and Atom, genetic information, Nanoparticles (NPs) are called nanoscale minutes and are one of the advanced secondary materials in a distinct category that can be produced and measured by the number of its inner pellets between (1-100 nanometers) (Khan et al. 2019), nanoscale minutes are characterized by their small size and increase their surface area (Gahlawat et al. 2016) Their small size enables them to enter organism cells easier and NPS shows vital effects and chemical reactions to different degrees and highly distinct qualities (Chen et al. 2006; Silva 2011)، Recent studies and research have focused on nanoscale minutes primarily in early diagnosis, treatment of diseases and delivery of medicines according to the characteristics of unique nanoscale minutes that can target toxic biophilia with great precision to cancerous tissue without causing damage to healthy body tissue (Jin et al. 2020).

Titanium is a shiny and solid element of its high temperature resistant and semi-conductive qualities for electricity, its melting point is as high as 1668C °, resistant to corrosion and rust and easily reacts with atmospheric air oxygen (Essalhi et al. 2017).

TiO2 is introduced by treating malignant tumors through its use in synthesis of vital compounds with antibodies (Ni et al. 2017) Nanomaterials also have a positive effect on stimulating productivity, livelihood, quality of animal products and feed as well as eggs nutrition.

Nanoscale applications have the potential to reduce the costs of poultry husbandry and improve production but have safety concerns for some nanoscale applications that hinder their rapid implementation. Extensive risk assessments must be undertaken to ensure the safety of nanoparticles before they are used by animals and humans (El Sabry et al., 2018).

Despite the benefits of nanoscale minutes, they have significant risks to health and determining the toxicity of nanoscale minutes is not sufficient to know their impact on organisms. A broad and continuous study to determine the toxicity of nanoscale minutes will be important for nanotechnology, thus determining the course of exposure to nanotoxins by reducing exposure to toxic nanoscale minutes (Buzea et al. 2007).

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2. Materials and Methods

The study was conducted on quail birds where 50 10-day-old chick was obtained from the Faculty of Agriculture and Forestry, Department of Livestock Production, University of Mosul. All the chicks were healthy and appearance-sound. Birds were raised in a suitable cage divided into two dimensions (60cm) length, (52cm) Width, (40cm) height, cage brushes with sawdust and exchanged the mulch between day and day with continuous cleaning care, birds placed under similar conditions of lighting, ventilation, heat during all stages of the experiment, birds were given tap water through the nuts, as well as the leafy of their nutrition, protein feed (Kesab, 2018), Turkey's manufacture packaged by Al-Nebras in Mosul consists of crushed legumes consisting of sacks, red lentils, beans, peanuts and sunspine.

The average lethal dose (LD_{50}) of titanium dioxide and detected to 60 mg/kg body weight was determined (Al-Mustafa and Al-Sultan, 2022). The experiments were designed using a bird 50 distributed to two groups and included a control group dosed with distilled water and an experimental group given an oral titanium dioxide concentration 35ppm dosed 10-day-old chicks and the dose was between day and day, At morning the water and leaf are lifted for an hour before dosing and returned after dosing. The autopsy took place 40 days after the dosage, 50 days after sexual puberty. The bird was placed in a sealed case containing a cotton piece saturated with chloroform. The birds were sedated from the control and treatment group. The testicle was extracted from the testes. The extracted organs were then stored in a sulfone cover and frozen until use.

Deoxyraipozzi DNA extraction

DNA was extracted according to the steps established in the extraction kit (AddBIO) and equipped by the Canadian company Promega, after which the electrical relay of DNA extracted from the testicle was performed for both the control group and the experimental group 35ppm and on the prepared agarose gel at a concentration (0.9g) and at a difference (100V),

Amplify DNA samples using PCR chain thermal reaction technology

The DNA amplification process (RAPD-PCR) involved the use of nine random prefixes as shown in Table 1.

(Table 1: Random prefixes designed, nitrogen base sequences each, show the binding temperature of each prefix)

ɪreference	Tm	)sequence(5 to 3	prefixes	No.
Istiak, et al., 2018	39	TGGACCGGTG	OPC-08	1
	39	AGGCGGGAAC	OPL-07	2
	39	ACCACCCACC	OPL-18	3
Eissa, et al., 2014	32	GTGATCGCAG	OPA-10	4
	36	AGGTGACCGT	OPA-18	5
	32	TGATCCCTGG	OPB-02	6
	36	CTGCTGGGAC	OPB-10	7
	36	AGATGCAGCC	OPE-06	8
	36	ACGGCGTATG	OPE-19	9

The thermal polymer device is set according to certain temperatures and time, as the PCR reactions begin with its temperature cycle (98°C) for two minutes, which is a preparatory thermal cycle for the first transcriptometer of the DNA tape, after which (35) thermal cycle begins with each cycle containing three steps and each step has a specific temperature as follows:

Step No. 1: It takes place at a temperature of (98°C) for 20 seconds and there is a dual tape dirty.

Step No. 2: It takes place at a temperature of (50°C) for 45 seconds, and there is a link to the primates of the site that complements it to the DNA tape.

Step 3: It is done at a temperature (72°C) for 2 minutes in order to start the elongation process of the principles and then a final thermal cycle gets a temperature (72°C), for five minutes in order to complete the elongation of the starter.

3. Results and Discussion

Figure 1 shows the integrity of the DNA sample extracted from the quail bird's testicle compared to the DNA Ladder used.

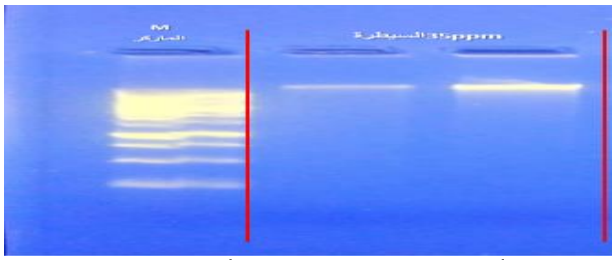

Figure 1: Shows DNA extraction gel.

This study also showed the effect of the TIO2NPs substance used in the experiment by focusing 35ppm on the DNA extracted from the quail's testicle in the treatment group compared with the DNA extracted from the control group, as illustrated in figure 2 (a, b.)

The starter (OPC-08) showed to the sample the treatment with TIO2NPs material at 35ppm three packages The first top package was pb450, the second was pb200 and the third was pb50, while his eye showed control and the same starter had three different packages: the first package up (pb 750) and the second package was (pb550), the third package down was the size (pb 440).

When the starter (OPL-07) showed three packages in the transaction sample ranged from (250pb) (150pb) and (100pb) compared to the control sample that showed two packages of the first size (pb300) and the second (pb200).

The starter (OPL-18) in the transaction sample showed one package size (200pb) while two packages appeared in the control eye and size (pb250) and size (220pb).

Between the first (OPA-10) of the transaction sample were three packages, the first (300pb) was the second (250pb) and the third (150pb), while only two packages appeared for the control sample, the first (275pb) and the second (100pb).

The starter (OPA-18) also revealed one package for both the transaction sample and the control sample, the size

(250pb) and (200pb) respectively, and two packages were observed when using the starter (OPB-02) in the first transaction sample (500pb) and the second (450pb) as well as in the first control sample (475pb) and the second package (445pb).

When using the starter (OPB-10) for the transaction sample, one package (200pb) was observed as well as one package for the control sample of the size (100pb). Also show the starter (OPE-06) for the transaction sample show one package size (250pb) and one package also for the control sample and size (100pb)

While the starter (OPE-19) appeared for the transaction sample, the first two packages (750pb) and the second (1000pb), while only one package appeared for the control eye (500pb).

The results of RAPD-PCR interactions showed a partial change in the DNA testicular sample of the titanium-treated quail at a concentration of 35PPM as in Figure 2 (a) and (b) Since this substance has the potential to partially alter DNA in a given area, it has been shown by RAPD-PCR, which is randomly designed and randomly associated in certain places, as the results of the control testicle and the transaction testicle with nano titanium have been associated with the same area but with a lower or more sequence relying on the division of marker (250-10000 pb), this shows that the DNA had mutations due to the effect of nano titanium and there was a clear difference between them.

These findings were in line with a study on the impact of nano titanium on European bass Dicentrachus labrax (Negro et al., 2015) where 1 mg/L of TIO2NPS is used individually and cadmium with 0.1 mg/L nano titanium is used for seven days The results showed that the stability of the genome mold had decreased after exposure to cadmium and titanium while only when exposure to titanium, He was solely responsible for altering chromosomes but was ineffective in terms of DNA damage, and in another vivo study (Kocco et al., 2015) The results are similar to RAPD-PCRVI indicators that the random prefix test had different results, giving seven unique packages to several members of the quail but the starter (OPE-19) Did not appear unique packages in a study on 270 chickens of quail.

A description of the genetic toxicity of TIO2NPS in Daniorerio zebrafish showed two weeks after adaptation, zebrafish populations were exposed to a concentration of 1,10 ug/L. For 7, 5, 14,21and 28 days, the assessment of TIO2NPS genetic toxicity potentials was initiated by RAPD-PCRF. The highest toxic effect was observed in the maximum concentration of nanomaterials 10 ug/L. With the rest of the three tests 14 and 21 days after exposure.

The results indicated that there are mechanisms that can reduce the genetic toxicity of TIO2NPS, as future studies are necessary to analyze the ability to repair DNA in cells and modify the response to nano titanium exposure.

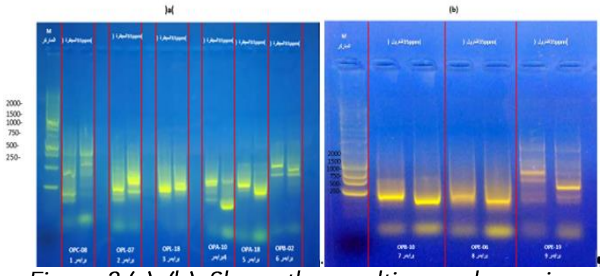

Figure 2 (a), (b): Shows the resulting packages in electric relay of random prefixes

4. Conclusions

Nanoparticles can be deduced from nanotyanium, when taken with 35ppm concentration they have a detrimental effect on DNA and have the potential to effect a change in its genetic composition.

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