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Histological and Biochemical Parameters Follow-up in Experimental rats Administrated *dexamethasone* and Treated with Green Synthesis Titanium Dioxide Nanoparticles Using (*Camillia sciences*) Extracts

Amal K. Abbas¹

Nada K. Abbas^{2*}

Ruaa M. Ali²

Lamia K. Abbas³

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Abstract:

In this study, Titanium Dioxide Nanoparticles were synthesized by an easy and eco-friendly technique (green synthesis) using green tea leaves (*Camillia sinensis*), Nanoparticles were analyzed using structural and optical analysis, the X-ray pattern showed that Titanium Dioxide NPs had a tetragonal structure with (Face Centered Tetragonal) FCT crystal structure, the UV-visible recorded an absorbance peak near 350 nm and calculated energy band gap was 3.5 eV, all measurements were proved the purity and Nano size of prepared Nanoparticles. Biochemical parameters evaluation also mentioned in this research, these analyzes showed that Titanium Dioxide nanoparticles in particular dose (50 mg/kg) have the ability to reduce blood glucose levels, improve liver functions and recover lipid levels in the animals body, and lastly, histological pancreatic parts were submitted to observe changes that occurred after dexamethasone and nanoparticles were injected.

Key words: Dexamethasone, Lipids, Liver enzymes, Titanium Dioxide Nanoparticles(TiO₂ NPs), X-ray diffraction (XRD).

Introduction:

There are various synthetic methods have been reported by researchers to synthesize nanoparticles mainly divided into three classes, physical, chemical and biological methods (1). In the conventional synthetic method, both physical and chemical often required reducing agent and stabilizing agent that are unfriendly and highly toxic to the surrounding environment. Besides, these methods are also costly, high energy consumption and the high possibility for the formation of toxic products (2). Nanoparticles appear high improvement in physical and chemical properties compared with bulk, such as energy gap (3). Varieties of living organisms are used as "biofactories" for the synthesis of different forms of nanoparticles. Plants give a specific option for the synthesis of nanoparticles among biological systems (4).

The use of environmentally benign material like plant extract (leave, flower, bark, seed, peels etc.), fungi, bacteria, and enzyme for the synthesis of nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemical for the synthesis protocol (5). Titanium dioxide (TiO₂) is an inert, nontoxic and inexpensive material whose high refractive index and high capability to absorb UV light make it an interesting white pigment and environmentally friendly catalyst and can be absorbed into the body by inhalation, ingestion and dermal penetration due to their small size (6), TiO₂ nanoparticles also can penetrate the liver cell (7).

Dexamethasone is an artificial corticosteroid similar to steroid hormone cortisol. Large quantities of corticosteroids administered for a long time to animals that cause infections such as diabetes (8), Dexamethasone has many side effects including the potential to increase blood glucose levels postoperatively, which is a known risk factor for complications after total joint arthroplasty (9). The use of plant extract is preferable as they are easily available; save to handle and possess a broad viability of metabolites, the choice of plant extracts to produce NPs is based on the added value of the

¹ Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

² Department of Physics, College of Science for Women, University of Baghdad, Baghdad, Iraq.

³ Department of Physics, College of Science, University of Baghdad, Baghdad, Iraq.

*Corresponding author: nadabbs@yahoo.com

*ORCID ID: <https://orcid.org/0000-0001-8573-4174>

biological material itself (10), biosynthetic procedures can provide Nanoparticles of a better size and morphology than several physicochemical preparation processes. Plant extracts can work as a reducing agent and as stabilizing agents in the synthesis of Nanoparticles (11, 12).

This study aims to prepare titanium dioxide nanoparticles and estimates its activity on the liver functions and lipid profile for experimental rats administrated dexamethasone drug.

Materials and Methods:

One mL of Deionized Water (DW) was mixed with Titanium Dioxide bulk particles (400-800 nm) (0.06M), then they placed on hot plate magnetic stirrer at 50 °C and 1500 rpm for few minutes. After that, (20 mL) of previously prepared green tea extract added to the solution gradually and allowed to cool for room temperature, the solution was centrifuged at 4000 rpm for 15 min. The precipitate formed was washed with DW many times and then centrifuged at 4000 rpm for 10 min. The obtained precipitate was dried at air oven for 2h at 60°C.

Experimental animals:

Forty eight Male adult Wister albino rats weighing (175-225g) and 10-14 weeks old housed in cages made from polypropylene under controlled conditions 25±5 °C and 12/12 hrs light/dark cycles, drinking water and Diet food were given *ad libitum*, the animals were reared and treated in University of Baghdad /animal house in college of science,.

Experimental animals had randomly divided into six groups, each group contained eight rats (n=8) treated for 30 days, and intended as follows: G1 the first group was served as negative control, G2 the second group was injected intraperitoneally with (0.5 mg/kg) dexamethasone sodium phosphate, G3 and G4 were injected intraperitoneally with (50 and 100) mg/kg TiO₂ Nanoparticles (13) and (0.5 mg/kg) of dexamethasone respectively, G5 was injected intraperitoneally with 50 mg/kg TiO₂ Nanoparticles and G6 injected intraperitoneally with 100 mg/kg TiO₂ Nanoparticles.

The Laboratory animals have fasted 24 hrs before dexamethasone injection; they were weighed and anesthetized by inhalation in a glass dome.

Biochemical analysis, statistical analysis and histological Examinations

When experience period end; blood samples collected from animals by heart puncture way, the blood was centrifuged at 4000 rpm for 10 min, serum was separated for test of several biochemical parameters such as blood glucose level (BGL) measured by commercially kit (Biosystem, Germany), cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Triglyceride (TG) and Low Density Lipoprotein Cholesterol (LDL-C) estimated using a commercially available kit (Linear kit, Spain), serum levels Alanine Aminotransferase (ALT), Aspartate Amino transferase (AST), Alkaline Phosphate (ALP) and Total Bilirubin (TB) were assessed using commercially available kit (Reflotron kit, Germany). Results were stated as means ± standard Errors. The Statistical Analysis System applied using one way (ANOVA) analysis of variance to verify the Least Significant Difference (LSD), All figures were drawn by the Statistical Package of Prism software 8.1.2 (Version 8.1.2, GraphPad, San Diego, CA, USA). The Probability (P) value ≤0.05 has been considered to be of statistical significance.

Pancreatic Samples were collected from animals after organs separation and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70%), cleared in xylene, and embedded in paraffin. 5 µm thick paraffin sections were prepared and then regularly stained with Hematoxylin and Eosin (H&E) (14) and then examined microscopically.

Results and Discussion:

Nano-powder structural characterization was recorded by Pro Penalty CAL with Cu-Kα radiation (1.5406 Å) XRD models, Tab.1, and Fig.1 illustrate X-ray diffraction (XRD) pattern of as synthesized TiO₂ Nanoparticles and standard X-ray pattern of TiO₂. Peaks obtained at 2θ values from 25.32° to 76.11° indicated tetragonal structure with FCT crystal structure of anatase TiO₂ nanoparticles (JCPDS No. 21-1272), The average crystallite size of TiO₂ was 11.82 nm calculated using Scherer relation (15) ($C.S = (0.9 \lambda) / (\beta \cos \Theta)$) where C.S is Crystallite Size measured in nanometers, β Full Width at Half Maximum measured in radian, λ wavelength of x ray measured in nanometers. And the lattice parameters of the pure TiO₂ nanoparticle is (a=b=3.79 Å, c =9.52Å) which equivalents to the lattice parameter of bulk TiO₂ (a=b=3.7850 Å, c=9.5196Å), broadening (Enlargement) of the peaks is due to the crystalline size of sample is very small, the obtained values is agreed with (16).

Table 1. The structural parameters of the as synthesized TiO₂ Nanoparticles

2θ (Deg.)	C.S (nm)	FWHM (Deg.)	d _{hkl} Exp. (Å)	d _{hkl} standard (Å)	(hkl)
25.32	9.2	0.877	3.517	3.585	(011)
36.97	5.7	1.398	2.431	2.427	(013)
37.80	39.8	0.200	2.379	2.376	(004)
38.59	5.7	1.398	2.332	2.3345	(112)
48.08	5.79	1.372	1.892	1.842	(020)
53.91	7.33	1.086	1.700	1.680	(015)
55.11	7.25	1.100	1.666	1.664	(121)
62.99	9.9	0.808	1.481	1.474	(024)
68.76	7.25	0.100	1.364	1.364	(116)
70.56	15.96	0.498	1.338	1.333	(220)
74.05	17.65	0.450	1.279	1.279	(017)
75.10	6.96	1.142	1.265	1.274	(125)
76.11	15.28	0.520	1.250	1.246	(031)

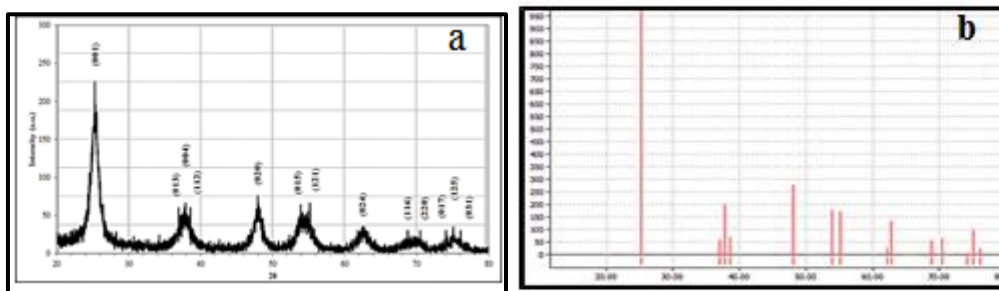


Figure 1. XRD pattern of the (a) as synthesized TiO₂ Nanoparticles (b) standard XRD peaks positions pattern of TiO₂

The optical behavior measured by VARIAN spectrophotometer (Cary 5000 Scan), absorbance and optical energy bandgap have been recorded for as-synthesized Titanium Dioxide Nanoparticles solution, Fig. 2 shows the maximum absorption depends on the size of the nanoparticles; calculated bandgap from Planck's relation (17) ($\Delta E = hc/\lambda$) was 3.5eV at wavelength Maximum 350nm corresponds to absorption peak, Where, 'h' represented the Planks constant, 'c' denoted speed of light and λ represented the maximum wavelength, From the current results, the present energy bandgap was shifted towards blue region spectra when this value compared to energy gap of bulk which may happened by the quantum confinement effect, and the Maximum peak is represent Surface Plasmon Resonance SPR.

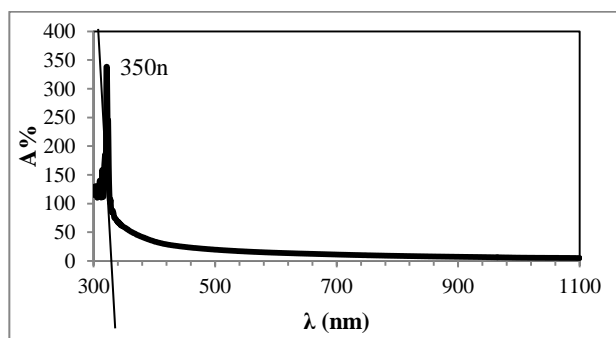


Figure 2. Absorption Spectrum of TiO₂ Nanoparticles

The biochemical results presented a significant differences between control and treated groups, Table 2 and Fig 3a indicated a significant increase ($P \leq 0.05$) of blood glucose level (BGL) in group which treated by dexamethasone (G2) compared with negative control group (G1), these results agreed with the previous studies (18) in which reported that dexamethasone caused tissue damage in pancreas which leads to decrease insulin secretion from Langerhans cells, oxidative stress is the reason of creation ROS, The generated ROS or free radicals is damage cellular membranes, proteins and nucleic acids. Chronic hyperglycemia is toxic to the body because of the auto oxidation of glucose that produces strong ROS hydrogen peroxide (H₂O₂) (19).

Nanoparticles treatment showed significant ($P \leq 0.05$) BGL decreases for 50 mg/kg, this reduction may be due to the ability of nanoparticles to delaying or inhibiting the absorption of glucose in the intestines (20), Stimulate insulin secretion from pancreas cells (21) the second dose of TiO₂ nanoparticles (G4) 100 mg /kg, wasn't significant because the high dose of TiO₂ Nanoparticles may be cause cytotoxicity that affecting cellular functions such as cell proliferation, differentiation and mobility resulting in apoptosis (22). (G5 & G6) which injected by nanoparticles were found to be near normal BGL range.

Table 2. Changes of Serum biochemical parameters in treated rats with TiO₂ Nanoparticles after 30 days, Each value represents the mean ± standard error of (n=8), The values found to be significant at P ≤ 0.05, (*P<0.02, **P<0.001, *P<0.0008, ****P<0.0001)**

Parameters	Means± SE						
	Groups	G1	G2	G3	G4	G5	G6
BGL (mg/dL)		113.2±6.4	395.2±29.7	167.7±2.9**	302.7±7.7	123.5±2.5	161.2±5.4**
ALT(IU/L)		35.7±1.6	66.2±4.7	40.2±1.3**	47±2.1	38.2±1.3	49±3.6
AST(IU/L)		27.2±4.4	48.2±3.5	35±1.6*	45.7±2.2	35±1.9	42±0.7*
ALP(IU/L)		151±17.8	383.2±30.7	222.2±5.8**	346.5±2.3	194±3.5	2315±24.6
TB(mg/dL)		0.33±0.01	1.42±0.08	0.61±0.04**	1.2±0.1	0.63±0.02**	0.93±0.03***
TC(mg/dL)		178.5±12.5	255±5.4	181.7±4.5**	213.5±5.7*	191±2.6	197.7±0.8
TG(mg/dL)		152.2±10	231.7±14.8	161.7±4.6*	195.2±2.5	161.7±20	177.7±2.8
HDL-C(mg/dL)		45±1.6	26±3	42±2*	29.7±2.1	43.2±1.3	38.2±0.8*
LDL-C(mg/dL)		91.2±8.1	172±13.1	110±2.1*	166.5±2.5	138.7±1.6*	146.2±4.1*

Table 2 and Figs. 3b, c, d and e, displayed increases in liver enzymes and TB in G2 which injected by 0.5 mg/kg dexamethasone, these raises recorded because of enzymes leakage from cytosol into the bloodstream because of hepatic damage associated with dexamethasone, ALT and AST levels increased is associated with insulin deficiency has been interrelated to increased gluconeogenesis (23).

After treating with 50 mg/kg these levels were decreases and their reduction were statically significant (P≤0.05) in G3, while G4 that treated with 100 mg/kg had no significant effects, G5 and G6 had normal levels. As shown in Table. 2 and Figs 3f, g, h and i; (G2) had TC increase attributed increasing the activity of (Lecithin-cholesterol acyltransferase) that responsible of cholesterol Absorption in intestines which stimulates in the

absence of insulin (24). Treatment with TiO₂ nanoparticles presented significant TC decrease (P≤ 0.05) in G3 and G4 compared with control group, the administration of high dose of TiO₂ NPs may deposit in the liver and leads to hepatic damage conformed by the fluctuations in hepatic marker.

This study also showed that dexamethasone was resulted an increases of TG and LDL-C compared to its level in G1, while there was a significant decrease (P≤ 0.05) when groups treated with nanoparticles this reduction may be occurs due to activation of lipase enzyme in the adipose tissue by nanoparticles, which acts to dissolve triglycerides into fatty acids that are absorbed by the cells. HDL-C was decreased after dexamethasone injection in G2 and there was a significant increase (P≤ 0.05) of its level in G3, these results are similar to (25) results.

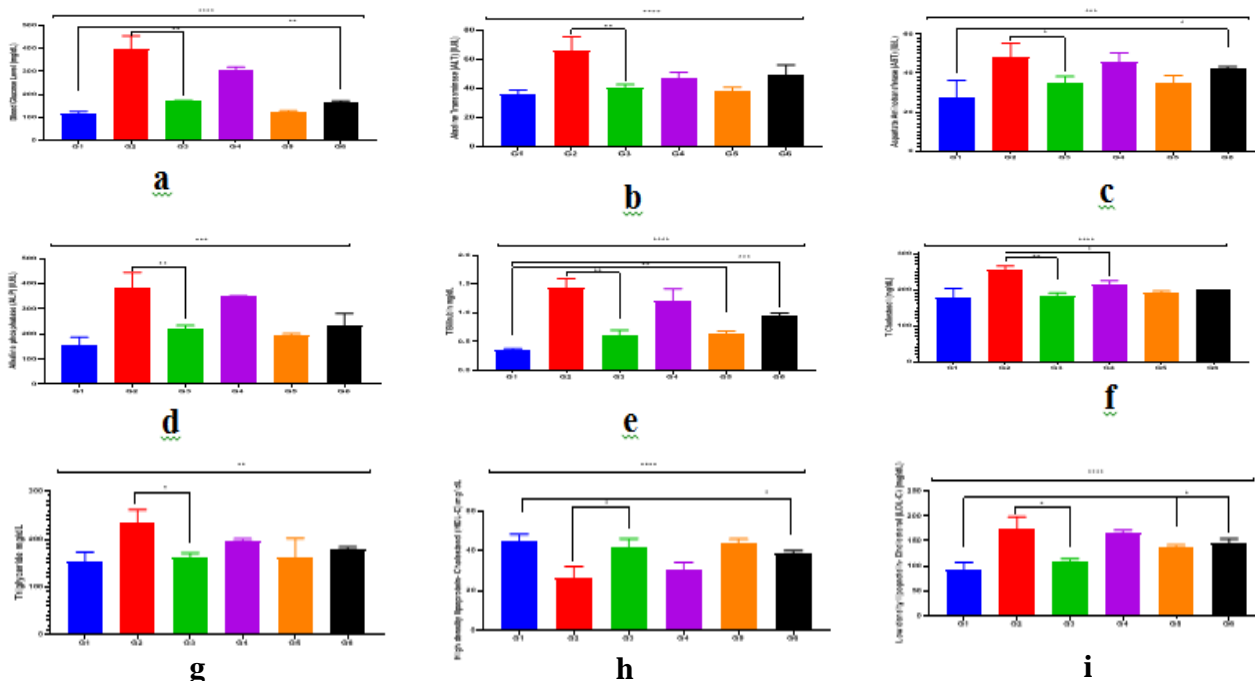


Figure 3. (a) Blood glucose mg/dL (b) Alanine Transaminase (c) Aspartate Aminotransferase (d) Alkaline Phosphatase (IU/L) (e) Total bilirubin (f) Cholesterol (g) Triglycerides (h) High density lipoprotein-cholesterol (i) Low density lipoprotein-cholesterol (mg/dL) for studied groups

Histological examination

The cells of pancreas in negative control rats were packed closely. When dexamethasone administrated in group two; the density of cells was significantly reduced. In case of Titanium Dioxide NPs treated in group three; it was observed that the spaces in cells produced by the dexamethasone induced necrosis is decreased, similar effects of alloxan on pancreatic cells were observed in previous studies (26).

The histological sections showed different alterations of rat's pancreas many histological changes were absorbed in pancreas section of (G2) that administrated by dexamethasone, such as necrosis and damage of endocrine cells of islet Langerhans with shrinkage of islets as in Figs.4 a, b and c, Dexamethasone induced necrosis is mediated by reactive oxygen species mediated lipid

peroxidation which causes bursting of plasma membrane of the cell and disturbance of osmotic balance this osmotic alteration ultimately leads the cell towards necrosis.

Also the histological section shows different change in pancreas of rats that administrated with dexamethasone and TiO₂ Nanoparticles (G3), the healing effect of Nanoparticles in Fig.5a photomicrograph it is observed that the empty spaces in islets created by dexamethasone significantly decreased; this indicates that Nanoparticles stimulates the regeneration of the damaged islets, Fig. 5b presented more damage and necrosis of endocrine cells and islets in comparison with dexamethasone treated animals, while Figs. 5c and d, shows that Nanoparticles kept normal shape of pancreatic cells and they look like normal.

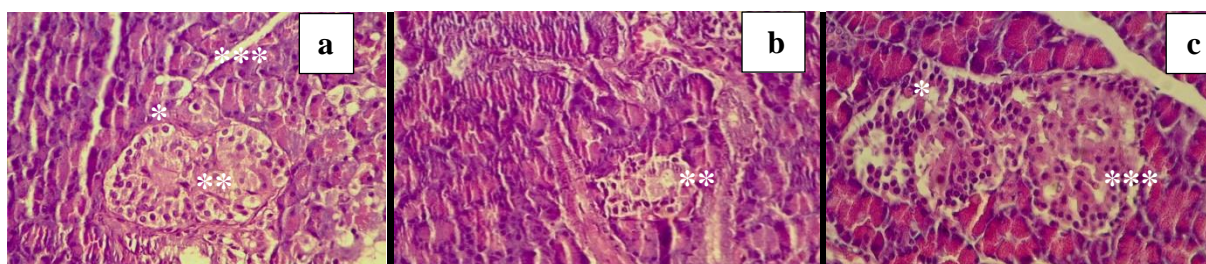


Figure 4. Cross sections of histological features of rat pancreas: (a) Photomicrograph section in the rat pancreas for Control group (G1), pancreatic lobules containing acini(*), islets of Langerhans (**), and unstained connective tissue septa (***).(b and c) Photomicrograph section of rats pancreas (0.5 mg/kg) dexamethasone (G2), showing damage and necrosis of endocrine cells of islet (*), Langerhans with shrinkage of islet (**), degenerated acini cells and islet of Langerhans (***) X400 (H&E)

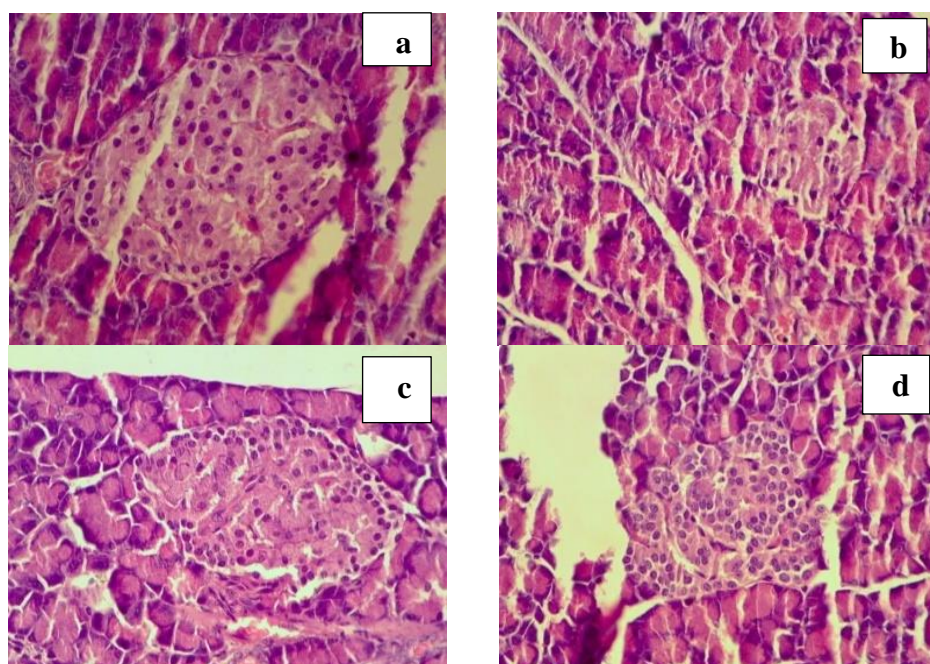


Figure 5. Cross section of pancreas cells (a) G3 (b) G4 (c) G5 (d) G6 X400 (H&E).

Conclusion:

This paper contains green synthesis of biocompatible Titanium Dioxide Nanoparticles by simple green procedure using green tea extract (*Camellia Sinensis*). The formation of nanoparticles was examined by applying structural and optical measurements, (50 mg/ kg) of TiO₂ nanoparticles provided a significant reduction against BGL after dexamethasone injection, using nanoparticles improved Liver enzymes, Bilirubin and lipids profile levels to confirm this results the histological staining of pancreatic section has performed, the other dose of titanium Dioxide NPs 100 mg/kg, didn't show activity on pancreas islets and it had no effect on liver function and body lipids.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

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متابعة بعض العوامل الكيموحيوية والنسجية في الجرذان المختبرية المعاملة بمادة الديكساميثازون والمعالجة بمادة ثنائي اوكسيد التيتانيوم النانوية المصنعة بطريقة التوليف الاخضر باستخدام مستخلص الشاي الاخضر

لمياء خضير عباس³

روى مثنى علي²

ندى خضير عباس²

امال خضير عباس¹

¹ قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.
² قسم الفيزياء، كلية العلوم للبنات، جامعه بغداد، بغداد، العراق
³ قسم الفيزياء، كلية العلوم، جامعه بغداد، بغداد، العراق.

الخلاصة:

في هذه الدراسة تم تحضير ثنائي اوكسيد التيتانيوم النانوي عن طريق تقنية سهلة وصديقة للبيئة هي طريقة التوليف الخضراء باستخدام اوراق الشاي الاخضر، وتمت دراسة خصائص هذه المادة عن طريق الفحوصات التركيبية والبصرية، حيث اظهرت نتائج حيود الاشعة السينية ان النظام البلوري لثنائي اوكسيد التيتانيوم هو رباعي وله شبكية مكعبية متمركزة الوجة، اما تحليلات الاشعة فوق البنفسجية- المرئية فقد اظهرت ان اعلى قمة لامتصاص كانت قرب 321 نانومتر وقيمة فجوة الطاقة لها التي تم حسابها عن طريق قانون بلانك كانت 3.8 إلكترون فولت، جميع القياسات اكدت الحجم النانوي ونقاوة المادة المحضرة. المعاملات الكيموحيوية اظهرت ان مادة ثنائي اوكسيد التيتانيوم النانوية ذات التركيز 50 ملغ/كغم كانت لها القابلية على تقليل مستوى السكر في الدم، التحسين من وظائف الكبد واسترجاع القيم الطبيعية للدهون في اجسام الحيوانات المختبرية المحقونة بمادة الديكساميثازون، واخيرا اظهرت المقاطع النسيجية للبنكرياس ان هناك تغيرات حصلت بعد حقن الديكساميثازون ومن ثم المعالجة بالمادة النانوية.

الكلمات المفتاحية: ديكساميثازون، الدهون، أنزيمات الكبد، جسيمات TiO₂ النانوية، XRD.