

# Histological Sections of Pancreas and Serum Biochemical Changes in Rats after *Dexamethasone* and Zinc Oxide Nanoparticles Injection

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## Abstract

Zinc Oxide Nanoparticles have been prepared using a simple green synthesis method by green tea extract (*Camellia sinensis*) as a reducing and capping agent, XRD measurement of Nano powder stated that Zinc Oxide had a hexagonal wurtzite structure, UV-Vis. The maximum absorption peak was approximately 318 nm and the energy gap was nearly 3.8eV calculated using Planck's equation, blood serum sugar levels, liver function after ZnO NPs injection for rats was recorded. Forty-eight adult male rats were used and separated randomly into six groups of eight (n=8) rats in each group. Results stated that blood glucose levels, liver enzymes were increased in groups that injected by dexamethasone while considerably modified in rats administered by the first dose (100 mg/kg) of ZnO Nanoparticles while the other dose (300 mg/kg) hadn't any significant effects.

**Keywords:** *Dexamethasone, XRD, Zinc Oxide NPs, AST, HDL-C*

## Introduction

recently, a strategic development has been frequently implemented in the context of a novel green synthesis technique that forms biocompatible nanoparticles because of the possibility of developing bio-nanomaterials with particular biological functionalities<sup>(1)</sup>. Today, non-toxic, environmentally friendly techniques have gained more importance because of their ability to generate a wide variety of nanomaterials to cover better suite biomedical applications<sup>(2)</sup> most of these apps are due to the fact that the Nanometer scale has considerably unique characteristics compared to the bulk. Nanoparticles have good surface bonding, The large surface-volume ratio in nanoparticles is the primary reason for a structural deformation that can influence the physical characteristics of nanoparticles<sup>(3)</sup> In particular, the synthesis of zinc oxide nanoparticles has gained considerable interest due to the fact that it has excellent characteristics for biomedical apps, zinc<sup>(4)</sup> plays a significant part in the preservation of blood sugar and activate more than three hundred of body enzymes.<sup>(5)</sup>, it is also known to maintain the structure of insulin and has an important roles in insulin biosynthesis, storage

and secretion<sup>(6)</sup>. There are several zinc transporters in pancreatic  $\beta$ - cells<sup>(7)</sup>. This report intended to investigate the ability of prepared Zinc Oxide by green synthesis to recover blood glucose, liver enzymes and lipids in rat's body after dexamethasone administration.

## Materials and Method

Zinc Acetate Dehydrate ( $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ ) (0.2 M) is mixed with 70ml of Deionized Water using a magnetic stirrer, stirring 1500 rpm a few minutes. Then 20 ml of the previously prepared green tea leaf extract was obtained from the stock solution (stored in the refrigerator) and gradually added. Now the blend was stirred continuously for 2 hours at 40°C. The solution was then cooled at room temperature and centrifuged twice at 4000 rpm for 15 min after a thorough washing and then dried at 60°C for 3 hours, lastly the produced ZnO nano-powder pale white colored using for further studies.

## Experimental Protocols and Grouping

(Forty-eight) Male adult Wister albino rats weighing (175-225 g) and (10-14) weeks old housed in cages

made of polypropylene under controlled conditions ( $25\pm 5$ ) ° C and 12/12 hrs light / dark cycles, drinking water and diet food were provided ad libitum, animals were grown and housed at the University of Baghdad / animal house in the college of science. The experimental animals were randomly grouped into six groups, each of which consisted of eight rats ( $n=8$ ) treated for 30 days and designed 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 (100 and 300) mg/kg ZnO NPs<sup>(8)</sup> and (0.5 mg/kg) of dexamethasone respectively, G5 was injected with 100 mg/kg ZnO NPs and G6 injected with 300 mg/kg ZnO NPs. The Laboratory animals were fasted 24 hrs before dexamethasone injection; they were weighed and anesthetized by inhalation in a glass dome.

### Biochemical assessment & Statistical Analysis

When the experiment period end; blood samples collected from animals by heart puncture, the blood 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 an available kit (Linear kit, Spain), serum levels Alanine Amino transferase (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  $\pm$  standard Errors. The

Statistical Analysis System applied one way (ANOVA) analysis of variance to verify (LSD) using statistical package of Prism software 8.1.2 (Version 8.1.2, GraphPad, San Diego, CA, USA). The Probability (P) value  $\leq 0.05$  has been considered to be of statistical significance. When pancreatic samples 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 micron thick paraffin sections were prepared and then regularly stained with Hematoxylin and Eosin (H&E)<sup>(9)</sup> and examined microscopically.

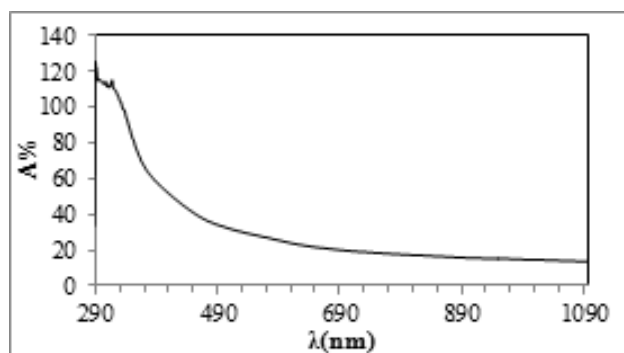
### Results and Discussion

Structural characterization of Nano-powder was recorded by Pro Penalty CAL with Cu-K $\alpha$  radiation (1.5406 Å). X-ray diffraction was indicating the structure and crystalline nature of as synthesized ZnO Nanoparticles. Results show all diffraction peaks are in good agreement with the international crystallographic data table; JCPDS card No. 01-075-0576. No impurities peaks can associate to the XRD spectrum of the gotten powder, showing high purity of ZnO products and the peaks intense of the samples proof to good crystallinity. The broadening of the diffraction peaks clearly indicates the presence of Nano-size, large broadening in X-ray diffraction lines occur when particle size is less than 100 nm, The peak width at half height using the Debye-Scherrer formula<sup>(10)</sup> gives indication for average crystallite size of green synthesized ZnO NPs, The calculated average crystallite size was 9.27 nm, result of X ray pattern agreed with<sup>(11)(12)</sup>.

**Table (1): The structural parameters of as synthesized ZnO Nanoparticles**

2 $\theta$ (Deg.)	G.S (nm)	FWHM (Deg.)	dhkl Exp.(Å)	dhkl standard (Å)	(hkl)
30.81	14.7	0.539	2.877	2.902	(100)
34.32	8.4	0.941	2.593	2.613	(002)
35.3	9	0.883	2.523	2.537	(101)
46.7	5.33	1.497	1.943	1.941	(102)
57.97	5.54	1.470	1.592	1.595	(111)
62.15	5.25	1.516	1.492	1.493	(013)
66.93	10.3	0.773	1.396	1.398	(201)
76.5	15.7	0.510	1.243	1.268	(202)

The optical behavior recorded by VARIAN spectrophotometer (Cary 5000 Scan), to explain the optical properties and energy gap value of ZnO NPs; UV-Vis absorption spectrum has been studied. Fig. (1) Shows the exciton maximum absorption is about 318 nm. Actually, energy absorbed in UV or visible region causes a change in the electronic excitation of the molecule, and hence results in corresponding change in its ability to absorb light in the UV-visible region of the electromagnetic radiation. The optical energy band gap for ZnO NPs was 3.8 eV has been calculated using Planck's relationship<sup>(13)</sup>.



**Figure (1): Absorption Spectrum of ZnO nanoparticles**

Table (2) shows blood glucose of the study groups, mean blood glucose level (BGL) of **G2** after 30 day administration of dexamethasone showed a significant

increasing compared with control group, the reasons behind increasing glucose is generated oxidative stress by sugar causes the generation of free radicals that attack and damage beta cells, thus inhibiting the formation and secretion of insulin, and then stops glucose metabolism and glycogen decomposition, Oxidative stress is involved in creation of ROS that damage cellular membranes, proteins and nucleic acids, the auto oxidation of glucose that produces hydrogen peroxide ( $H_2O_2$ ), a strong ROS<sup>(14)</sup>, these results similar to previous study<sup>(15)</sup> that reported the dexamethasone cause tissue damage in pancreas.

Treatment with 100 mg/kg of ZnO NPs were responsible of significant reduction to BGL ( $P \leq 0.05$ ) may be due to that Zinc has been elucidated to be a potent metal that Improves glucose utilization and metabolism through its potent influence on enhancement of hepatic glycogenesis through actions on the insulin signalling pathway<sup>(16)</sup>, interestingly, on the basis of Umrani and Paknikar<sup>(17)</sup>; ZnO did not possess the risk of hypoglycemia in living organisms so it can act as an insulin secretion, more researches have the same results revealed that ZnO NPs could increase serum insulin level in diabetic animals<sup>(18)</sup>.

**Table (2): Changes of Serum biochemical parameters in treated rats with CeO<sub>2</sub> Nanoparticles after 30 days, Each value represents the mean± standard error of (n=8), The values were found to be statistically significant at  $P \leq 0.05$ , (\* $P < 0.01$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0008$ , \*\*\*\* $P < 0.0001$ )**

Groups Parameters	Means± SE					
	G1	G2	G3	G4	G5	G6
BGL (mg/dL)	113.2±6.4	395.2±29.7	130.2±18.9**	236.7±5.1*	110.25±0.8	121.25±6.4
ALT(IU/L)	35.7±1.6	66.2±4.7	33.7±1.6*	46.5±0.6	41.2±0.6	43.7±1.6*
AST(IU/L)	27.2±4.4	48.2±3.5	34.5±2.3*	58±3	31±1.6	38.2±1.2
ALP(IU/L)	151±17.8	383.2±30.7	124±7.5**	317.5±8.5	159.5±12.7	238.2±20.5*
TB(mg/dL)	0.33±0.01	1.42±0.08	0.54±0.02**	1.27±0.04	0.45±0.06	0.62±0.1
TC(mg/dL)	178.5±12.5	255±5.4	139.7±13.8**	232.7±5.9*	164±4.2	173.2±5.5
TG(mg/dL)	152.2±10	231.7±14.8	170.5±2.2*	203.7±3.8	157±1.9	165.7±2.9
HDL-C(mg/dL)	45±1.6	26±3	46.2±2.3*	29±1.4	40.2±2.2	35±1.7
LDL-C(mg/dL)	91.2±8.1	172±13.1	115.2±3.9*	163.5±5.1	101.7±2.4	113.7±2.4

ALT and AST are very sensitive enzymes and have been closely connected with hepatic damage these levels enzymes will rise when liver is in dysfunction (19) (20), Maiti et al. (21) have suggested that increases in the actions of ALT, AST, and ALP in the serum of diabetic rats as a consequence of hepatic damage, ALT and AST levels increased is associated with insulin deficiency has been interrelated to increased gluconeogenesis during hyperglycemia (22).

The results showed there was a significant increase in liver functions parameters such as ALT, AST and ALP in experimental animals compared with negative control group (G1) table (2), increases of liver functions parameters levels possibly due to hepatic damage and increase synthesis of these enzymes in the liver (23), and a significant decrease ( $P \leq 0.05$ ) with liver enzymes when animals treated with (100 mg/kg) of ZnO NPs while there was no statistically significant effect of 300 mg/kg dose for infected rats respect to G2. Increases of Bilirubin levels may be attributed to the changes in cell membrane permeability with change of effective stress of the membrane under the oxidative stress (24), ZnO nanoparticles have reduced Bilirubin levels in G3; G5 and G6 were nearly the normal ranges.

G2 had a significant increase in cholesterol; this increasing attributed to high exposure of free radicals which stimulate the rate limiting enzyme hydroxyl-methyl glutaryl which is responsible for liver synthesis (25). When these animals treated with 100 mg/kg of ZnO NPs, a significant recovery in cholesterol levels occurred. Higher levels of Triglycerides (TG), LDL-C and reduction in HDL-C levels in G2 is possibly due to a direct consequences of conversion white adipocytes to

brown adipocytes (26), or may be attributed to decrease the activity of lipoprotein lipase which is responsible for TG removal. However, triglyceride and LDL-C levels ( $P \leq 0.05$ ) decreased was evident. With the exception of raises of serum HDL level.

Histological section in (Fig. 2a) shows pancreatic lobules containing acini (\*), islets of Langerhans (\*\*\*) and unstained connective tissue septa (\*\*\*) for control group. Fig. (2b and c) showed damage and necrosis of endocrine cells of islet (\*), Langerhans with shrinkage of islet (\*\*), degenerated acini cells and islet of Langerhans (\*\*\*) and presented different alterations of rat's pancreas and many histological changes were detected in pancreas section of (G2) that administrated by dexamethasone, such as necrosis and damage of endocrine cells of islet Langerhans with shrinkage of islets, 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. The histological sections showed several modified in rats pancreas of rats in groups that treated with 50 mg/kg of ZnO Nanoparticles (G3), whereas group that treated with (100 mg/kg) (G4) had no clear changes after dexamethasone and nanoparticles injection, in fig. (3a) the section shows animal from G3 had a normal histological structure of islets of Langerhans of pancreas which consist of  $\alpha$  and  $\beta$  cells, in between capillaries blood vessels, while fig. (3b) section shows no effects of Nanoparticles on pancreatic cells, abundant necrosis, degenerated acini and damage of endocrine cells, G5 and G6 in fig. (3c and d) appears look like normal pancreatic structure.

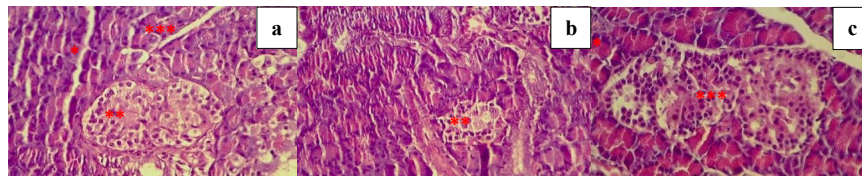


Figure (2): Cross sections of pancreas (a) Control group G1, (b and c) G2 X400 (H&E)

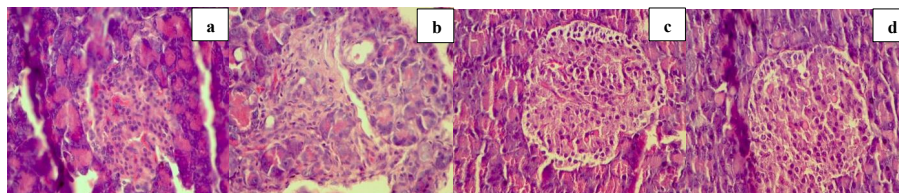


Figure (3): Cross section of pancreas (a) G3 (b) G4 (c) G5 (d) G6 X400 (H&E).

## Conclusions

Biocompatible nanoparticle synthesis was produced by using green tea extract (*Camellia Sinensis*) method for the preparation of Zinc Oxide nanoparticles. Successful formation of nanoparticles by examining its structural and optical properties was investigated. Results indicated that blood glucose concentrations, liver enzymes and lipids were enhanced in dexamethasone-injected groups, while significantly altered in rats administered 100 mg / kg dose of ZnO NPs and the other dose (300 mg / kg) did not significantly improve BGL, liver enzymes and lipids.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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