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STUDY OF ZNO NANOPARTICLES ON THYROID HORMONES, TESTOSTERONE LEVEL AND TESTES HISTOLOGY

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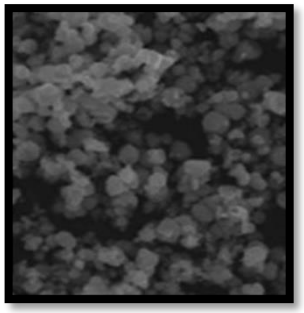
Original Research Article

ABSTRACT

This study examines hormone secretions in a rabbit model to identify the effects of oral administration of ZnO nanoparticles on the thyroid gland and testes. For this purpose, thirty healthy rabbits were acclimatized and divided into control, experimental group 1 (EG1) and 2 (EG2). These groups were examined for 50mg/Kg and 75mg/Kg nanoparticle dosages. Experimental groups lose weight, while control doesn't. Both groups had non-significantly lower Thyroid-stimulating hormone (TSH), triiodothyronine (T3) and Thyroxine (T4) levels. EG1 and EG2 had considerably lower testosterone levels on the 20th and 10th days of trials, respectively. In the experimental group, nanoparticles caused seminiferous tubule degeneration and germinal epithelium sloughing or depletion. Histology was normal in the control group. This present study provides a glimpse into the toxicity of nanoparticles; a more in-depth analysis using various animal models is needed.



Keywords: ZnO nanoparticles, thyroid-stimulating hormone, triiodothyronine, thyroxine, histology.

Introduction:

ZnO nanoparticles are multifunctional inorganic materials with unique chemical and physical properties [1]. It is the most commonly used nanomaterial [2]. The application of zinc oxide nanoparticles in different areas of science and technology shows that it is an indispensable substance but is equally important to animals and humans due to long time exposure to higher

concentrations that may be harmful to the living systems [3]. Since these nanoparticles have good antibacterial activities, solubility and chemical stability, their application in packing, agriculture, and biomedicine has increased [4].

ZnO nanoparticles are incorporated in industrial products and there is much concern about their eco-toxicological impact [5]. ZnO nanoparticles are hexagonal, spherical, cuboidal, or cylindrical [6]. Various research projects have been carried out to explore the hazardous effects of these nanoparticles. Nanoparticles, in vitro toxicity is due to their accumulation in a cell leading to oxidative stress, inflammation and ultimately, cell death [7]. It has been reported that the gastrointestinal tract is the main route of nanoparticles. These particles may be ingested through diet container dissolution or inhaled [8]. ZnO nanoparticles, when administered orally, enter the gastrointestinal tract. In the small intestine, these are absorbed in the blood and are distributed by systemic circulation to various organs. Since these are very small in size, these nanoparticles can easily penetrate the cell membranes [9].

Many investigations have revealed that thyroid hormones have an important role in cell differentiation and growth metabolism [10]. Thyroid-stimulating hormone (TSH) affects the metabolism of testosterone, where decreased testosterone levels lead to diminished sexuality [11]. For normal gonad development and spermatogenesis, the cells in the testes, like Leydig and Sertoli cells, are very important [12]. The aim and objective of the present study were to investigate the changes in serum levels of TSH, T3, T4 and testosterone in rabbits that were given ZnO nanoparticles orally. Along with the hormonal studies, histological changes in the testes of rabbits were also observed.

Materials and Method:

The study was carried out to find out the effects of oral administration of ZnO nanoparticles on serum levels of Thyroid stimulating hormone (TSH), Triiodothyronine (T3), Thyroxine (T4) and testosterone. The histological effects on testes were also studied. This study was conducted at the Physiology Lab of the Zoology Department, Lahore College for Women University.

Experimental Animals:

Thirty adult male rabbits were purchased from Tollinton Market Lahore and then transferred to the animal house of the Zoology Department, Lahore College for Women University Lahore. The weight was approximately 1 kg to 1.5 kg.

Acclimatization:

All animals were allowed to acclimatize to the laboratory conditions for seven days before starting the experiment. They were kept in cleanly separated steel cages with metal network covers under the climate-controlled condition of the animal house with an optimum temperature. They were exposed to 12- 14 hours of light and darkness and allowed free access to ad libitum and water.

Animal Care and Maintenance:

Rabbits were housed separately in clean wire floored cages in the lab. Rabbits were fed a normal, nutritionally balanced diet. Their food and water consumption was checked daily. The rabbits were provided carrots, cucumber, berseem fodder, wheat, various fruits and vegetables, and certain kind of grasses. All experiments were carried out following the guidelines of the Ethical Committee, Lahore College for Women University.

Animal Grouping and Nanoparticles Dosage:

All animals were randomly categorized into three groups; the first group was the control group, including untreated rabbits who were not given nanoparticles. The experimental group (EG1) of rabbits was the low-dose group which was given 50 mg/ kg. The experimental group (EG2) was a high-dose group treated with 75 mg/kg. The experimental groups were given nanoparticles for twenty days, and parameters were detected on the experiment's 1st, 10th and 20th day.

ZnO Nanoparticles Synthesis:

Synthesized and characterized ZnO nanoparticles were obtained from Physics Department, Lahore College for Women University Lahore. The SEM image of these nanoparticles showed their spherical and polygonal shapes. Some of these particles also show faceting. The nanoparticle's size was about 20 nm and 70 nm. It was prepared by a solvothermal process [13]. Following this method, 150 mM urea and 50 nm zinc acetate were dissolved in 160 ml of ethylene glycol and deionized water with a volume ratio of 1:1. The solution was stirred for about 30 minutes. It was then transferred to an autoclave for 14 hours at about 180 ^{0}C. It was cooled down to room temperature. ZnO nanoparticles were collected and washed well with deionized water. After centrifugation, the nanoparticles were dried at about 60 ^{0}C.

Determination of Weight:

The body weight of each rabbit was determined in kilograms by digital weight balance, Redmond digital scale model no ZT7400 18.

Blood Collection and Serum Separation:

Blood samples were collected from each rabbit on the 20th day of the experiment. Upto 5ml blood sample was drawn from each rabbit from the marginal vein of the right ear by applying alcohol. Blood samples were collected in EDTA tubes. It was allowed to clot at room temperature. Later it was subjected to centrifugation at 3000 rpm for 15 minutes. The serum was stored in cryovials at – 20 ˚C.

Elisa for TSH, T3, T4 and Testosterone Level:

The serum TSH, T3, T4 and testosterone levels were measured by ELISA (enzyme-linked immunoassay) kits according to the respective kit protocol. This assay employs the competitive inhibition enzyme immunoassay technique. ELISA is aimed at a specific antigen in a collection competing with the same antigens, which are covered to the multi-well plate for the first antibodies.

Histological Study of Testes:

Testicular morphology was also evaluated. The rabbits were sacrificed on the 20th day of the experiment after taking the blood samples. The testes were removed immediately and fixed in phosphate buffer containing 4% formalin. The preserved organ was processed, dehydrated with alcohol, cleared with xylol, infiltrated, and embedded with paraffin wax. Parrafin wax blocks were sectioned according to the desired thickness with a rotary microtome using a disposable blade. Sectioned slides were stained with Hematoxylin and Eosin to visualize cells' general structures and components [14]. Observation of histological sections was performed by OPTIKA microscope (magnification 40X).

Statistical Analysis:

Values obtained from ELISA were calculated in mean and standard error to mean. All parameters were subjected to a one-way analysis of variance (ANOVA). Turkey's HSD test was used to determine the significant difference in mean data. The data were presented as a mean with

a standard error mean of $P<0.05$, which was considered significant and data was evaluated by SPSS Software (16 version). The graphs were plotted in Microsoft Excel (2018).

Results:

The following observations were recorded during this study.

Body Weight:

Body weight in the control group ranged from 1.09-1.36 kg. Experimental group 1 showed non-significant variations. A non-significant decrease in body weight was observed in Experimental group 2 (Table 1) (Fig. 1).

Hormone Levels:

TSH in the control group has not shown any significant change. TSH level was non-significantly decreased in EG1,2.23±0.04 U/L to 1.45±0.02 U/L and EG2 2.26±0.07 U/L to 1.28±0.02 U/L. T3 level, when measured in the control group, has not shown changes. It was 3.08±0.02 U/L on the first day and 3.18±0.03 U/L on the last day of the experiment (Table 1) (Fig. 2). T3 level of EG1 ranged 3.02±0.06 U/L to 2.18±0.01 U/L. A non-significant decrease was observed in EG2, 3.11±0.25 U/L, 2.08±0.01 U/L, and 1.31±0.05 U/L on the 1st, 10^{th} and 20^{th} day of the experiment (Table 1) (Fig. 3). T4 level in the control group ranged from 3.16±0.00 U/L to 2.98±0.41 U/L. A non-significant decrease was observed in T4 levels of both the experimental groups. The values were found to be 3.14±0.55 U/L, 2.79±0.09 U/L, 2.08±0.07 U/L in EG1 and 3.14±0.12 U/L, 2.26±0.06 U/L and 1.72±0.06 U/L in EG2 respectively (Table 1) (Fig. 4). Testosterone levels of the control group were found to be 6.35±0.41 U/L, 6.33±0.48 U/L and 6.36±0.52 U/L. In experimental group 1, a significant decrease in testosterone level was observed on the 20^{th} day of the experiment (2.78±0.44 U/L). In experimental group 2, a significant decrease was observed on the experiment's 10th and 20th day of 2.89±0.16U/L and 2.29±0.13 U/L, respectively (Table 1) (Fig. 5).

Histology of Testes:

Histopathological testing indicated normal testicular histology in rabbits in control. Testis of the control mature rabbits showed uniform seminiferous tubules and interstitial tissues containing Leydig cells. Histological examination on the 20^{th} day of the experiment revealed few alterations in the testicular cells of animals in experimental group 1. Zinc oxide nanoparticles caused atrophy of the seminiferous tubules in some cells, characterized by vacuolar degeneration and desquamation of spermatogonial cells lining the seminiferous tubules. On the 20th day, the animals treated with 75mg/kg of ZnO nanoparticles showed significant degenerative changes in their testes. It triggers necrosis in testicular cells. Due to the high dose, degeneration of seminiferous tubules has been observed. The germinal layer was thinner in the adjacent tubule and there was also sloughing or depletion of the germinal epithelium. There were no sperms in the cavity of some tubules. Some cells had destroyed nuclei, significantly losing most organelles (Fig. 6-8).

Table 1: Mean ± SEM of parameters in control and experimental groups.

Parameters	Control (n=10)			Experimental Group 1 (EG1) (n=10) (50mg/ kg)			Experimental Group 2 (EG2) (n=10) (75 mg/kg)		
	1st Day	10th Day	20th Day	1st Day	10th Day	20th Day	1st Day	10th Day	20th Day
Body Weight (Kg)	1.09± 0.00	1.15± 0.01	1.36± 0.02	1.35± 0.01	1.28± 0.07	1.29± 0.06	1.53± 0.01	1.38± 0.04	1.13± 0.03
TSH (U/L)	2.25± 0.02	2.24± 0.05	2.26± 0.04	2.23± 0.04	1.89± 0.03	1.45± 0.02	2.26± 0.07	1.75± 0.02	1.28± 0.02
T3 (U/L)	3.08± 0.02	3.11± 0.02	3.18± 0.03	3.02± 0.06	3.17± 0.01	2.18± 0.01	3.11± 0.25	2.08± 0.01	1.31± 0.05
T4 (U/L)	3.16± 0.00	3.00± 0.23	2.98± 0.41	3.14± 0.55	2.79± 0.09	2.08± 0.07	3.14± 0.12	2.26± 0.06	1.72± 0.06
Testosterone (U/L)	6.35± 0.41	6.33± 0.48	6.36± 0.52	6.37± 0.39	4.48± 0.16	2.78± 0.44*	6.31± 0.41	2.89± 0.16*	2.29± 0.13*

Values represent the Mean ± SEM of animals.

*P<0.05 indicates a significant difference as compared to the control.

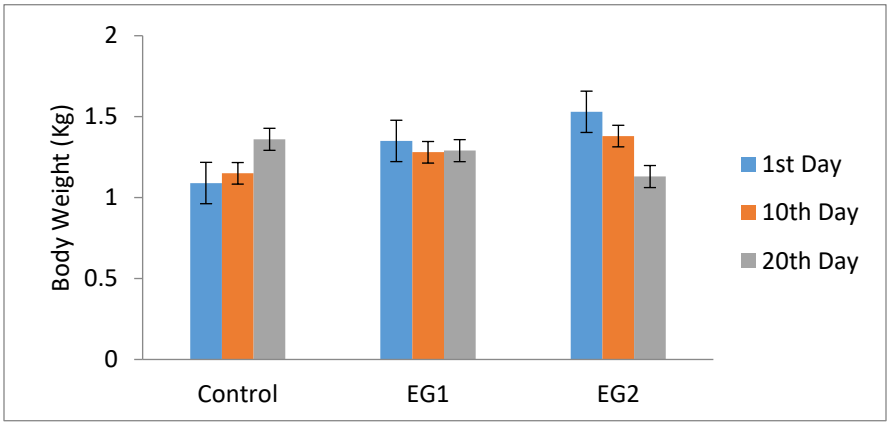


Fig. 1: Body weight (Kg) (Mean ± SEM) of control and experimental groups 1 & 2.

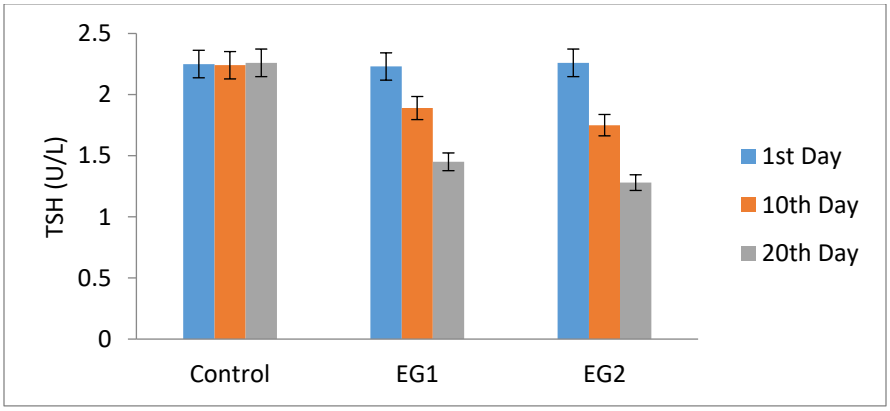


Fig. 2: TSH (U/L) (Mean ± SEM) of control and experimental groups 1 & 2.

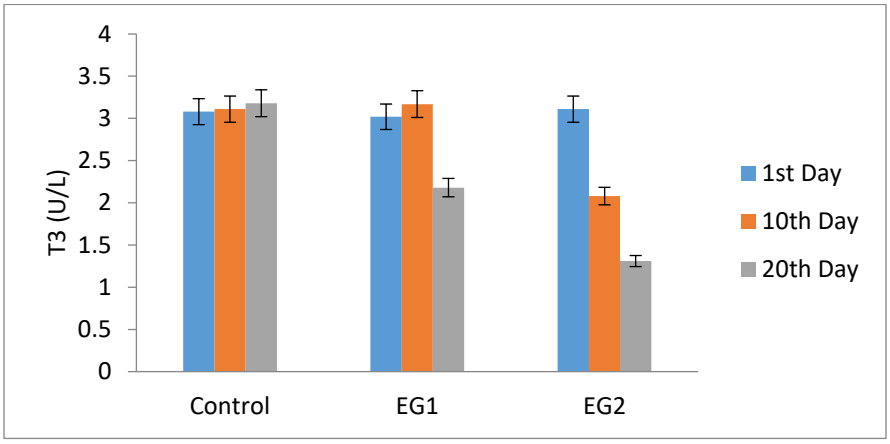


Fig. 3: T3 (U/L) (Mean ± SEM) of control and experimental groups 1 & 2.

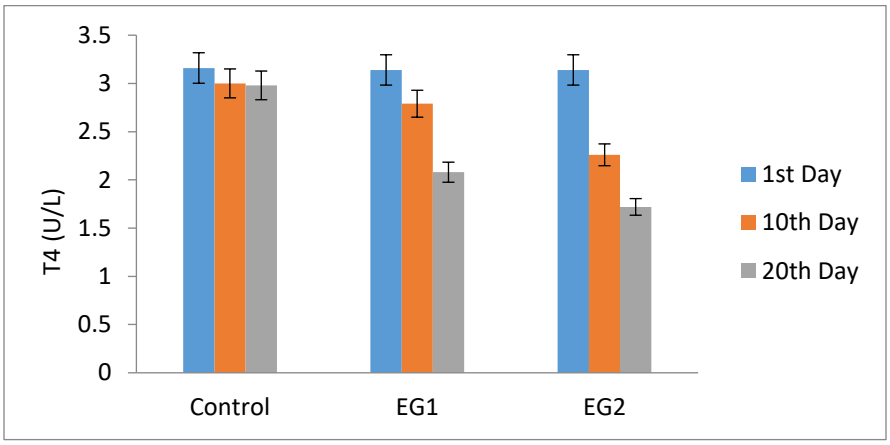


Fig. 4: T4 (U/L) (Mean ± SEM) of control and experimental groups 1 & 2.

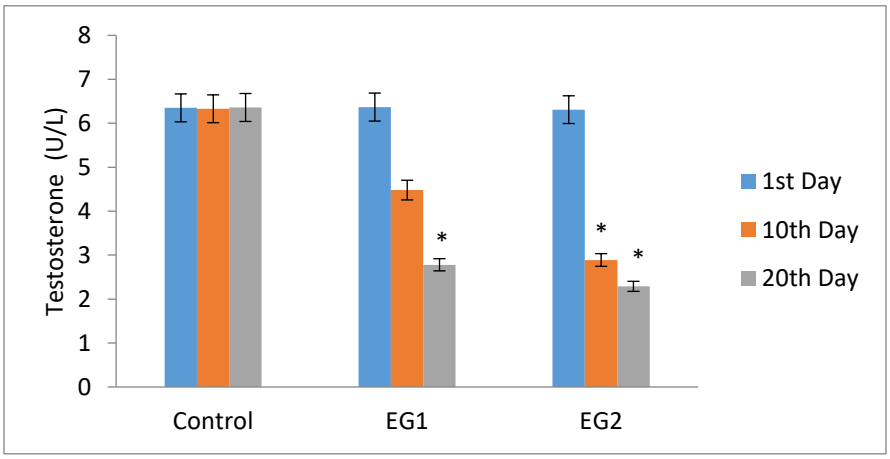


Fig. 5: Testosterone (U/L) (Mean ± SEM) of control and experimental groups 1 & 2.

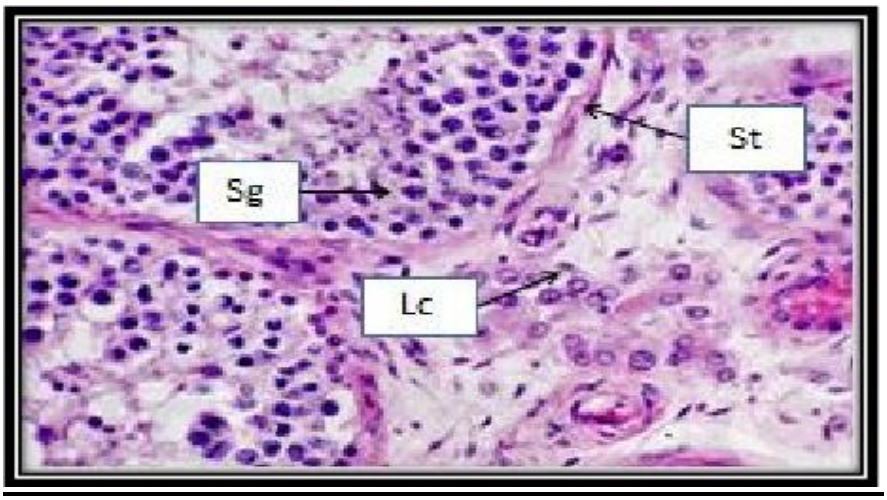


Fig. 6: Cross-section of rabbit testes of the control group at 40X
Key: St: Seminiferous tubules, Lc: Leydig cells, Sg: Spermatogonia

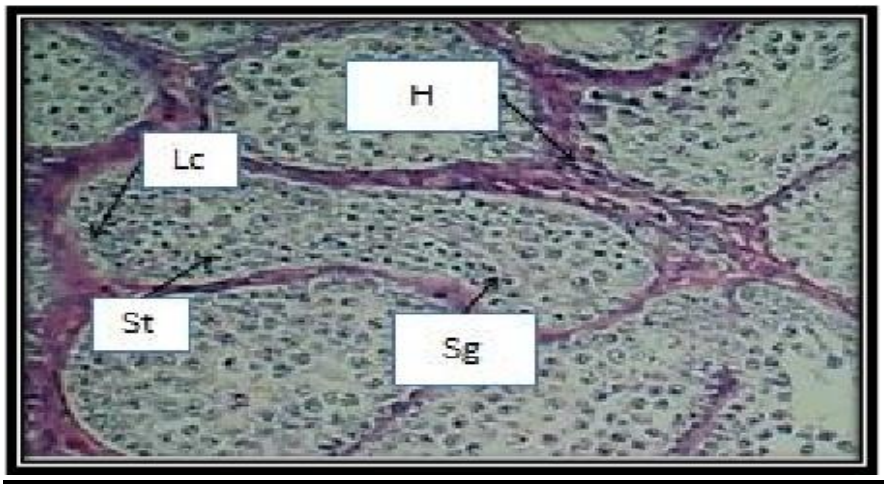


Fig. 7: Cross section of rabbit testes of Experimental group 1 at 40X
Key: Lc: Leydig cells, Sg: Spermatogonia, St: Seminiferous tubules, H: Haemorrhage

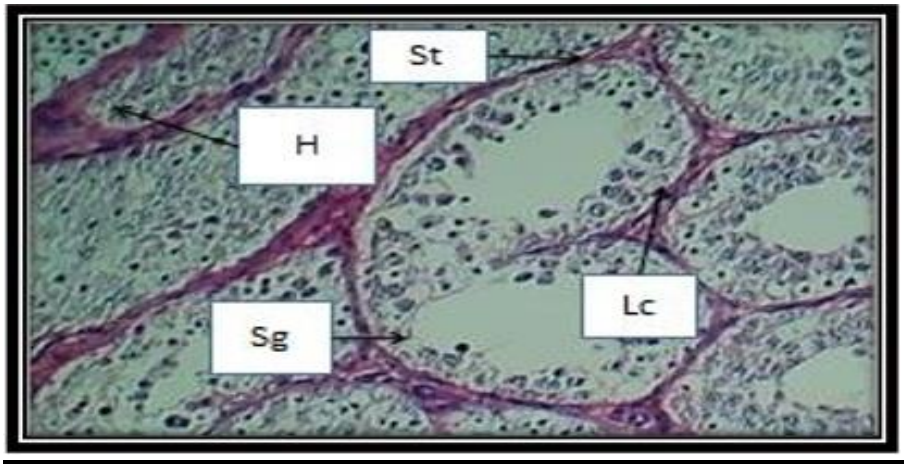


Fig. 8: Cross section of rabbit testes of Experimental group 2 at 40X
Key: H: Haemorrhage, Sg: Spermatogonia, Lc: Leydig cells, St: Seminiferous tubules

Discussion

The body weight of the control group has no significant change throughout the study. There was no particular change in EG1, whereas experimental group 2 showed a reduction in body weight. A considerable body weight reduction was also reported in earlier studies on rats [15]. Levels of TSH were nearly the same in the control group while non-significantly decreased in both the experimental groups. Levels of T3 and T4 hormones have not exhibited much change in the control group and EG1. In EG2, T3 and T4 levels were reduced. A study on rats observed significant decreases in levels of TSH, T3 and T4 while using a 200 mg/Kg dose [16].

Testosterone level was significantly decreased in experimental groups at the end of the experiment. A similar finding was reported on testosterone levels using ZnO nanoparticles in rats [1]. Histological examinations revealed that the control rabbit's testis showed no significant histological structure alterations in the current study. Minor changes have been observed in the testis of EG1. In EG2, the testis showed degeneration of seminiferous tubules and germinal epithelium. Cells with destroyed nuclei have been seen. Histopathological reports presented earlier have revealed low sperm count, Sertoli cell apoptosis and thinner spermatogenic epithelium in mice that were treated with ZnO nanoparticles [17].

Author's Contribution: F.A. conceived the idea; F.A., Z.B., & A.N[4]., designed the simulated work or acquisition of data; Z.B., & A.N[4]., executed simulated work, data analysis or analysis and interpretation of data. N.A., S.H., wrote the basic draft. A.N[6]., did the language and grammatical edits or critical revision. A.N[6]., conducted the histopathological study.

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