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ZnO Nanoparticles induced behavioral and hormonal alterations in *Oryctolagus cuniculus* and comparative ameliorative effects of methanolic and ethanolic corn extracts

Momil Liaquat^{1*}, Tahira Ruby², Kashif Ali^{3*}^{1*}Institute of zoology, Bahudddin Zakria University Multan, Pakistan. Email: momilliaquat00@gmail.com²Bahauddin Zakariya University, Multan Pakistan. Email: tahira.ruby@bzu.edu.pk^{3*}Division of Science and Technology, Department of Zoology, University of Education Lahore, Pakistan. Email: Kashif.ali@ue.edu.pk

Abstract

Zinc oxide nanoparticles (ZnO-NPs) are increasingly used in various applications, but their potential systemic toxicity remains a concern. This research was conducted to investigate the therapeutic potential of the methanolic and ethanolic corn extracts on zinc oxide nanoparticles (ZnO-NPs) induced toxicity in adult rabbits of both sexes. Preparation of the powder extract was done by extracting ground powder with ethanol and methanol at room temperature, followed by filtration and drying. Phytochemical analysis of both maize extracts revealed a diverse range of phytochemical constituents. The ethanolic extract showed a comparatively broader phenolic compound and phytochemical profile than the methanolic extract, with several phenols and flavonoid derivatives, daidzin and scopolet. Adult male and female rabbits were separated into two experiments, further were sub-divided into six groups as: group A (control), B treated with ZnO nanoparticles alone (300 mg/kg BW), C and D treated with ZnO-NPs (300 mg/kg) and methanolic extract of corn at two levels (150 and 300 mg/kg) and E and F treated with ZnO-NPs (300 mg/kg) and ethanolic extract of corn (150 and 300 mg/kg) for 24 days. ZnO-NPs exposure produced marked behavioral disturbances, such as locomotor and exploratory activity and Grooming Behavior, Body weight, food intake and hormonal alterations LH, TSH, T3, T4, decreased significantly ($p < 0.05$), and FSH levels increased significantly ($p < 0.05$). Methanolic extract showed partial protection, whereas ethanolic extract showed greater amelioration, specifically at 300 mg/kg, with restoration of behavioral response and hormonal assay. Overall, ethanolic maize extract demonstrated greater protective potential against ZnO-NP-associated toxicity, which may be due to its bioactive compounds, and can be further used against toxic elements.

Keywords: zinc oxide, hormonal assay, nanoparticles, phytochemicals, therapeutic potential.

Introduction:

Nanotechnology has gained significant public interest owing to its extensive applications in medicine, industry, public health, agriculture, business, and particularly with the assimilation of nanoscale structures into larger material structure and components, as well as the development of new and enhanced materials (Pasupuleti et al., 2012). The rising production and utilization of nanoparticles (NP) have elicited concerns over their potential harm to human and environmental health (Cazenave et al., 2019). Zinc oxide nanoparticles (ZnO-NPs) have expanded substantial interest due to their distinctive physicochemical characteristics, such as high ultraviolet (UV) absorption capabilities, surface area, antibacterial efficacy, and chemical stability. These traits have resulted in their extensive utilization across various biomedical, industrial and commercial sectors. Zinc oxide nanoparticles are frequently utilized in electronic gadgets, food packaging, sunscreens, cosmetics, agricultural goods, paints, textiles and pharmaceuticals. (Sirelkhatim et al., 2015). The NP can get into the body by finding its way through different pathways including inhalation, ingestion, or dermal route and when absorbed they can diffuse across the biological barriers to reach other vital organs such as the liver, kidney, brain, and intestine. Too much exposure can lead to cellular homeostasis disruption, overproduction of Reactive Oxygen Species (ROS), oxidative stress, inflammation and cellular damage (Sun et al., 2024). One of the main

mechanisms involved in tissue damage by nanoparticles is oxidative stress. Hence, more focus has been paid to naturally occurring phytochemicals, which are potential ameliorative agents. Phenolic acid and flavonoids in plants show antioxidant activity and can prevent oxidative damage by free-radical scavenging and by increasing the antioxidant defenses within organisms (Muscolo et al., 2024).

ZnO-NPs can cause behavioral and food intake alterations, as well as disrupting endocrine homeostasis. ZnO-NP exposure has been reported to induce changes in reproductive hormones, such as luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone. These changes may result in disruption of the hypothalamic–pituitary–gonadal axis. Biopesticides, like ZnO-NPs, also influence the level of thyroid hormones such as triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) which affects the regulation of the thyroid endocrine system (Mozaffari et al., 2020; Luaibi & Zayed, 2020). These hormonal imbalances can occur due to oxidative stress, inflammatory responses and disruptions of the endocrine tissues and steroidogenic pathways. Thus, the evaluation of reproductive hormones and thyroid hormones is another sign of systemic effects of ZnO-NP exposure.

Maize (*Zea mays* L.) is a commonly used cereal and a rich source of bioactive compounds especially phenolics and antioxidants which are mostly found in bound form as ferulic acid. The compounds are essential in maintaining the stability of cell membranes, ROS scavenging, and cells against oxidative stress, inflammation, and enzyme leakage (Van Hung, 2016). These bioactives are extracted by different solvents such as methanol and ethanol, and ethanol is commonly favored because of its greater efficacy in extraction of phenolic compounds, its biological safety, and capacity to maintain the structural and activity of the antioxidants. Although it has been extensively studied on the toxicity of ZnO nanoparticles, little has been found on the possibility of corn-based extracts to counteract the effects of NPs on oxidative stress and tissue damage. This research examined dose-dependent protective property of the methanolic and ethanol corn extracts against ZnO NP-induced behavioral, feed intake and hormonal alterations in adult rabbits. This study hypothesized that, corn extracts, especially ethanolic extracts will inhibit behavioral and hormonal alterations and normalize functioning in a dose dependent manner, which shows that the extracts can be used as a natural therapeutic intervention in nanoparticle induced toxicity.

Material and methods

ZnO Nanoparticles preparation

Zinc oxide nanoparticles (ZnO NPs) were made through the dispersion of the particles in bi-distilled water in order to create a stock suspension of the nanoparticles. The ZnO NPs were obtained through the Institute of chemistry, The Islamia University of Bahawalpur. The suspension was made right before administration to maintain a standardized suspension and chemical stability.

Corn Extracts Preparation

Grounded corn cobs (mesh size 60) were used to extract corn (*Zea mays*) using a solution of 60 per cent aqueous ethanol and methanol as solvents. The extraction was carried out at ambient temperature at a ratio of 1 kg cobs to 7L of the solvent. The extracts were filtered to remove solid particles and dried by spray using maltodextrin (0.5 -1 kg/100 L extracts) as an agent. Temperature inlet and outlet were kept at 180 °C and 85 °C respectively according to the protocol in Pedreschi and Cisneros (2007). The powdered extracts obtained were kept in appropriate conditions until the point of use.

Animal handling and captivity

In the present study all the procedures and protocols were adopted under the guidelines of National Bioethics Committee (NBC) of Pakistan and ethical permission was obtained from the institute of zoology, Bahauddin Zakariya University, Pakistan via Ref. N0. Zool/115. Dated:5-11-2023. The Rabbits were placed in typical cages in a climate-controlled area (27 °C) and fed on a nutritionally balanced diet and made water ad libitum. The 7 days acclimatization period was implemented to ensure that stress was minimized and that the participants adapted to the environment in the laboratory before the experiment was started.

Phytochemical analysis of extract

Qualitative Phytochemical analysis of methanol and ethanol corn extracts for the presence or absence of phytochemicals was carried using standard procedures given in report of Santhi and Sengottuvel (2016). The extracts were screened for phenols, flavonoids, steroids, terpenoids, saponins, tannins, glycosides, and carbohydrates using the following qualitative tests:

Ferric chloride test for phenols: 0.4ml of FeCl₃ solution was taken in the test extract. The presence of phenolic compounds was indicated by the development of a characteristic colour.

Sulfuric acid test for flavonoids: A few drops of sulfuric acid (H_2SO_4) were added to the test extract, and the resulting colour change was observed.

Liebermann–Burchard test for steroids: 5 mL of the extract was thoroughly shaken with 2 mL of chloroform and then mixed with 3 mL of sulfuric acid/acetic acid reagent appropriate to the steroids being tested. Characteristic colour development was indicative of the presence of steroids.

Salkowski test for terpenoids: To the extract, 2 mL of chloroform was added and treated carefully with 3 mL of H_2SO_4 . The characteristic colour that formed at the interface was thought to represent the terpenoids.

Frothing test for saponins: 4 mL of distilled water was added to about 0.4 mg of extract and vigorously shaken. The froth was persistent, which suggested the presence of saponins.

Ferric chloride test for tannins: 2 mL of the extract was taken, mixed with 2 mL of distilled water and then a few drops of FeCl_3 solution was added. A typical colour change was observed, which was the signature of the tannins.

Nitroprusside test for glycosides: Two millilitres of extract were mixed with hydrochloric acid (HCl), sodium nitroprusside in pyridine and ammonium hydroxide (NH_4OH) solution and subjected to Characteristic colour was noted as a positive reaction.

Molisch test for carbohydrates: About 5 mg of the extract was placed in 5 mL of distilled water and two drops of molisch reagent were added. Then 1 mL of H_2SO_4 was carefully poured down the side of the test tube. Violet ring formation proved the carbohydrate present in the formation.

Administration of toxicant and Test extract to Animals (Grouping and Treatment)

The experimental animals were randomly allocated into six groups (A-F), with both male and female rabbits included in each of 2 experiments. Group A served as the untreated control group, while Group B received ZnO-NPs (300 mg/kg BW). Groups C and D received ZnO-NPs at 300 mg/kg BW in combination with methanolic corn extract (MCE) at doses of 150 and 300 mg/kg BW, respectively. Groups E and F received ZnO-NPs at 300 mg/kg BW together with ethanolic corn extract (ECE) at doses of 150 and 300 mg/kg BW, respectively for 24 days. The same treatment doses were administered to both male (Experiment No.1) and female rabbits (Experiment No.2).

Observational scoring scale:

Behavioral observations were daily conducted during the study period. Each rabbit was tested separately in its home cage for a predetermined time of observation under similar environmental conditions. Behavioral parameters, including locomotory activity, exploratory and grooming behavior. The behavioral scoring criteria were as follows:

- +++ = Normal behavior (comparable to the control group)
- ++ = Moderate improvement
- + = Mild improvement
- = Markedly impaired behavior or absence of normal behavioral response

The behavioral scores obtained from Groups A–F were tabulated and used for qualitative comparison of the effects of zinc oxide nanoparticle-induced toxicity and the protective efficacy of the methanol and ethanol corn extract.

Assessment of body Weight and Food Intake

During the investigation, the body weight and food intake of the experimental rabbits were observed to assess the overall health status and the impacts of zinc oxide nanoparticles (ZnO-NPs). The body weight of each group of rabbits was recorded using digital weighing balance at the onset of the experiment (Day 0) and at weekly intervals up to the conclusion of the experimental period. Body weight was recorded in grams (g) under similar environmental conditions and preferably at the same time of day to minimize fluctuations. The acquired numbers were utilized to measure the changes in the body weight across the study period. Each rabbit was fed a known amount of regular rabbit food each day. After 24 h the residual feed was collected and weighed. Daily food intake was estimated by subtracting the weight of residual feed from the total amount of feed provided. Daily food intake was indicated in g/day.

Determination of hormonal alteration:

Rabbits FSH, LH, TSH, T3, T4 and Testosterone (T), were determined in the serum using their corresponding ELISA kits from MyBioSource.com for the research purpose (Alkaladi et al., 2020).

Statistical Analysis

The data was presented as a mean and standard deviation (SD). A one-way analysis of variance (ANOVA) was employed in SPSS software (version 20.0) for inter-group comparisons. The importance of the differences was deemed statistically significant at $p < 0.05$.

Results**Phytochemical analysis of methanol and ethanol corn extracts**

The Zea mays extract revealed a diverse range of phytochemical constituents as shown in Table 1. The phytoconstituents identified in the ethanolic extract included phenolic compounds, phenolic acids, flavonoids, is flavonoids, hydroxycoumarins, fatty acid-related compounds and steroidal derivatives. The methanolic extract contained phenolic acids, flavonoids, flavan-3-ols, flavonols, amide derivatives and heterocyclic compounds. The ethanolic extract showed a comparatively broader phytochemical profile than the methanolic extract, with several flavonoid derivatives, phenolic acids, daidzin and scopoletin

Table 1. Qualitative Phytochemical Analysis of Zea mays Extract

Phytochemical	Test applied	Methanolic Extract	Ethanolic Extract
Phenols	Ferric chloride test	+	+++
Flavonoids	H ₂ SO ₄ / Shinoda test	+	++
Tannins	Lead acetate test	+	+
Saponins	Frothing test	+	+
Terpenoids	Salkowski test	+	++
Steroids	Liebermann–Burchard test	+	+
Glycosides	Nitroprusside test	+	+
Carbohydrates	Molisch test	+	+

Whereas + = Present normal amount, ++ = Present great amount

Behavioral observation

Behavioral observations revealed noticeable differences among the experimental groups. Rabbits in the control group (Group A) exhibited normal locomotory, exploratory, and grooming activities, corresponding to a +++ behavioral score. In contrast, rabbits exposed to zinc oxide nanoparticles (Group B) showed marked impairment in normal behavioral responses, including reduced locomotory and exploratory activities and altered grooming behavior, resulting in a – score. The scoring criteria used for the assessment of locomotory activity, exploratory activity, and grooming behavior are presented in Table 2 and 3, for male and female rabbits respectively. The ethanolic extract gave better improvement when compared to Group C and D. Overall, the behavioural observations showed that there was more improvement in the treated groups, especially in the higher dose group, which were approaching the control group.

Table No 2: Scoring of Behavioral observation across treatment groups of male rabbits.

Parameters	Treatment Groups					
	A(control)	B(ZnO-NPs 300mg/kg BW)	C(ZnO)+MCE 150mg/kg BW)	D(ZnO)+MCE 300mg/kg BW)	E(ZnO)+ECE 150mg/kg BW)	F(ZnO +ECE 300mg/kg BW)
Locomotory Activity	+++	–	–	+	++	+++
Exploratory Activity	+++	–	+	+	+	+++
Grooming Behaviour	++	–	–	+	++	++

Table No 3: Scoring of Behavioral observation across treatment groups of female rabbits.

Parameters	Treatment Groups					
	A(control)	B(ZnO-NPs 300mg/kg BW)	C(ZnO)+MCE 150mg/kg BW)	D(ZnO +MCE 300mg/kg BW)	E(ZnO)+ECE 150mg/kg BW)	F(ZnO)+ECE 300mg/kg BW)
Locomotory Activity	+++	–	+	+	++	+++
Exploratory Activity	++	–	–	+	++	++
Grooming Behaviour	+++	–	+	+	+	+++

ZnO-NPs=zinc oxide nanoparticles; MCE= methanolic corn extract; ECE= ethanolic corn extract. Scoring: +++ normal behavior; ++ Moderate improvement; += Mild improvement; – = absence of normal behavioural response

Analysis of ZnO- NPs and corn extract on body weight, food intake of male and female Rabbits:

The effect of ZnO-NPs exposure and Zea mays extract on body weight and food intake in male and female rabbits are presented in figure 1-4. A reduction in body weight was observed in the ZnO nanoparticle-treated group (Group B) compared with the normal control group (Group A) indicating an adverse effect of ZnO nanoparticle exposure on body weight. Treatment with methanolic and ethanolic *Zea mays* cob extracts showed varying degrees of improvement in body weight. The ethanolic extract-treated groups (Groups E and F) exhibited a comparatively improved body-weight pattern toward the control group in both male and female rabbits, as shown in Figures 1 and 2.

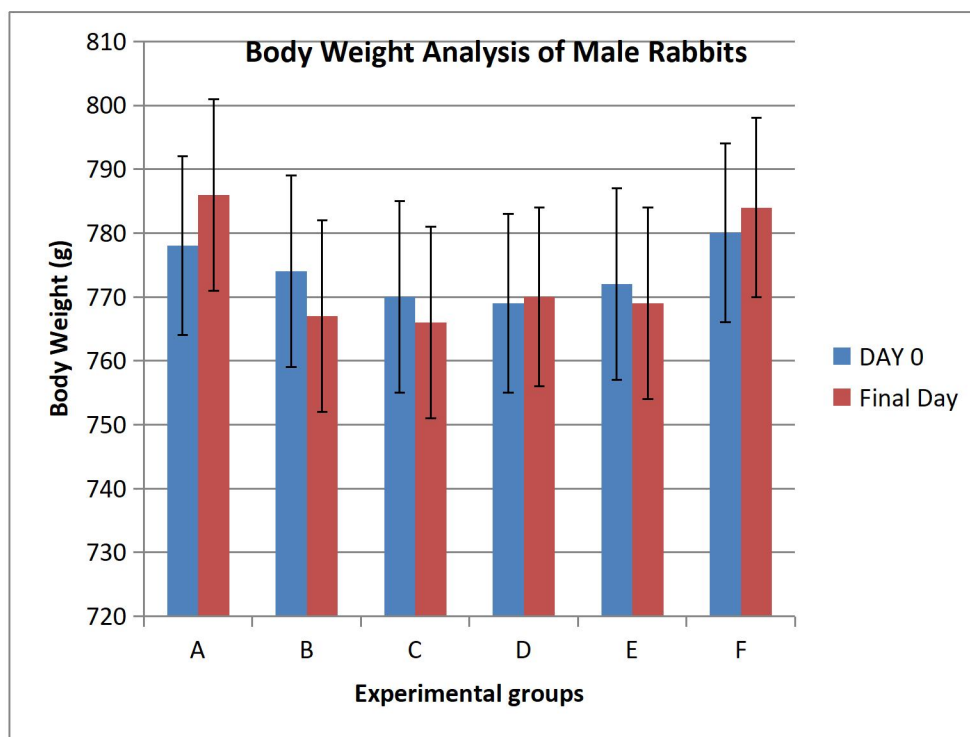


Figure 1. A (Control); B (ZnO 300mg/kg BW); C&D ZnO (300mg/kg BW)+MCE(150+300mg/kg BW); E & F ZnO (300mg/kg BW) + EEC (150+300mg/kg BW). Represents body weight of male rabbits.

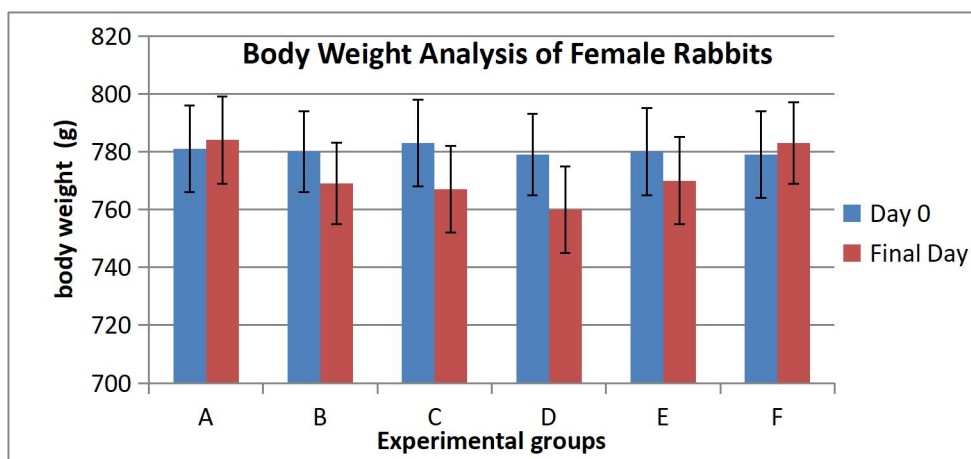


Figure 2 A (Control); B (ZnO 300mg/kg BW); C&D ZnO (300mg/kg BW) +MCE(150+300mg/kg BW); E & F ZnO (300mg/kg BW) + EEC (150+300mg/kg BW). Represents body weight of female rabbits.

Food intake also differed among the experimental groups. The control animal's maintained relatively consistent food consumption throughout the experimental period, whereas reduced food intake was observed in the ZnO-NPs treated group (Group B) in both male and female rabbits. Food intake was improved in the treatment group receiving Zea mays cob extracts as compared with the group receiving only ZnO-NP with the ethanolic extract treated group having a relatively larger improvement when compared with the control pattern (Figures 3 and 4). In conclusion, it is observed that exposure to ZnO-NP was linked with lowering of body weight and food consumption while supplementation with extracts from Zea mays had ameliorative effect wherein the ethanolic extract had a relatively higher ameliorative effect on the above mentioned parameters.

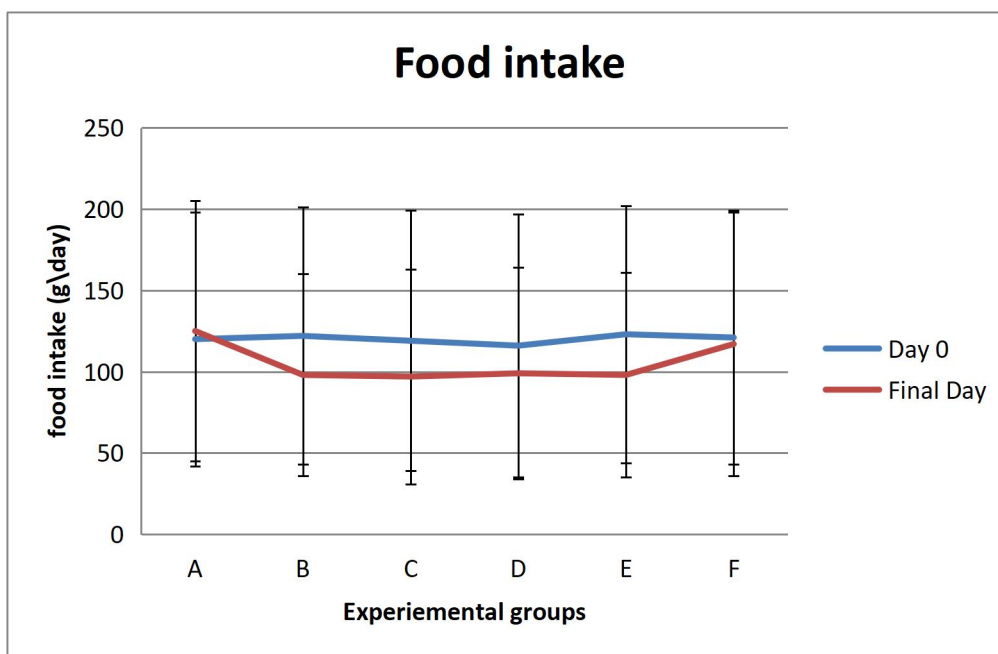


Figure 3 A (Control); B (ZnO 300mg/kg BW); C&D ZnO (300mg/kg BW) +MCE(150+300mg/kg BW); E & F ZnO (300mg/kg BW) + EEC (150+300mg/kg BW) represents food intake (g/day) of male rabbits.

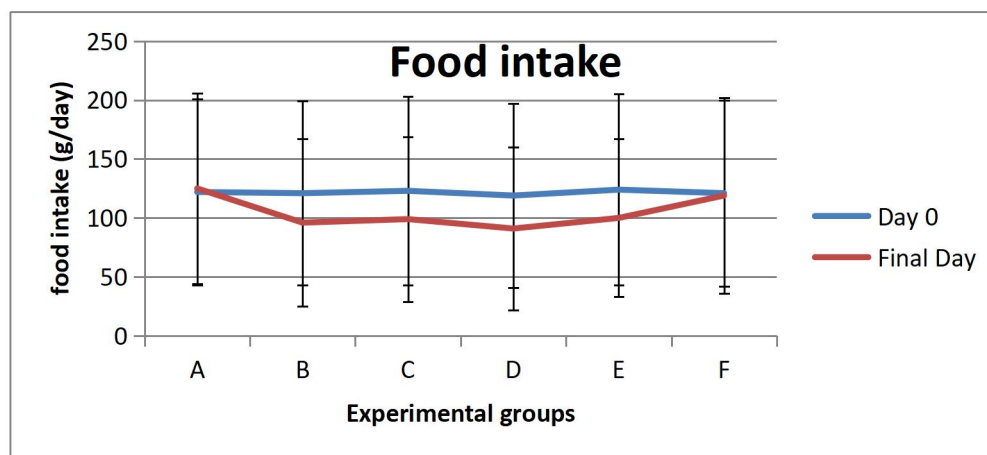


Figure 4. A (Control); B (ZnO 300mg/kg BW); C&D ZnO (300mg/kg BW) +MCE(150+300mg/kg BW); E & F ZnO (300mg/kg BW) + EEC (150+300mg/kg BW) represents food intake (g/day) of female rabbits.

Hormonal Studies

The exposure to ZnO-NPs and Zea mays extract on reproductive and thyroid hormones in male and female rabbits after 24 days of exposure are presented in table 4 and 5. In male rabbits, ZnO-NP exposure significantly altered LH and FSH levels. LH decreased from 5.83 ± 0.49 in the control group to 2.39 ± 0.94 in the ZnO-NP-treated group (B), whereas FSH increased from 4.72 ± 0.52 to 6.91 ± 0.50 . Treatment with methanolic and ethanolic extracts reversed these alterations which was comparatively more with ethanolic extract. LH increased to 5.15 ± 0.73 and 5.63 ± 0.37 in Groups E and F, respectively, while FSH decreased to 4.83 ± 0.74 and 5.29 ± 0.56 . Exposure to ZnO-NP also caused significant changes to thyroid hormones in male rabbits. T4 decreased from 110.01 ± 1.02 in Group A to 91.44 ± 0.87 in Group B, while T3 decreased from 310.36 ± 0.96 to 261.23 ± 1.07 . The values of T4 and T3 were increased towards the normal values after treatment with the extracts with values of 107.90 ± 0.36 and 306.19 ± 0.55 in Group E respectively. Also, following the administration of ZnO-NPs, the level of TSH decreased in Group B (0.14 ± 0.01) when compared with Group A (0.01 ± 0.01). However, Group E (0.14 ± 0.01) and Group F (0.01 ± 0.01) had higher levels of TSH after the administration of ethanolic extract compared with Group B. Furthermore, the level of TSH was higher in Groups E and F after the administration of ethanolic extract as compared to Group B (0.09 ± 0.01).

For female rabbits, there were no significant differences between the experimental groups in terms of LH. The mean FSH level was significantly reduced to 5.01 ± 0.83 after exposure to ZnO-NPs, compared with the control group with a mean of 6.81 ± 0.76 . Treatment with the extracts produced partial improvement, with FSH reaching 5.84 ± 0.77 in Group E. ZnO-NP exposure markedly reduced T4 and T3 from 119.59 ± 1.20 and 320.04 ± 0.81 in the control group to 84.76 ± 1.16 and 250.11 ± 1.02 , respectively. Both the extracts induced the improvement of these thyroid hormones, with the ethanolic extract showing more response; the concentration of T4 rose to 114.85 ± 0.91 and T3 raised to 311.06 ± 0.70 in the Group E. In Group B, TSH concentration decreased from 0.10 ± 0.01 to 0.07 ± 0.01 after being exposed to ZnO-NP, while in Group E it was 5.70 ± 0.41 , and in Group F it was 5.77 ± 0.37 .

Overall, exposure to ZnO-NP resulted in changes in reproductive and thyroid hormone levels, and more notably in thyroid hormone in both females and males. A treatment with the Zea mays cob extracts caused an improvement of the altered hormonal parameters, showing that generally the ethanolic extract had a higher ameliorative potential than the methanolic extract.

Table 4: Toxic effects of zinc oxide nanoparticles and ameliorative effect of corn extract on the hormonal profile of male rabbits after 24 days of treatment.

Parameters	Groups					
	A(control)	B (ZnO 300mg/kg BW)	C(ZnO(300mg/kg BW) +MCE 150mg/kg BW)	D(ZnO(300mg/kg BW) +MCE 300mg/kg BW)	E(ZnO(300mg/kg BW)+ECE 150mg/kg BW)	F(ZnO(300mg/kg BW)+ECE 300mg/kg BW)
LH	5.83 ± 0.49^a	2.39 ± 0.94^c	3.02 ± 0.74^c	4.34 ± 0.43^b	5.15 ± 0.73^{ab}	5.63 ± 0.37^a

(mIU/mL)						
FSH (mIU/mL)	4.72±0.52 ^c	6.91±0.50 ^a	6.10±0.63 ^b	5.42±0.47 ^{bc}	4.83±0.74 ^c	5.29±0.56 ^c
T4 (ng/mL)	110.01±1.02 ^a	91.44±0.87 ^f	98.20±0.47 ^d	96.96±0.78 ^e	107.90±0.36 ^b	103.44±0.71 ^c
T3(ng/mL)	310.36±0.96 ^a	261.23±1.07 ^f	284.93±0.86 ^d	277.85±0.98 ^e	306.19±0.55 ^b	301.91±0.55 ^c
TSH (µIU/mL)	0.14±0.01 ^{ab}	0.09±0.01 ^c	0.1±0.01 ^c	0.15±0.05 ^a	0.12±0.02 ^{bc}	0.11±0.01 ^c
Testosterone (ng/mL)	7.69±0.69 ^{ab}	5.94±0.46 ^d	6.25±0.60 ^{cd}	7.00±0.72 ^{bc}	7.91±0.61 ^a	7.53±0.57 ^{ab}

Values are presented as mean ± SD (n = 5). Different superscript letters within a row indicate significant differences among groups (p < 0.05).

Table 5: Toxic effects of zinc oxide nanoparticles and ameliorative effect of corn extract on the hormonal profile of female rabbits after 24 days of treatment.

Parameters	Groups					
	A(control)	B (ZnO 300mg/kg BW)	C(ZnO(300 mg/kgBW) +MCE 150mg/kg BW)	D(ZnO(300 mg/kgBW) +MCE300 mg/kg BW)	E(ZnO(300 mg/kg BW)+ECE 150mg/kg BW)	F(ZnO(300 mg/kg BW)+ECE 300mg/kg BW)
LH (mIU/mL)	4.66±0.65 ^a	3.98±0.65 ^a	3.96±0.68 ^a	4.03±0.73 ^a	4.64±0.62 ^a	4.79±0.37 ^a
FSH (mIU/mL)	6.81±0.76 ^a	5.01±0.83 ^b	5.62±0.60 ^b	4.95±0.87 ^b	5.84±0.77 ^{ab}	5.63±0.74 ^b
T4 (ng/mL)	119.59±1.20 ^a	84.76±1.16 ^e	97.73±0.94 ^d	98.86±1.13 ^d	114.85±0.91 ^b	111.75±0.92 ^c
T3(ng/mL)	320.04±0.81 ^a	250.11±1.02 ^f	285.62±0.73 ^e	287.69±0.99 ^d	311.06±0.70 ^b	305.11±0.66 ^c
TSH (µIU/mL)	0.10±0.01 ^{ab}	0.07±0.01 ^c	0.10±0.01 ^{ab}	0.11±0.01 ^{ab}	0.12±0.01 ^a	0.10±0.01 ^{ab}
Testosterone (ng/mL)	6.03±0.64 ^a	4.78±0.51 ^c	5.04±0.66 ^{bc}	5.08±0.75 ^{bc}	5.70±0.41 ^{ab}	5.77±0.37 ^{ab}

Values are presented as mean ± SD (n = 5). Different superscript letters within a row indicate significant differences among groups (p < 0.05).

Discussion

Zinc is a crucial trace mineral in the human body as well as in animals, and zinc oxide (ZnO) is often utilized as a nutritional or therapeutic supplement (Wang et al., 2017). In the present study, ZnO-NPs caused multisystem changes in adult male and female rabbits manifested by behavioral changes, changes in body weight, food consumption and hormonal alterations. The results validate the toxicological potential of the investigated exposure dose of ZnO-NPs. High surface reactivity, dissolution to Zn²⁺, generation of ROS, induction of oxidative stress, inflammation and mitochondrial dysfunction that have been reported as the potential mechanisms that lead to the toxicity of ZnO-NPs (Sharma et al., 2011).

The exposure of ZnO-NPs caused marked behavioral changes in rabbits such as decreased locomotor, exploratory activity and changed grooming behavior when compared to the control group. Such behavioral abnormalities are likely a consequence of excess generation of ROS, oxidative stress, neuro-inflammation and neuronal impairment induced by ZnO-NPs. The decrease in body mass and feed consumption seen in the ZnO-NP treated animals suggests a systemic effect on body physiology in response to the exposure to nanoparticles. The decrease in body weight observed could be due to reduced feeding but the present results must be considered with reference to the dose and exposure period. Some previous studies show that biological effects of ZnO-NPs are dose and exposure-dependent; lower exposure level may have nutritional benefit while a higher exposure level may have adverse effects as reported by Farag et al. (2024). Similar behavioral deficits following ZnO-NP exposure have been reported by Abbasalipourkabir et al. (2015) and

Sharma et al. (2009) who demonstrated that ZnO-NPs cause oxidative damage to the normal functioning of the nervous system. The improvement in body weight, food intake, and behavioral responses following maize cob extract treatment suggests attenuation of these systemic effects.

The endocrine findings also support systemic toxicity of ZnO-NPs. The exposure of ZnO-NP significantly induced changes in LH, FSH, and testosterone in males but FSH and testosterone levels were the most significant changes observed in females (LH was not significantly different among female groups). Changes in reproductive hormone levels have been previously reported by Ahmed et al. (2025) after ZnO-NPs exposure, and the response of each individual hormone is different depending on the dose, exposure time, species and experimental conditions. Exposure to ZnO-NP has been linked to changes in LH, FSH and testosterone in male rodents and reproductive tissue damage and changes in steroid hormones have also been reported in female rodents (Mozaffari et al., 2022;Pinho et al., 2020). The results presented here, in combination with the sex-specific effects reported in previous studies, indicate that the endocrine activities of ZnO-NPs might be sex-specific. There was a significant change in thyroid hormones as reported by Hosseini et al. (2019) in both female and male. Exposure to ZnO-NP resulted in a decrease in T3 and T4 (along with changes in TSH), while the maize extracts changed the parameters closer to the control. ZnO-NPs have been shown to cause similar reductions of both T3 and T4, as well as in the levels of TSH and T, in experimental animals, suggesting a disruption of thyroid and reproductive endocrine regulation. The relatively constant decrease in T3 and T4 in both sexes of rabbits is an important observation of the present study and suggests that the effect of ZnO-NPs on the endocrine system was not limited to the reproductive axis.

The current research designates the effectiveness of the ethanolic corn extract to a synergistic action between the characteristics of ethanol as a solvent in extracting plant-based components and the phyto transcript of corn. According to Colombo et al. (2021) and Ramírez-Esparza et al. (2024), ethanolic corn extracts are rich in phenols, flavonoids, anthocyanins, and carotenoids. All the compounds have been extensively reported to neutralize reactive oxygen species, modulate inflammatory pathways, and reduce lipid peroxidation (Elsayed et al., 2021; Colombo et al., 2021). Other studies by Elsayed et al. (2021) and Cai et al. (2019) have explained the antioxidant power and enzyme-inhibitory capacity of corn extracts, which are relevant in inflammatory and metabolic disease cases, thus highlighted its protective and ameliorative effects. Ethanol preserves the integrity and bioactivity of these phytoconstituents throughout the extraction and storage (Ramírez -Esparza et al., 2024), which is also supported by its intestinal absorption and systemic bioavailability (Verediano et al., 2022). ZnO-NP may cause toxicity in biological systems manifested in the form of oxidative stress, inflammation, and cellular damage (Fatima et al., 2024). Phytochemicals found in corn prevent cellular oxidative stress, DNA damage, protein oxidation and apoptosis, which offers cytoprotection (Hosseini et al., 2024). Verediano et al. (2022) also indicated that corn extract contained micronutrients and cofactors helpful in strengthening of enzymatic antioxidant defenses, which serves as a strong justification for its use in treating ZnO-NP toxicity.

Conclusion

The study was aimed to assess the ameliorative potential of phenolic and other phytochemical compound extracted from natural source against toxicity of zinc oxide (ZnO) in rabbits with special reference to gender wise differences. The phytochemical analysis revealed the presence of various bioactive phytochemicals in the corn extract, indicating its potential as a rich source of phenolic and other possible antioxidant properties. The results of the experiments showed that there was a negative effect on physiological and behavioral variables such as body weight, food consumption and hormonal alterations after exposure to ZnO NPs. Analysis based on gender did not disclose any significant difference in the reaction of male and female rabbits to the toxicity generated by zinc oxide or to the treatment with the phenolic compound. The administration of the phenolic compound produced equal protective benefits in male and female rabbits, reflected in similar improvements in the behavioral, physiological and hormonal parameters. The results of the present experiment indicate that the phenolic compound of natural origin has significant protective effects against zinc oxide induced toxicity in rabbits.

Author Contributions

Conceptualization and study design [Tahira Ruby and Kashif ali]. Animal handling, experimentation and sample collection [Momil Liaquat]. Phytochemicals analysis, behavioral observation and hormonal Analysis [Momil Liaquat and Kashif ali]. Statistical analysis [Kashif Ali]. Manuscript preparation and original draft writing [Momil Liaquat]. Critical revision and final approval [Tahira Ruby and Kashif ali]. All authors have read and approved the published version of the manuscript.

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Conflicts Of Interest

The authors have no conflict of interest.

Data Availability

The raw data that support the conclusions of this article are available upon reasonable request from the corresponding author.

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