Ameliorative Role of *Moringa oleifera* Plant Extract against Zinc Oxide Nanoparticles Induced Sperm and Sex Hormones Abnormalities in Male Albino Rats

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***Abstract*—Nanoparticles (Nps) have a relatively greater toxicity compared to large sized materials. Therefore materials at the nano size are highly reactive. Furthermore, due to the widespread application of zinc oxide nanoparticles (ZnO Nps) in different industries such as food additives, biosensors, resin production and electronic equipment, it is necessary to evaluate the toxic effects of this nanoparticle in biological systems, specially its effects on the male sperm and sex hormones (testosterone & FSH). *Moringa oleifera* (M.O.) extract has been shown to have strong antioxidant activity and decreases oxidative stress. Thus, the present investigation was designed to evaluate the toxic effects of ZnO Nps on sperm morphology and sex hormones (testosterone &FSH) and/or the ameliorative role of M.O. plant extract *in vivo* using male albino rats.**

The results of the present work revealed that the abnormalities in the sperm morphology and testosterone level induced by ZnO Nps were alleviated by the administration of M.O. plant extract (but no significant change in FSH level was observed).

***Abbreviations*--- Nps (Nanoparticles); ZnO Nps (Zinc oxide nanoparticles); ZnO (Zinc oxide);TH (Testosterone hormone); FSH (Follicle stimulating hormone) ; M.O. (*Moringa oleifera*) and G.(Group).**

***Keywords*—Nanoparticles, Zinc Oxide, *Moringa oleifera*, sex hormones, male albino rats.**

I. Introduction

A nanoparticle is a nano-object with all three external dimensions in the size range of approximately 1–100 nm[(1)]. These nanoparticles may enter into body through various ways such as skin, inhaling or food [(2)]. Because of their small size, it can penetrate into the cell membrane and interfere in important cell functions [(3)]. Zinc oxide nanoparticles (ZnO Nps) is one of the most commonly used types of nanoparticles [(4)]. It is a non organic substance and white powder that is widely used as an additive in different materials and productions including plastics, ceramics, glass, cement, tires, lubricants, dyes, sunscreens, glues, pigments, food and batteries etc. [(5)]

Moringa oleifera tree (drumstick tree) is a small tree of about 13m tall and 35 cm in diameter with an umbrella-shaped open cap belonging to the Moringaceae family cultivated in the tropical belt. Moringa leaves are an excellent source of antioxidants as it contains vitamin A, vitamin C, vitamin B, calcium, protein, potassium and plant pigments. Due to *Moringa oleifera* (M.O.) numerous uses it has been called tree of life in many cultures of the world [(6)]and[(7)].

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II. Material and Methods

A. Experimental animals

Twenty male albino rats (*Rattus norvegicus*) were used for the present study. The animals were obtained from the Animal House of the Faculty of Veterinary Medicine, Zagazig University, Egypt. Their weights ranged from 200-220g each**.** The animals were kept in cages under conditions of temperature 22±10 °C, with12h light and dark cycle and access to water and food *ad libidum*. The rats were housed in this condition for 2 weeks prior to the experiment for adaptation.

B. Zinc oxide nanoparticles

They were purchased from Sigma- Aldrich (St.louis MO,USA).The aqueous suspension of ZnO Nps was given orally in a dose level of (7.5mg/kg) which was selected on the basis of literature [(8)].

C. Moringae extract

The plant was collected from the pharmacognosy experimental farm, Faculty of Pharmacy, Zagazig University, Egypt in March ,5kg was extracted by maceration at room temperature -3times each time 24hour by 70% ethanol. The total extract was concentrated under reduced pressure. The total extract was 250g. 36g from the extract was dissolved in 900ml distilled water. The extract was given orally at a dose level of 150mg/kg b.wt as described by [(9)].

Experimental Design: The study was performed on twenty mature male albino rats (*Rattus norvegicus*), divided into four main groups, each group consisted of five rats as the following:

1)The control group: Animals received distilled water orally for 45 successive days.

2)The M.O. group: Animals treated orally with aqueous suspension of M.O. extract in a dose of 150mg/kg b.wt daily for 30 successive days using stomach tube.

3) The ZnO Nps group: Animals received orally aqueous suspension of ZnO Nps in a dose of 7.5mg/kg b.wt daily for 15 successive days using stomach tube.

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4) The ZnO Nps plus M.O. group: Animals received orally aqueous suspension of ZnO Nps in a dose of 7.5mg/kg b.wt daily for 15 successive days then were administered with aqueous suspension of M.O. extract in a dose of 150mg/kg b.wt daily for 30 successive days using stomach tube. At the end of the experiment, blood was collected from the retro-orbital vein of all animals [10]. Serum was harvested and subsequently used for the determination of hormonal assay of TH and FSH. Haematological analysis was carried out in the laboratories of Clinical Pathology Department at Zagazig University Hospital.The animals were sacrificed and the sperm smears were obtained from the caudae epididymes of their testes for estimation of the frequency of abnormally shaped sperms.

Methods:-

I) Estimation of the frequency of abnormally shaped sperms:

Collection of epididymal sperm smears: The sperm smears were obtained from the caudae epididymes of the testes of adult control and treated males. The caudae epididymes were cut into small pieces in 1ml saline solution. Sperm smears were obtained from the resulting suspension and they were stained by Feulgen nuclear stain. Approximately 1000 sperm cells were microscopically examined for each rat. A binocular microscope with X10 eyepices and X100 oil immersion objective lenses were used for this study. Abnormally shaped sperm cells were recorded randomly and microphotographs were taken whenever necessary.

II) Hormonal assay:

The assay used for the determination of TH and FSH in serum was enzyme linked immunosorbent assay (ELISA), using available kit purchased from Diagnostic Laboratories Inc (England). The assay was according to the method of [11].

Statistical analysis: Data were collected, arranged and reported as mean ± standard error of mean (S.E.M) of four groups (each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS/ version (15.0) .The statistical method was one way analyzes of variance ANOVA test (F-test) and if significant differences between means were found, Duncan's multiple range test (Whose significant level was defined as ($P<0.05$) was used according to [12] to estimate the effect of different treated groups.

III. Results

I- Estimation of the Frequency of Abnormally Shaped Sperms:

Table (1) and fig.(B) showed that the frequency of abnormally shaped sperms in the testes of male albino rat significantly were increased ($p<0.05$) after the oral administration of aqueous suspension of ZnO Nps in a dose of 7.5mg/kg b.wt daily for 15 successive days .The percentage of total deformed sperms reached 7.24% compared with 2.34% in control group. Meanwhile, ZnO Nps plus M.O. group elucidated a significant ($p<0.05$) decrease in total deformed sperms reached 3.74% versus 7.24% in ZnO Nps group. Although the ameliorative role of M.O. extract against ZnO Nps spermatotoxicity, the total deformed sperms increased significantly as compared with the control group, but the effect was much less intense compared with ZnO Nps treated group.

The sperm shape abnormalities involved either head or tail and/or head and tail regions. In the present investigation, the percentage of deformed head region from testis of male rats was significantly increased in ZnO Nps group to reach1.14% versus 0.02% in control group (fig.A). However, ZnO Nps plus M.O. group elucidated a significant decrease in total deformed head region reached 0.12% compared with 1.14% in ZnO Nps group .Moreover, the change scored was non significant compared with control group.

In the tail region, the administration of ZnO Nps to male rats resulted in a significant increase in the frequency of total deformed tail region with percentage reached 6.04% compared with2.32% in control group (fig.A). Meanwhile, the treatment of animals with the combination of ZnO Nps plus M.O. extract revealed a significant decrease in the frequency of sperms with deformed tail region reached 3.62% versus 6.04% in ZnO Nps group. At the same time, the result showed significant increase in the total deformed tail region compared with control group, but the effect was much less intense compared to ZnO Nps group. On the contrary, ZnO Nps administration to male albino rats afforded non significant changes in the percentage of total deformed head and tail regions when given alone or in its combination with M.O. extract.

II- Effect on TH and FSH levels:

Table (2) and figure (B) showed that the administration of the same dose of ZnO Nps mentioned above to male albino rats induced a significant ($p<0.05$) increase in the level of serum TH in all animals after fifteen days of oral administration of these Nps with percent change reached 88.8%. Moreover, ZnO Nps plus M.O. group elucidated a significant ($p<0.05$) decrease in TH level (36.8%) compared with ZnO Nps (88.8%) and these results illustrated the ameliorative role of M.O. extract against abnormal TH level induced by ZnO Nps .On the contrary, ZnO Nps administration to male rats afforded non significant changes in the level of FSH when given alone or in combination with M.O. extract.

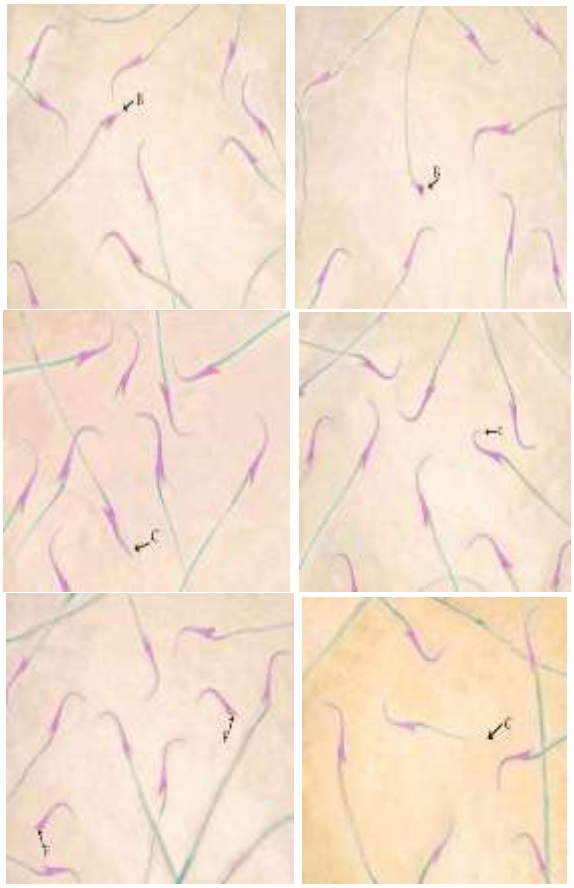

Fig. (A): different sperms from testes of male albino rats orally administered with aqueous suspension ZnO Nps in a dose of 7.5mg/kg b.wt daily for 15 successive days showing:N=Normal sperm ,A=Sperm without head, B= Triangular shaped head(left), Irregular shaped head(right), C=Straight acrosome, D=Sharply curved acrosome, F=Sperm without tail and G=shortening in tail region

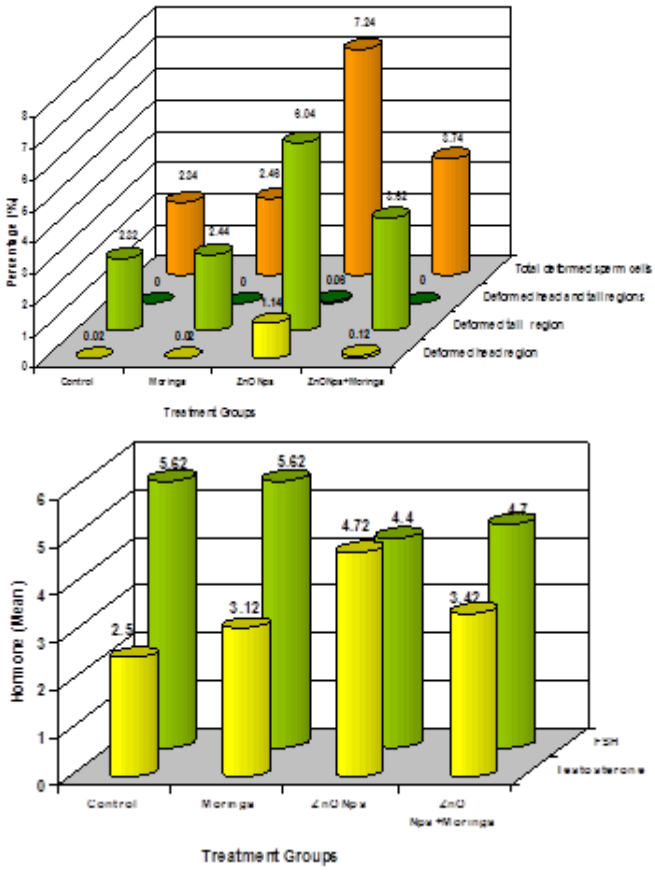

Fig.(B): Comparison between the effect of ZnO Nps (7.5mg/kg b.wt), M.O extract (150mg/kg b.wt) and their combination on the frequencies of deformed sperms from the testes(left) and the level of serum TH (ng/ml) and FSH (mIU/ml) of male albino rats(right).

TABLE (1): COMPARISON BETWEEN THE EFFECT OF ZNO NPS , M.O EXTRACT AND THEIR COMBINATION ON THE FREQUENCIES OF DEFORMED SPERMS FROM THE TESTES OF MALE ALBINO RATS

Groups	No. of rats	No of examined sperm cell 1000/rat	Deformed sperm cells									Total deformed sperms		
			Deformed head region			Deformed tail region			Deformed head and tail regions					
			No	%	Mean±SE	No	%	Mean±SE	No	%	Mean±SE	No	%	Mean±SE
1-Control G.	5	5000	1	0.02	0.20 ±0.20^{b}	116	2.32	23.20±1.49^{c}	0	0	0.00±0.00^{a}	117	2.34	23.40±1.63^{c}
2-Moringa G.	5	5000	1	0.02	0.20±0.20^{b}	122	2.44	24.40±1.32^{c}	0	0	0.00±0.00^{a}	123	2.46	24.60±1.43^{c}
3-ZnO Nps G.	5	5000	57	1.14	11.40±1.56^{a}	302	6.04	60.40±2.42^{a}	3	0.06	0.60±0.40^{a}	362	7.24	72.40±0.74^{a}
4-ZnONps+M.O. G.	5	5000	6	0.12	1.20±0.20^{b}	181	3.62	36.20±0.66^{b}	0	0	0.00±0.00^{a}	187	3.74	37.40±0.81^{b}

TABLE(2):COMPARISON BETWEEN THE EFFECT OF ZNO NPS, M.O EXTRACT AND THEIR COMBINATION ON THE LEVEL OF SERUM TH (NG/ML) AND FSH (MIU/ML) OF MALE ALBINO RATS.

Treated Groups	Hormones				
	No. of Rats	Testosterone		FSH	
		Mean±SE	% change	Mean±SE	% change
Control G.	5	2.50±0.14^{b}	-	5.62±0.75^{a}	-
Moringia G.	5	3.12±0.49^{b}	24.8↑	5.62±0.20^{a}	0
ZnO Nps G.	5	4.72±0.26^{a}	88.8↑	4.40±0.41^{a}	21.70↓
ZnO Nps+ Moringa G.	5	3.42±0.52^{b}	36.8↑	4.70±0.12^{a}	16.37↓

Means within the same column in each category carrying different litters are significant at ($p \leq 0.05$) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically. Similar letters are non significant on the statistical level ;% Change=(X_1-X_2/X_1)*100 ; X_1=Control mean ,, X_2= Treated mean.

IV. Discussion

The present investigation was designed to evaluate the toxic effects of ZnO Nps on sperm morphology and sex hormones (testosterone &FSH) and/or the ameliorative role of M.O. plant extract *in vivo* using male albino rats.

The results of the present study revealed that, ZnO Nps (7.5mg/kg b.wt daily for 15 successive days) can cause spermatotoxicity evidenced by a significant increase ($p<0.05$) in the frequency of abnormally shaped sperms in rat epididymis with percentage reached 7.24% compared with 2.34% in control group. These results are in conformity with [13] who concluded that zinc oxide lead to significant changes in sperm quality and quantity in adult male wistar rats . Zinc oxide Nps have spermatotoxicity in human sperm and may lead to infertility after exposure [14]. Also, it has cytotoxic actions on testicular germ cells in a dose dependent manner [15]. In addition[16] supported that the sperm percentage of head DNA showed a progressive reduction with increasing concentrations of ZnO Nps . So, zinc oxide Nps are capable of inducing genotoxic effect in human sperm and that effect is enhanced by its cytotoxicity. Also, a concentration-dependent induction of sperm DNA damage was observed in human spermatozoa treated with different doses of zinc oxide nanoparticles[17].

In our opinion, ZnO Nps spermatotoxicity may be induced mainly by its ability to produce reactive oxygen species (ROS) and free radicals. The imbalance between antioxidant defense and free radicals production causes a condition that is named oxidative stress and may cause toxicity and sperm damage. This result seem to be conceivable with that obtained by [18] who recorded that several *in vitro* studies have assessed the potential adverse health effects of NPs pointing out their ability to produce reactive oxygen species, release toxic ions and cause oxidative damage .

In our view, ZnO Nps toxicity may also be due to its very small size which makes the particles very reactive and can penetrate easy into the sperm heads and tails affecting their morphology. This view is greatly supported by [19] who concluded that nanoparticles do not have any special problem in passing the physiological barriers due to their small size so it can pass through cell membrane easily and even pass through blood-brain barrier and blood-testes barrier, so it can affect all of the body [2,3, 20].

Regarding the effect of *Moringa oleifera* extract, according to the present work treatment with M.O. after ZnO Nps administration afforded a significant decrease in the frequency of total deformed sperm to reach 3.74% compared with 7.24% in ZnO Nps alone. At the same time, the increasing in frequency of total deformed sperm was still significant compared with control group, but the effect was much less intense compared with ZnO Nps treated group and it may be avoided by using longer period of M.O. extract administration. This result is also in accordance with [21] and [22] who showed that exposure to chromium and *M. O.* extract significantly enhanced the sperm parameters compared to rats exposed to chromium alone .Also, *M. O.* leaf extract had a protective effect against the infertility-induced by electromagnetic radiation in rats. This was manifested by the improvement in sperm parameters [23]. The administration of ethanol leaves extract of M.O.to male rats ameliorated the sperm motility, sperm count, normal morphology and viability [24] .

From the present study, it is clear that M. O. extract reduced the percentage of sperm abnormalities induced by ZnO Nps by about 50% and this mainly is attributed to its anti-oxidant activity and free radical scavenging activity as M.O. leaves are excellent source of vitamins and essential micronutrients with powerful antioxidant activity. These findings are completely supported by [25] who proved that the sperm cytoplasm contained very low concentrations of scavenging enzymes therefore an increase in the antioxidant enzyme system levels by M.O. treatment can favour the reproductive process and also enhances spermatogenesis.

In the present study, the administration of the same dose of ZnO Nps mentioned above to male albino rats induced a significant ($p<0.05$) increase in the level of serum TH in all animals after fifteen days of oral administration with percent change reached 88.8%↑ compared with control group .This also may be due to oxidative stress mediated by increased free radical generation and depletion of antioxidants causing disturbances in Leydig cells secretion of TH. These results are in full agreement with [26] who recorded that ZnO Nps caused significant and dose-related increase in TH level in rats. NPs increase free radical levels inside the cells and cause tissue damages. Also, [27] added that most of the adverse effects of NPs on male reproductive function are mainly due to modification of the testicular structure, impairment of spermatogenesis and alteration in the biosynthetic and catabolic pathways of testosterone.

It seems apparent from the present result that, treatment with M.O. after ZnO Nps administration significantly decreased the abnormal TH level to reach 36.8% compared with 88.8% in ZnO Nps alone . This may be explained by M.O. as antioxidant restored the altered levels of testosterone hormone. This result is in accordance with that reported by [28] who reported that the antioxidants present in the leaves of the plants, M.O. acting in concert with the antioxidant system present in the epididymis preserved and enhanced the process of spermatogenesis ostensibly by its ability to reverse the levels of FSH and LH and ultimately testosterone. Moreover, [29] and[30] added that M.O. treated rats restored the hormonal status and improve the male sexual function .Where, the leaves of this plant contain a profile of important trace elements and are a good source of proteins, vitamins, beta-carotine, amino acids and various phenolics [31]. On the contrast, ZnO Nps administration to male rats afforded non significant changes in the level of FSH when given alone and in its combination with M.O. extract. On the same bases [32] recorded that oral administration of zinc oxide nanoparticles to wistar rats showed non significant difference in the sexual hormone level (FSH) between control and treated group . Also, M.O. does not affect serum FSH level but enhances seminiferous tubule, epididymis, testis and seminal vesicle in male mice [33].

From the above results, it is worth mentioning that the combination between M.O. leave extract and ZnO Nps

treatment afforded clear ameliorative effects against ZnO Nps induced sperm shape abnormalities and abnormal testosterone level.

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