RESEARCH ARTICLE

Effects of anxiolytic doses of ZnO nanoparticle on ECG parameters in restraint and non-restraint ovariectomized female rats

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ABSTRACT

Objective(s): According to the effects of nano-ZnO on physiological behaviors in animals, in the present study we have evaluated the effects of nano-ZnO anxiolytic doses on electrocardiogram (ECG) sings changes in the restraint and non-restraint ovariectomized (OVX) female rats.

Methods: Female rats (160-180 g) were divided into: control (non-OVX + saline) and OVX groups including: control(saline), nano-ZnO 1, 2.5 and 5*mg/kg*, stress of 90 or 180 min + saline and stress of 180 min+ nano-ZnO 2.5*mg/kg*. Elevated plus maze (EPM) and hole board apparatus were used to measure of anxiety-like behaviors, thirty min after intraperitoneally injections or stress induction. ECG parameters and serum zinc level were measured in all groups.

Results: Ovariectomy induced anxiety and restraint stress in OVX rats increased it in the hole board test. Nano-ZnO 2.5 and 5 *mg/kg* improved anxiety-like behaviors in the EPM test. In the stressed rats, nano-ZnO 2.5 *mg/kg* improved anxiety-like behavior in the hole board apparatus. Nano-ZnO increased zinc level in a dose-dependent manner in the non-restraint OVX rats. Nano-ZnO 1 *mg/kg* decreased QRS amplitude and all doses decreased QT interval to the level of non-OVX group. Nano-ZnO 5 *mg/kg* alleviated QTc to the level of non-OVX group. Stress of 90 min increased QT interval while stress of 180 min decreased it. Nano-ZnO after stress induction could alleviate heart rate, R-R- interval and QRS interval to the level of non-OVX group.

Conclusion: It seems that nano-ZnO rather than could improve anxiety, lleviated ECG parameters in the restraint and non-restraint OVX rats.

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INTRODUCTION

It has been shown that in laboratory animals ovarian hormones depletion may development the depression and anxiety-like behaviors in the stressful situations [1, 2]. Also, postpartum progesterone withdrawal can induce depression and increase anxiety in the laboratory animals [3]. These indicate the important roles of ovarian hormones on mode and behaviors in the female genus. On the other hands, zinc as an important element is necessary for neural and hormonal activities in the body [4, 5]. Zinc supplementation may be effective in reducing anger and depression in a young woman [6]. Zinc ions can affect estrogen secretion from adrenal glands and help to maintain serum calcium and phosphorus contents at the normal level in long-term ovariectomized rats, also zinc supplementation to ovariectomized rats can be useful in restoring the calcium mechanism [7]. It has been shown that in mice, ovariectomy could raise hippocampal synaptic vesicle zinc level, whereas estrogen replacement could lower it [8].

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Today, metal oxide nanoparticles have been used in medicine, pharmacy and drug delivery systems [9, 10]. Previously, we have shown that nanoparticles of zinc oxide (nano-ZnO), as a new source of zinc ions, can affect some behavioral parameters in animal models including; anxiety-like behaviors, pain perception and memory [11-13].

Zhai et al. (2018) have reported that in the female mice, nano-ZnO can affect pre- and post-natal O'ogenesis related processes and result in increased DNA damage and decrease the ovarian follicle reserve [14]. Also, our study on female rats has indicated that acute injection of nano-ZnO could reduce acute somatic pain and induce analgesic effects in them [13].

On the other hands, there are some studies on zinc effects in cardiovascular functions and it has been shown that high dietary zinc intake is associated with a greater incidence of cardiovascular disease in women and zinc level can affect cardiovascular functions [15, 16]. Is has been shown that, magnesium oxide nanoparticles could induce changes on cardiovascular system, including heart rate and electrocardiographic indexes, but there is not clear study about the effects of nano-ZnO on ECG parameters [17].

Since, stress can trigger both atrial and ventricular arrhythmias in ECG [18], and also can induce anxiety-like behaviors in the rodents [11, 19, 20]; so that, in the current experimental work we have tried to investigate the effects of nano-ZnO on anxiety-like behaviors and ECG parameters in the ovariectomized female rats under acute stress condition.

MATERIALS AND METHODS

Animals and grouping

Female Wistar rats (160-180g) were randomly divided into eight groups of control (non-OVX+ saline), OVX + saline, OVX + nano-ZnO 1, 2.5 and 5 *mg/kg*, OVX + stress of 90 min (ST 90 min) + saline, OVX + stress of 180 min (ST 180 min)+ saline and OVX + ST 180 min + nano-ZnO 2.5 *mg/kg*. All doses of nanoparticle and stress times were selected based on our previous works [11, 12, 21]. Animals were provided from animal house of Veterinary Science of Shahid Chamran University of Ahvaz and kept in a room under standard laboratory conditions (ambient 23±2 °C temperature, 12-h light-dark cycle). Nano-ZnO (USnano, Co., USA) suspension was prepared by sonication in saline and was shacked before every injection to reduce aggregation. Control groups just received saline 1 *mL/kg* and all components injected intraperitoneally (i.p.). Thirty minutes after injections, first behavioral test was performed and in every group, 6-7 animals were used. All experiments were performed in an animal physiology laboratory and approved by the Ministry of Science, Research and Technology committee in the Shahid Chamran University of Ahvaz (EE/96.24.3.88369/scu.ac.ir).

Surgery procedure and stress induction

All subjects were anesthetized by i.p. administration of ketamine hydrochloride and xylazine (60/4 *mg/kg)*, and then ovaries were removed bilaterally. During the surgery and after that the animals were treated with topical antibiotic at the site of surgery. Ovariectomized female rats were placed in a community cage for ten days recovery period. A control operation, during which the ovaries were just touched with forceps, was performed on the control group. To minimize hormonal influence in the control group, animals were kept in a cage for more than 2 weeks before the begging of the experiments. Acute stress was induced by animals placing into the plexiglas tubes for 90 and 180 min. Animals were received i.p. injections, after restraint times [11].

Behavioral tests

Two different unconditional models were used to measure of anxiety-like behaviors.

Elevated plus maze (EPM)

The wooden elevated plus maze made was elevated 50 *cm* above the floor and was consisted of two equal open and two closed arms, which have 40 *cm* walls, and with a central square. The subjects were placed in the center square facing an open arms and were explored the maze freely for 5 min. In this apparatus, anxiety-like behaviors parameters including; the percentage of time spent in open arms (%OAT) and the percentage of open arm entries (%OAE) were calculated according to the below formula:

%OAT= (time spent in open arm/time spent in open + closed arm) ×100

%OAE= (number of open arm entries/ number of open + closed arm entries) ×100.

Locomotor activity was evaluated by counting total entries in the both open and closed arms [22].

Hole board

Animals passed the hole-board test thirty minutes after the EPM test. The apparatus was

elevated 50 *cm* above the ground on a chair and was made of a flat square disk (35×35 *cm*) that has 16 regulate holes (4×4 *cm*), which diagonal of each hole was 3 *cm*. Animal first sits in the center of the apparatus and its behavior evaluated during 5 min and recorded by a camera. In this apparatus, each animal was used just for one and there was no any prior instruction or learning. Two anxiety indexes from this apparatus including, latency time (time that animal for the first time plunged its head in one of the holes) and head-dipping (count of the animal inserts the head into the hole in 5 min) were recorded. In this method, if latency time was low and head entries in the holes were high these show anxiety increased and the inverse of these cases shows anxiety reduced [23].

Electrocardiogram parameters (ECG recording)

After hole board tests, all animals anesthetized with a i.p. injection of mixture of ketamine hydrochloride and xylazine (60/4 *mg/kg)* and ECG parameters including; heart rate (beat/min), R-R interval (sec) QRS interval (sec), QT interval (sec), QTc (sec), QRS (mV) and T amplitude (mV) were recorded by Bio-Amp from lead II during in 2 min and monitored by a Power Lab system (AD-Instruments, Australia).

Assessment of serum zinc concentration

At the end of ECG measurement, blood was collected by cardiac puncture in all animals and serum zinc content was analyzed by an atomic absorption spectrophotometer (Avanta, GBC, Australia) and expressed as a *μg/ mL* of the serum.

Data analysis

All data were analyzed by using SPSS 16 software and were expressed as the mean ± SEM. One way ANOVA with the Tukey post hoc was used for comparison among multiple groups and unpaired student t-test was used for the comparison between two groups. Pearson correlation between anxiety parameters and serum zinc level changes was evaluated by SPSS 16 software. In all cases difference with p value of <0.05 were considered significant.

RESULTS

Anxiety-like behaviors experiments in the EPM and hole board tests

Fig. 1 is the scanning electron microscopic (SEM) image of dry powder of nano-ZnO that was provided before the study.

Results of the EPM test showed that the nano-ZnO 2.5 and 5 increased %OAT ($F(3,24)=10.90$, $P<0.05$) (Fig. 2A).

In the EPM apparatus, stress could partially increase anxiety level, although it was not significant (OAT ($F(2,18)=2.90$, $P>0.05$) and OAE ($F(2,18)=2.75$, $P>0.05$) (Fig. 2B). Also, nano-

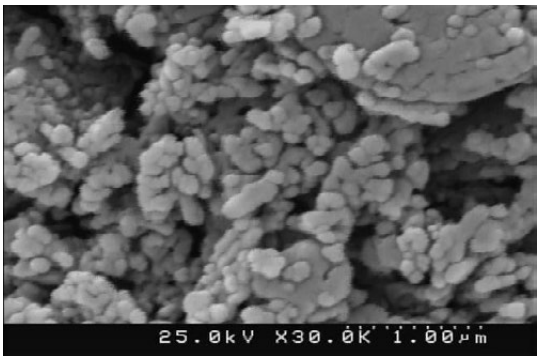

Fig.1. Nano-ZnO image provided by the SEM.

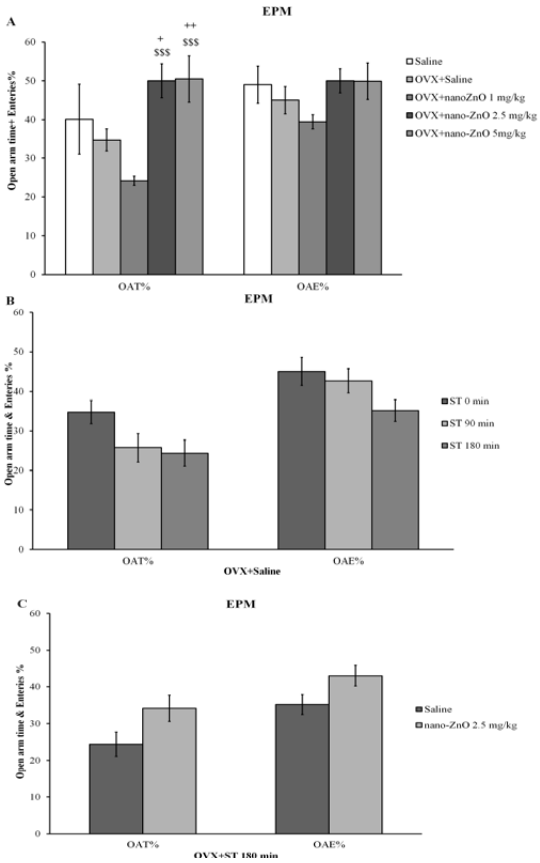

Fig.2. Anxiety- like behaviors in the EPM test. +P<0.05 and ++P<0.01, are in comparison with the OVX + saline. $$$P<0.001, is in comparison with the OVX + nano-ZnO 1. OVX= Ovariectomized, ST= Stress.

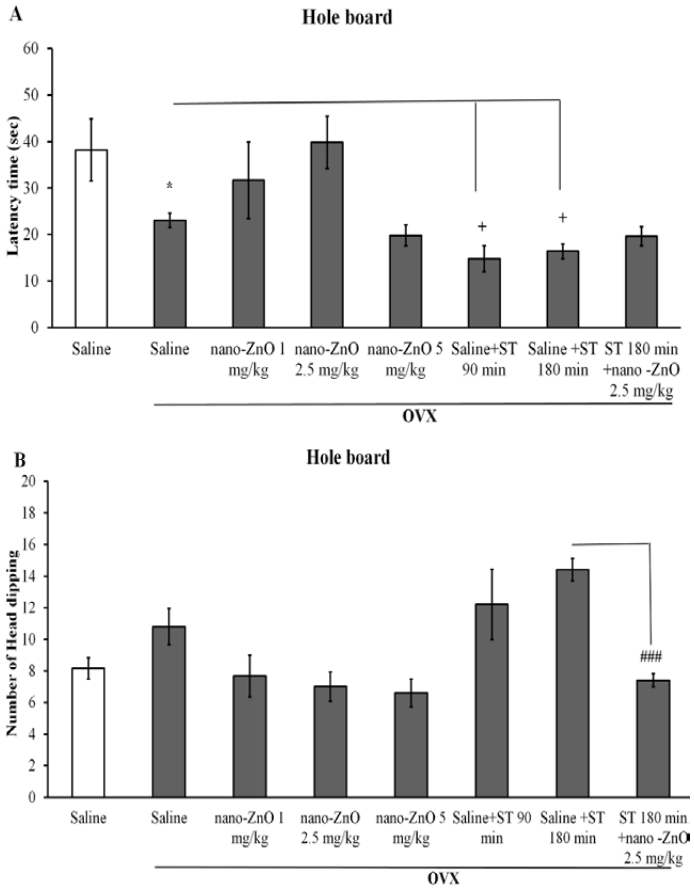

Fig.3. Anxiety-like behaviors in the hole board test. * P<0.05, is in comparison with the saline. +P<0.05, is in comparison with the OVX+ saline. ###P<0.001, is in comparison with the OVX+ saline+ ST 180 min. OVX= Ovariectomized, ST= Stress.

ZnO could not change anxiety parameters (OAT (t(12)=2.006, P=0.067) and OAE (t(12)=2.005, P=0.068)) in the stressed rat (Fig. 2C).

On the other hands, hole board test showed that ovariectomy significantly decreased latency time of the first head dip in comparison with control (non-OVX) group (t(12)=2.22, P=0.046) and nano-ZnO injection, especially in the dose of 2.5, could partially increase it (F(3,24)=3.048, P>0.05) (Fig. 3A). Also, stress in both time decreased latency time and induced anxiety in hole board test (F(2,18)=4.41, P<0.05) (Fig. 3A). Nano-ZnO decreased number of heads dipping in the hole board test and improved anxiety in the stressed rats (t(12)=8.51,P<0.001) (Fig. 3B). Locomotor activities did not change in the EPM test (Fig. 4).

Assessment of serum zinc concentration

Data in Fig. 5 show that nano-ZnO 2.5 and 5 (F(3,20)=7.145, P<0.05 and P<0.01, respectively) increased serum zinc level while it did not change in all stressed rats without (F(2,15)=1.004, P>0.05) and with nano-ZnO administration (t(10)=0.67, P=0.51).

Statistical analysis showed that the Pearson correlation between serum zinc concentration and anxiety parameters in the EPM (OAT% (R= 0.312, P=0.031)) and hole board tests (number of head dipping (R= -0.286, P=0.049)) were significant.

Assessment of ECG parameters

Fig. 6 shows a typical recording of heart rate and ECG that were recorded by Bio-Amp from lead II.

Data in Table 1 show that in OVX animals all ECG parameters including; heart rate (beat/min) (t(10)=3.10, P=0.011), R-R interval (sec) (t(10)=2.995, P=0.0135), QRS interval (sec) (t(10)=2.92, P=0.0152), QT interval (sec) (t(10)=5.500, P=0.0003), QTc (sec) (t(10)=5.43, P=0.0003), and T amplitude (mV) (t(10)=2.562,

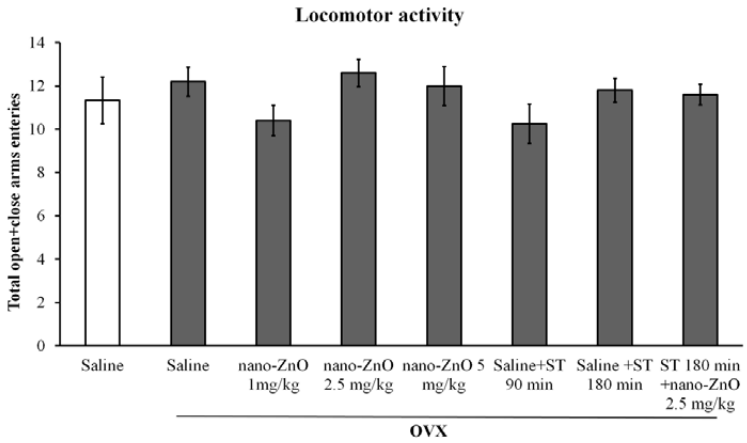

Fig.4. Locomotor activity in the EPM test. There were no significant differences between all groups. OVX= Ovariectomized, ST= Stress.

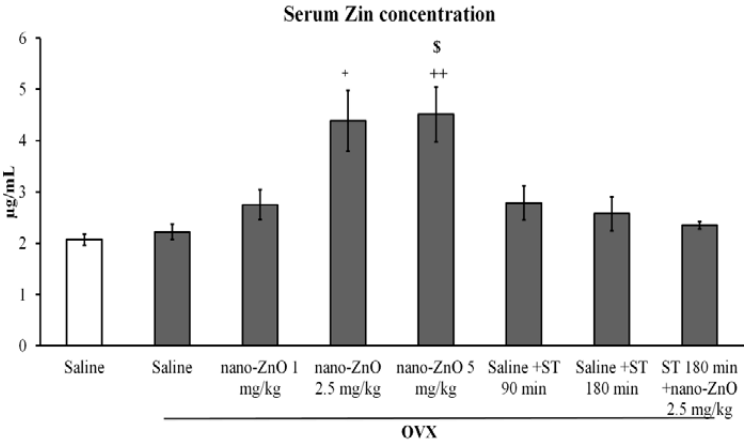

Fig. 5. Assessment of serum zinc concentration. + P<0.05 and ++P<0.01, are in comparison with the OVX+ saline. $P<0.05, is in comparison with the OVX+ nan-ZnO 1. OVX= Ovariectomized, ST= Stress.

Fig. 6. A typical recording of heart rate and ECG.

Table 1. Assessment of ECG parameters

Groups	Heart rate (beat/min)	R-R interval (sec)	QRS interval (sec)	QT interval (sec)	QTc (sec)	QRS (mV)	T amplitude (mV)
Control (Saline)	273.7333 ±7.216	0.220767 ± 0.006064	0.013523 ± 0.00013	0.044057± 0.000308	0.09408333±0.0 01553	0.8038± 0.027	0.169067± 0.01189
OVX+ Saline	245.74± 5.417*	0.24488 ± 0.005295*	0.01823 ± 0.001605*	0.07234 ± 0.005133 ***	0.145±0.009235 ***	0.7459 ± 0.035	0.22278± 0.01726*
OVX+ nano-ZnO 1 *mg/ kg*	238.08± 9.341	0.25436 ± 0.009964	0.015658± 0.00117	0.045343± 0.002406 +++	0.1085725±0.01 443	0.4110 ± 0.0758++	0.1775± 0.008347
OVX+ nano-ZnO 2.5 *mg/ kg*	245.8± 5.386	0.245023 ± 0.005477	0.020203± 0.002503	0.047512± 0.00044 +++	0.12774±0.0151 7	0.74925 ± 0.01525$$	0.194567± 0.0166
OVX+ nano-ZnO 5 *mg/kg*	225.76± 6.448	0.26718 ± 0.00808	0.015292± 0.000351	0.048222± 0.000626 +++	0.096675±0.001 652+	0.673336± 0.064 $	0.18704± 0.006946
OVX+ ST 90 min	259.94± 11.651	0.23362 ± 0.01032	0.01497± 0.000527	0.08766± 0.0005 ++	0.1552666±0.01 225	0.71499 ± 0.03132	0.19492± 0.02072
OVX+ ST 180 min	253.24± 6.49	0.23792 ± 0.006158	0.014726± 0.000714	0.048033± 0.000337 +++, &&&	0.117152±0.014 17	0.8685± 0.03799 &	0.21328± 0.01475
OVX/ ST 180 + nano ZnO 2.5	265.4± 5.381 #	0.2267 ± 0.004798#	0.014152± 0.000626#	0.048663± 0.000513	0.1208925±0.01 279	0.743983± 0.03104	0.20096 ±0.015

*P<0.05, **P<0.01 and ***P<0.001 are in comparison with the saline. +P<0.05, ++ P<0.01 and +++P<0.001, are in comparison with the OVX+ saline. #P<0.05, is in comparison with the OVX+ nano-ZnO 2.5. $P<0.05 and $$P<0.01, are in comparison with the OVX+ nano-ZnO 1. &P<0.05 and &&&P<0.001, are in comparison with the OVX+ ST 90 min. OVX= Ovariectomized, ST= Stress.

P=0.028) changed, while QRS (mV) did not change even though decreased partially (t(10)=1.303, P=0.22). On the other hands, all doses of nano-ZnO decreased QT interval and reach to the level of non-OVX animals (F(3,20)=19.768, P<0.001). Nano–ZnO in a dose-dependent manner could decrease QTc and reach to the non-OVX animals that it was significant in nano-ZnO 5 (F(3,20)=3.446, P<0. 05). Stress of 90 min increased QT interval while stress of 180 min decreased it (F(2,15)=45.27, P<0.01 and P<0.001, respectively).

Nano-ZnO 1 decreased QRS complex (F(3,20)=8.963, P<0.01) and stress of 180 min significantly increased it (F(2,15)=5.406, P<0.05) in comparison with stress of 90 min.

At the end, even though stress induction or nano-ZnO 2.5 injection, alone could not change heart rate, R-R interval and QRS interval, but the simultaneous presence both of them increased heart rate (t(10)=2.57, P=0.027), decreased R-R intervals (t(10)=2.516, P=0.030), QRS interval (t(10)=2.345, P=0.0410) and neutralized the effects of each other on QT interval.

DISCUSSION

This study has indicated that ovariectomy induced anxiety-like behaviors and changed all ECG recorded parameters; these may be related to the sex hormones effects and their importance on anxiety and cardiovascular functions [24- 26]. It has been indicated that, in the women menstrual cycle can affect heart rate, QT and QTc intervals [27].

Acute injection of nano-ZnO in the OVX female rat could improve anxiety-like behaviors in non-restraint situation in the EPM test while in stressed group just in the hole board test could improve anxiety that these results can be due to the differences of these two tests [11, 23]. Previously, we have showed that nano-ZnO could improve anxiety-like behaviors in the EPM test in the restraint and non-restraint male rats [11, 21, 22].

In this work, zinc level did not change in OVX rats, but nano-ZnO increased its level in non-stressed rats. Our previous studies have been demonstrated that there is a positive correlation between anxiety-like behavior and zinc level in the male rats and probably changes of other elements contents such as magnesium, calcium and iron levels in their systemic circulation and hippocampus area can be responsible for behavioral changes following nano-ZnO administration in the male rats [11, 21].

Stress could also change QT interval in the OVX rats. As mentioned before, stress can trigger both atrial and ventricular arrhythmias and lead to ECG parameters changes such as QT interval and T wave alteration [18]. Also, it has been shown that acute mental stress can increase heart rate, decrease PR interval, QT interval and prolong QTc interval in the humans [28]. These mentioned researches

also confirm our results.

In this study, nano-ZnO effects on ECG parameters in the restraint and non- restraint OVX female rats were evaluated. Nano-ZnO in lowest dose decreased QRS amplitude, also in all doses significantly decreased QT interval in non-restrain OVX rats and reached to the level of non-OVX animals.

In mammalian cardiomyocytes zinc plays an important role in excitation-contraction coupling [29]. It has been shown that the excess zinc ion influences cardiomyocyte electrical activity, although it does not affect the resting membrane potential of them, decreases the time to peak action potential amplitude and affects calcium current [30].

Also, zinc improves cardiac muscle cells action and reduces arrhythmias in the rodent [31]. Zinc-pyrithione exposure, in the isolated papillary muscle strips of the rat heart could prolonge action potential repolarization phase and slowdown in both contraction and relaxation rates of twitch activity probably by endogenous generation of reactive nitrogen species [32]. Yi et al, (2013) have been shown that in the isolated cardiomyocytes zinc ions could suppress cardiomyocyte systolic function and enhance relaxation function by lowering intracellular calcium levels [33]. Previously we have been shown that the nano-ZnO could increase serum calcium concentration in the male rats [11]. Therefore, probably zinc ions released from nano-ZnO or calcium level changes could affect the cardiomyocyte electrical activity and change ECG parameters such as QT interval. Even though the nano-ZnO injection or stress induction alone could not change heart rate, R-R and QRS interval, but an injection of nano-ZnO after induction of stress could significantly reach these parameters to the non-OVX rat levels. The anxiolytic dose of nano-ZnO showed a multiplier effect with the stress effects on cardiovascular functions.

CONCLUSION

It seems that the nano-ZnO in anxiolytic doses can alleviate some ECG parameters in OVX female rats, as well interfere with stress effects on the ECG parameters probably through changes in the transient or current of ions channels of cardiac cells. The exact mechanisms of nano-ZnO effects on ECG parameters changes need to more investigation.

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CONFLICT OF INTEREST

There is no conflict of interest.

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