Nano and Conventional ZnO with Aerobic Exercise have Effects on Some of Hematological Parameters in Male Rat

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Abstract: According to the role of exercise and some elements like zinc on hematological parameters, the aim of this study was investigation the effects of endurance exercising with conventional zinc oxide (cZnO) and/or zinc oxide nanoparticles (ZnO NP) on hematological parameters in adult male Wistar rats. In this study sixty Adult male Wistar rats were divided into groups: controls (receiving saline, without physical activity), physical activity, receiving cZnO and/or ZnO NP (1mg/kg i.p.) daily (5 days in a week) for 6 weeks with and without physical activity. 30 minutes after injection, physical activity groups were doing daily physical activity protocol. After 24 hours of the last practice, animals were became anesthetized and whole blood was gotten directly from their heart and blood samples were transferred to laboratory in order to measure hematologic parameters. Data showed that exercise caused meaningful decrease in red blood cells, hemoglobin and hematocrit amount lonely, in comparison with control group. Also using cZnO and ZnO NP causes meaningful increase in red blood cells, hemoglobin and hematocrit amount in comparison with control group and exercising group. For other parameters no difference was seen between groups ($p<0.05$). Our study showed that aerobic exercises may cause improvement in some hematological parameters. We didn't see meaningful difference between cZnO and ZnO NP alone and with exercise on hematological parameters. It seems that interaction between zinc oxide supplements and exercise on hematological parameters need to more studies.

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1. Introduction

Zinc is one of the most important elements and the fourth essential element in the human body (Ebadi and Pfeiffer, 1984) which has important roles in operation of cells such as enzyme operation (Vallee and Auld, 1990), metabolism of nucleic acid (Miller et al., 1967; brown et al., 1985) and cell massage transmission (McNulty and Taylor, 1999). Also zinc is essential for physiological processes including growth (Prasad, 1985), lipid metabolism (Cunnane, 1988) and brain operation (Endre, 1975).

In the last years, athletes have been using different methods for improvement their performances. Some researchers reported that the existence of zinc in muscles causes increases in power and much endurance (Isaacson and Sandow, 1978), and using zinc as a supplementary causes athletic performance improvement (Krotkiewski et al., 1982), also lack of that causes reduction in physical operation (Golub et al., 1994). On the other hand athletes optimal performance depends on a lot of physiological factors, that blood factors are one of the most important of them (Mercer and Densmore, 2005). Studies on Various blood indexes showed different results; in last research projects the effect of physical activities on parameters of hematology indicates decrease, increase or no change in these parameters (Wu et al., 2004; Fujitsuka et al., 2005; Havil et al., 2003; Robson et al., 2007).

Physical activity can cause the body excrete zinc too much by urinating and sweating that it lead to a decline in the deal of zinc (Anderson and Guttman, 1988). Lack of zinc brings a lot of anemia (Ece et al., 1997; El Hendy et al., 2001; Podel, 1984). Few studies have engaged in the effects of zinc supplements on blood parameters in athletes body. Cordova and et al (1993) have shown that zinc supplements increase the some blood parameters in rats. Southern and Baker (1983) have reported that in broiler chicks zinc supplements cause blood parameters reduce. On the other side, Donmez et al (2002) shown that zinc supplements have no effect on blood parameters in broiler chicks.

Todays, many of food production corporations prefer to use salt gotten from main elements like zinc oxide than salt gotten from their sulfide form, because it decreases toxic effect of these compounds in long time usage (Hardy et al., 1999). In

last years the use of Nano scale materials has developed quickly, and nanoparticles of oxidized elements like zinc oxide nanoparticles (ZnO NPs) are used in different useful industrial, hygienic, etc. the wide variety of nanoparticles application has caused these compounds to go through human's life system and environment very fast (Handy et al., 2008). In addition, there is no report about the effects of cZnO and ZnO NP on blood parameters alone and with exercising, so the goal of this study is to compare effects of these compounds with and without exercising on blood factors.

2. Material and Methods

Animal care: Sixty adult male wistar rats (initial weight 185 ± 20g) were used and housed in groups of 4 per cage and kept under standard laboratory conditions (temperature 22 ± 2°C, 12/12 h light - dark cycle). After one week they were randomly divided into six homogenous groups (n =10) and treated daily (5 days week) for 6 weeks as follows saline-control group, groups that received saline with exercise, groups that received cZnO (Merk Co; Germany) and ZnO NP (Lolitech Co; Germany, < 50 nm) (1mg/kg intraperitoneally (i.p.)) with and without exercise.

Supplementation: For injection of ZnO NP and cZnO, they were dispersed in saline %0.9, by ultrasonic bath (S2600) for 15 minutes. All drugs injected as a volume of 10 ml/kg. Thirty minutes after injection, training groups allowed exercising.

Exercise training protocol: Treadmill training began by familiarizing the rats with the apparatus for 4 days by placing them on the motorized-driven treadmill. The training group exercised 5 days/week for 6 weeks. Whole period exercise was divided into the three steps: introductory stage: in the first week rats exercised on a treadmill at a speed of 10 m/min. The angle of inclination was 0%, and a running time was 10-15 min/day, overload stage: in the second and third weeks the speed was increased to 12 to 28 m/min with a 0% gradient, and the duration was increased to 15 to 60 min/day and maintains stage or stabilizes the work intensity: in the four to six weeks the speed remained constant at 28 m/min, the angle of Inclination was %0, and the exercise duration was 60 min/day..24 hours after the last exercising, while rats were completely fast (didn't eat anything for 12 hours), they became anesthetized by ether, and their blood was gotten directly from their heart and poured in the test tubes including EDTA, and was delivered to laboratory by cell counter machine.

Statistical analyses:

Data are presented as Mean ± SEM. Statistical differences were determined by Anova followed by the student Newman Keuls post hoc test. $p<0.05$ was considered as significant level.

Table 1. training protocol

Stages	Speed(meters /minute)	Exercise time(min)
Introductory stage	10	10-15
Overload	12-28	15-60
stabilize	28	60

3. Results

Data analyzing showed that after six weeks, exercising caused meaningful decrease of red blood cell average, hemoglobin and hematocrit. (Table 2)

Also the result of blood parameters of receiver groups (cZnO and ZnO NP) with no exercising showed that number of red blood cells of these groups had meaningful increase in comparison with control group ($p<0.05$), but there was no meaningful difference between zinc supplementary receivers and zinc nanoparticle receivers. For other parameters of hematology (Hb, Hct, Wbc, Plt, Mcv, Mch, Mchc) no meaningful difference was seen. ($p<0.05$) (Table 3). Blood parameters results of the groups with exercising together with zinc supplementary and zinc nanoparticles are shown at table 4.

The results of table 4 showed that number of red blood cells in groups of cZnO with exercising and groups of ZnO NP with exercising has meaningful increase in comparison with exercising group alone ($p<0.05$), and also hemoglobin and hematocrit amount in these groups had meaningful increase in comparison with exercise. In other hematological parameters (Wbc, Plt, Mcv, Mch, Mchc) there were no noticeable differences among all groups. ($p<0.05$).

Table 2. Hematological parameters in control group and exercise group. Values are given as mean ± SEM. student t-test was used to comparison between groups. *P<0.05 in compared with saline control group

Group / **Parameters**	**Saline control**	**Saline Exercise**
Rbc counts (×106/µL)	7.122±0.959	*6.808±0.564
Hematocrit (%)	38.240±4.403	*33.060±0.818
Hb values (g/dl)	14.330±0.847	*12.650±1.036
Mcv volume (fL)	53.650±1.290	53.01±0.741
Mch (pg)	17.810±1.350	17.910±0.667
Mchc (g/dL)	33.360±2.154	33.380±1.798
Wbc (×103/L)	8.612±0.895	11.450±4.885
Plt (×103/µL)	797.90±61.150	738.20±117.90
Numbers(N)	10	10

Table 3. Table3. Hematological parameters in control group and supplemental groups without exercise. Values are given as mean ± SEM. :*P<0.05 in compared with saline control group.

Gr / Par	Saline Control	cZnO	ZnO NP
Rbc	7.122±0.959	*7.837±0.444	*7.875±0.260
Hct	38.240±4.403	38.731±0.558	38.634±0.705
Hb	14.330±0.847	14.754±0.355	14.942±475
Mcv	53.650±1.290	50.980±2.113	51.235±2.382
Mch	17.810±1.350	18.760±1.029	18.20±0.802
Mchc	33.360±2.154	34.940±1.533	34.860±0.890
Wbc	8.612±0.895	10.895±4.214	9.883±2.727
Plt	797.90±61.150	752.20±264.95	798.30±105.238
(N)	10	10	10

Table 4. Hematological parameters in exercise group and groups on supplemental diets with exercise. Values are given as mean ± SEM. *P<0.05 in compared with saline exercise group.

Gr / Par	Saline Exercise	cZnO + Exercise	ZnO NP + Exercise
Rbc	6.808±0.564	*7.176±0.561	*7.153±0.116
Hct	33.060±0.818	*37.992±1.052	*36.905±0.896
Hb	12.650±1.036	*13.992±0.394	*13.985±0.176
Mcv	53.01±0.741	51.90±1.635	52.090±0.952
Mch	17.910±0.667	17.960±1.259	18.960±0.871
Mchc	33.380±1.798	34.720±1.240	34.930±1.615
Wbc	11.450±4.885	12.780±1.912	11.580±2.493
Plt	738.20±117.90	643.80±197.860	797.50±79.248
(N)	10	10	10

4. Discussions

Results of this study showed that exercise after 6 weeks caused significant decrease in erythrocyte index, our results are similar to the studies of Fujitsuka et al and Schumacher et al but there are opposite to results of Wu et al (2005; 2002; 2005).

Maybe reduction of red blood cells in this study is because of two factors. 1) Reduction of red blood cells precursors. 2) red blood cells demolition because of foot mechanical impact and damage to the old red blood cells in whirling small flows and probably abdomen-intensity bleeding. On the other side the explanation of hematocrit reduction can be due to the increase in plasma amount (Mellion, 2003), and the probable reason for hemoglobin reduction can be related to blood volume. We should notice the probably damage to red blood cells too.

Data showed, after six weeks, cZnO and ZnO NP caused significant increase in number of red blood cells in comparison with control group, but there were no differences in hemoglobin and hematocrit volume.

About using zinc supplements, some studies have shown that using zinc sulfate had no effect on number of red blood cells(Donmez et al., 2002), while some other studies have shown that in the lack of zinc, volume of erythrocyte indexes (hemoglobin and hematocrit) decrease (El Hendy et al., 2001), and zinc supplements can improve these indexes (Khaled et al., 1999).

Shortage of zinc probably has some effect on anemia outbreak, because the mixture of iron citrate and zinc increases consistency of iron in red blood cells, and level of albumen, according to these studies, zinc is essential for expanding red blood cells (Huber and Cousins,1993; Baltaci et al., 2003).

Studies have shown that metallothionein synthesis in precursor cells that are sensitive to erythropoietin in marrow, it has been demonstrated that zinc increased production of metallothionein in marrow of rats (Gyoffy and Chan, 1992). Parameters related to the body metabolism such as phosphorus and albumin of serum decrease in shortage of zinc and erythropoietin of serum, and all of these parameters become better after receiving of zinc (Nishiyama et al., 1996). Zinc has roles in some aspects of blood making, such as enzyme systems that participate in DNA synthesis (Thymidine kinase and DNA polymerase) (Prasad and Oberleas, 1971) and also Zinc – Finger (GATA – 1) is essential for erythropoiesis (Prasad and Oberleas, 1971; Labbaye et al., 1995). Lukaski (2005) has shown that while playing sports, low levels of zinc plasma is related to reduction of carbonic anhydrase in RBC activity and in metabolic disorder responses.

As following in table 3, number of red blood cells and hemoglobin and hematocrit amount in groups that receiving ZnO NP with exercises showed meaningful increase in comparison with exercising group alone. It has been reported that zinc sulfate increases number of blood cells in rats with swimming exercises and zinc shortage causes reduction of blood cells(Baltaci et al., 2003). On the other hand, it has been shown that using zinc sulfate and zinc picolinate with exercise causes significant increase in number of red blood cells, hemoglobin and hematocrit (Kilic et al., 2004; Polat, 2011)in compared with exercise group alone. The important thing in physical activities is that oxygen requirement increases (Brun, 2002). The increase in erythrocyte of people who got shows, probably zinc can make better delivering oxygen into tissues by increasing red blood cells and help athlete's performance (Kilic et al., 2004).

Hemoglobin and hematocrit in groups that received ZnO NP and/ or cZnO with exercise were considerably more than exercise group alone as the same as red blood cells were higher. These consequences are in conformity with reports of hematocrit and hemoglobin increase in rats with swimming exercises by using zinc sulfate(Baltaci et al., 2003; Cordova et al, 1993).

In this study, there weren't noticeable differences in Mchc,Mch and Mcv in ZnO NP and/ or cZnO receiver groups in compared with saline control groups. Also we didn't see a significant difference in number of white blood cells in all groups that doing exercises. Several studies have reported increase in number of white blood cells and blood platelets after exercising while a few studies have reported decrease or no significant change to number of white blood cells and blood platelets. Wu et al showed that long time exercising causes increase in Wbc and Plt (Wu et al., 2004), but Robson et al didn't see significant difference after short time physical activity(Robson et al, 2007).

There were no noticeable differencse in number of Leukocytes and platelets between ZnO NP or cZnO receiver groups and exercise group. It has been shown that zinc sulfate and zinc picolinate with exercising causes increase in number of Leukocytes (Kilic et al., 2004; Polat, 2011), while Cordova et al (1993) and Baltaci et al (2003) reported that there is no noticeable difference in leukocyte amount between rat that swimming exercising and receiving zinc sulfate.

It has been shown that zinc sulfate doesn't have noticeable change on the number of platelets(Kilic et al., 2004; Polat, 2011) while Baltaci et al reported that in rats that have received zinc sulfate with exercising number of Platelets significantly decreased. (Baltaci et al., 2003). In this study there were no significant differences between cZnO receiver and ZnO NP receiver groups with and without exercising in platelets amount.

This study, show that both of ZnO NP or cZnO have equal positive effects on athletics' physical performance that this probably can be related to nanoparticles faster excretion from body in spite of faster assimilation and dissemination and this need to more investigation (Muhlfeld et al., 2007).

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