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Original Article

Effect of Sodium Cyanide-induced Tissue Hypoxia on Reproductive Capability of Male Mice and the Protective Effect of Ethyl Pyruvate

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Abstract

The present study aimed to assess the effect of tissue hypoxia induced by sodium cyanide (NaCN) on male mice fertility and the protective role of ethyl pyruvate (EP). A number of 30 adult mice were assigned to three groups: 1) a control group, 2) a treatment group treated with 2 mg/kg of NaCN, and 3) a treatment group treated with 2 mg/kg of NaCN, along with 40 mg/kg EP (NaCN+EP). After 35 days, animals were anesthetized and serum, sperm, and tissue samples were taken. The results demonstrated a significant decrease in sperm quality, reproduction potency, and anti-oxidant potential, as well as an increase in lipid peroxidation in the NaCN group ($P<0.05$). Moreover, the use of EP effectively restrained the disastrous effects of tissue hypoxia. It can be concluded that EP can moderate the complications resulting from tissue-hypoxia that is related to testes parameters.

Keywords: Ethyl pyruvate, Cyanide, Mice, Reproduction potency, Sodium, Tissue hypoxia

Effet de l'Hypoxie Tissulaire Induite par le Cyanure de Sodium sur la Capacité de Reproduction des Souris Mâles et Effet Protecteur du Pyruvate d'Ethyle

Résumé: La présente étude visait à évaluer l'effet de l'hypoxie tissulaire induite par le cyanure de sodium (NaCN) sur la fertilité des souris mâles et le rôle protecteur du pyruvate d'éthyle (PE). Un certain nombre de 30 souris adultes ont été réparties en trois groupes: 1) un groupe témoin, 2) un groupe de traitement traité avec 2 mg/kg de NaCN, et 3) un groupe de traitement traité avec 2 mg/kg de NaCN, ainsi que 40 mg/kg PE (NaCN + PE). Après 35 jours, les animaux ont été anesthésiés et des échantillons de sérum, de sperme et de tissu ont été prélevés. Les résultats ont démontré une diminution significative de la qualité du sperme, de la puissance de reproduction et du potentiel anti-oxydant, ainsi qu'une augmentation de la peroxydation lipidique dans le groupe NaCN ($P<0.05$). De plus, l'utilisation du PE a efficacement restreint les effets désastreux de l'hypoxie tissulaire. On peut conclure que le PE peut modérer les complications résultant de l'hypoxie tissulaire liée aux paramètres des testicules.

Mots-clés: Pyruvate d'Ethyle, Cyanure, Souris, Puissance de Reproduction, Sodium, Hypoxie Tissulaire

1. Introduction

Some diseases and complications can result in hypoxia in the whole body on tissue or cellular scales. Among these complications, we can refer to parasitic diseases (hematophagous parasites), anemia induced by toxins (phenylhydrazine) or deficiencies (vitamin B12 or iron), hemolytic anemia, hematologic conditions (e.g. hemophilia), respiratory engaging infections (bacterial, viral, or fungal), as well as chemical, industrial, and military agents. In general, hypoxia, as major stress, can threaten body health and even the reproductive system. In the preceding decade, some studies addressed growth, evolution, and reproduction under hypoxic conditions (Saxena, 1995; Verratti et al., 2008).

It was previously demonstrated that hypoxia impairs reproduction potency in rats and rhesus monkeys, as well as humans, by reducing sperm count and motility (Gosney, 1984; Saxena, 1995; Verratti et al., 2008). Morphologic studies on animals revealed that hypoxia results in the degeneration of germinal epithelium (Saxena, 1995). All these studies are in agreement with the fact that hypoxia interferes with the natural spermatogenesis process; nonetheless, there is a paucity of information on the level or phase of this interference (Liao et al., 2010). It is hypothesized that hypoxia reduces sperm count through an increase in germ cell apoptosis (Park et al., 2002; Assinder et al., 2007).

Based on the related studies, higher levels of hypoxia delay maturation in female rats (Elia et al., 1985). In male rodents, hypoxia blocks the synthesis and release of gonadotropins (Khmel'nitskii and Tararak, 1991). Chronic hypoxia can also decrease testosterone levels in plasma (Fahim et al., 1980). In a similar vein, chronic hypoxia blocks spermatogenesis in rats and rhesus monkeys (Saxena, 1995). In their study, Kalantari Hesari et al. (2015) indicated that hypoxia induced by hemolytic anemia after phenylhydrazine injection considerably affects reproductive capabilities in males.

Cyanide is a chemical compound that contains the functional group C≡N, and nitriles are organic cyanides with the formulation R-CN in which the CN-functional group is highly toxic and induces histotoxic hypoxia. Cyanide products are used in photography, laboratory reactions, industry, pesticides, and rodenticides [HCN], and they can be found in some fruits, such as bitter almond, apple seeds, plum, and peach seeds (Vetter, 2000).

Cyanides induce tissue hypoxia through various mechanisms: they restrain the respiratory center in the brain and reduce respiratory depth, decrease ventricular outflow by myocardial suppression, inhibit dissociation of O_2 from hemoglobin. Moreover, some cyanides compete with oxygen in binding to hemoglobin iron or even the iron in mitochondrial cytochrome oxidase C, leading to impaired cellular respiration and subsequent cellular death. The toxic potential and lethal quantity of cyanide are 0.10 and 0.50 gr, respectively (Pieniazek et al., 2006). This toxic material is rarely observed in surface and underground waters, although abundant use of these chemicals, especially in plating industries and their leak through wastewater, has led to environmental pollution. Even small amounts of this highly toxic anion can be lethal to humans and fish.

Cyanide anion is an inhibitor of the enzyme cytochrome c oxidase in the electron transport chain (residing in the mitochondrial membrane of eukaryotic cells). Cyanide binds to the heme subunits of this protein and hinders the transfer of electrons from cytochrome C to oxygen. Therefore, cyanide disturbs the electron transport chain in the manner that cells would not be able to produce Adenosine Triphosphate (ATP) through aerobic processes (Pieniazek et al., 2006). The role of environmental cyanogens in Mare Reproductive Loss Syndrome (MRLS) was demonstrated in a study that assessed the association between black cherry plants (Prunus serotina) and the prevalence of this syndrome in central Kentucky (Dirikolu et al., 2003). It is also hypothesized that exposure to such plants as cassava that harbor cyanides, elevates the occurrence of some disorders, such as

teratogenicity (Singh, 1981).

Ethyl pyruvate (EP) as a synthetic antioxidant has gained assiduous interest in recent years. Pyruvate performs a mediating role in metabolism and is a product of glycolysis, as well as a substrate of the tricarboxylic acid cycle (Vander Heiden et al., 2009). Pyruvate is also of great help in the detoxification of Reactive Oxygen Species (ROS) inside cells and is an anti-inflammatory agent (Wang et al., 2009). There is a paucity of information about the effects of cyanide on male reproductive organ, and no protective agent has been introduced against the possible deleterious effects of hypoxia. Therefore, the current study aimed to investigate the effects of histotoxic hypoxia induced by the injection of Sodium cyanide (NaCN) on the reproductive potency of mice and histomorphometric evaluation of testes. The EP was selected for its antioxidant properties (Mousavi et al., 2010) to determine its effectiveness in decreasing the deleterious effects of induced tissue hypoxia.

2. Material and Methods

A number of 30 adult male Balb/c mice weighing 25-30 gr were randomly assigned to three groups (one Control group and two experimental groups) and were kept in standard conditions in the abundance of food and water. Before the commencement of the experiment, animals were accustomed to the environment with 12 hours of daylight and 12 hours of darkness for 2 weeks. The minimum number of required animals to obtain valid results was determined. The pain and discomfort of animals were controlled according to General Principles and Guidelines for Care and Use of Experimental Animals.

2.1. Chemicals

In the present study, a dosage of 2 mg/kg NaCN (~¼ of LD50) (Sigma-USA-Aldrich- 71431) dissolved in distilled water was used to induce hypoxia in mice (Mathangi and Namasivayam, 2000). The EP (40 mg/kg) (Sigma-USA-Aldrich E47808) was employed to moderate the hypoxic effects of NaCN (Yang et al., 2009). Ketamine (Medica Company-Germany) and xylazine (Alfasan Company-Netherlands) were employed for anesthetization and euthanization processes, respectively.

2.2. Experimental Design

Each group contained 10 adult male mice. Control and experimental groups were treated as follows:

1. Control group received normal saline (9% NaCl) intraperitoneally in constant volumes.

2. Sodium cyanide recipient group (NaCN) received an intraperitoneal injection of 2 mg/kg of NaCN (~¼ of LD50) dissolved in distilled water (Mathangi and Namasivayam, 2000).

3. NaCN plus EP (NaCN+EP) recipient group received the same dosage of NaCN with an additional dosage of 40 mg/kg of EP in the same manner after their daily injection of NaCN (Wang et al., 2009).

The animals were weighed and anesthetized using a cocktail of ketamine (90 mg/kg) and xylazine (10 mg/kg) 35 days after the first injection. In this stage, blood sampling was carried out by cardiac puncture, the samples were centrifuged, and the supernatant serum content was stored at -20 degrees Celsius for future examinations. The animals were then euthanized by cervical dislocation, and epididymal sperm was retrieved for further reproductive evaluations, such as sperm analysis (sperm count, motility, viability, nuclear maturation, and DNA defects). The reproductive potential was assessed using in-vitro fertilization (IVF) in Human Tubal Fluid (HTF).

2.3. Spermatological Evaluations

Hemocytometer was used to enumerate average sperm count in unit volume and constant dilution of 2:100 of semen to distilled water. Sperms were counted in 25 central squares and multiplied by 5×10^5. To assess sperm motility, they were diluted in 2:100 ratio of sperm to HTF medium, and motile and non-motile sperms were detected in 5-6 microscopic fields envisaged under a light microscope (Olympus CX23). The viability of sperms was assessed by eosin-nigrosin staining which was conducted by the placement of 20

µL of sperm sample on a glass slide, addition of 40 µL suspension of eosin and nigrosin stains, and preparing a smear before scoring.

Sperm DNA fragmentation was determined by acridine orange staining, and nuclear maturation was discerned by aniline blue staining (Rezvanfar et al., 2013). Sperm was first diluted in phosphate-buffered saline (PBS) and centrifuged. The supernatant liquid was cast away, and sedimented sperm was gathered to be washed again. The process was repeated three times before preparing a smear of sperms on a slide. After drying in open air condition, the samples were fixated by immersion in acetone and were finally stained either with acridine orange or aniline blue using the same immersion method.

In preparation for the IVF assessment, four healthy female mice were acquired per male mouse. Superovulation was induced by the administration of Pregnant Mare Serum Gonadotrophin (PMSG) (10 IU) three days before the commencement of the experiment, followed by Human Chorionic Gonadotropin (HCG) (10 IU) injection 48 h after PMSG administration. Thereafter, 13 h after HCG injection, they were sacrificed to collect the oocytes from the oviduct in the presence of HTF culture medium. The oocytes were fertilized and kept under 5% CO_2 pressure and 37°C for one week and evaluated during this period. The conducted evaluations included determining the percentage of fertilized oocytes, bicellular oocytes, blastocysts, and different types of arrests in embryonic development. In the present study, arrested embryos were subdivided into three categories: Type I where the majority of the embryonic cells were lysed, Type II where lysis was observed in about half of embryonic cells, and Type III where although the majority of cells were normal, the development of embryo was arrested.

2.4. Histopathological Evaluation

Testes were weighed after separation from surrounding tissues, and their total volume was calculated according to the liquid displacement method. Testicular tissue was then fixated in Bouin solution for further histological and morphometric evaluations. The histological assessment included visual evaluation of capsule shape, interstitial tissue (edema or hyperemia), and seminiferous tubules. The morphometric analysis included thickness of testicular capsule, the diameter of seminiferous tubules, the height of germinal epithelium, Leydig cell count (in circles of 50 µm radius - an area of ~7853 µm2), number of Sertoli cells (per seminiferous tubule), and assessment of spermatogenesis and spermiogenesis regarding spermiogenesis index (SI), repopulation index (RI) and tubular differentiation index (TDI) factors (Kalantari Hesari et al., 2015). Four testes were selected in each group for tissue sectioning. Animals' blood serums were used to evaluate Total Antioxidant Capacity (TAC), lipid peroxidation (MDA), and testosterone level (Kheradmand et al., 2013).

2.5. Statistical Analysis

The data were analyzed in SPSS software (version 19). Data distribution was examined by Kolmogorov-Smirnov (K-S) test to ascertain normality. This data was then analyzed by parametric tests. T-test was used to compare morphometric results among group pairs, and one-way analysis of variance (ANOVA) was employed to compare several groups. Tukey post-hoc test was used following ANOVA whenever necessary. The comparison of population proportions was carried out in Minitab software to evaluate IVF results. A p-value less than 0.05 was considered statistically significant, and the results were demonstrated in mean±SE (Standard Error) format.

3. Results

3.1. Bodyweight and Testicle Weight and Volume

The three groups did not differ in bodyweight, as well as testicular weight and size (Table 1).

Table 1. Average bodyweight and testes volume and weight in Control and treated mice groups

	Control	NaCN	NaCN+EP
Bodyweight (gr)	30.22 ±0.89	28.91 ±1.13	30.88 ±0.95
Testis weight (mg)	112.25 ±13	95.50 ±11	114.25 ±10
Testis volume	0.09 ±0.01	0.08 ±0.01	0.10 ±0.01

No significant difference was observed among groups.

3.2. Sperm Quality

The results about the investigation of total sperm counts displayed that sperm count was decreased in both experimental groups, while the group treated with NaCN+EP had a higher sperm count, as compared to the NaCN group (P<0.05). The control group significantly differed from both experimental groups (P<0.05) (Table 2). The evaluation of DNA defects and sperm maturation showed no statistically significant difference, whereas sperm viability and motility were significantly decreased in the NaCN group, compared to the NaCN+EP group (Table 2).

Table 2. Comparative evaluation of sperm parameters in control and treatment groups

	Control	NaCN	NaCN+EP
Total count of sperm	67×10^6 ±215.50^a	46×10^6±115.21^b	65×10^6±98.45^c
Sperm viability (%)	74.25±4.50^a	52.25±3.10^b	71.75±3.56^a
Sperm motility (%)	90.50±3.90^a	66.75±4.30^b	85.25±2.95^a
Sperm with intact DNA (%)	99.25±1.01	97.75±0.85	98.50±0.94
Immature sperm (%)	8.25±0.98	10.00±1.15	8.75±1.37

Different letters indicate significant differences among groups (P<0.05).

3.3. Histological Study

Histological study of testicular sections revealed normal seminiferous tubules in control and NaCN+EP groups with sufficient homogeneous blood supply and no edema. In the NaCN-treated group, seminiferous tubules had lost their homogeneously scattered formation, and structural defects (such as the appearance of vacuoles among spermatogonial cells and numerous degenerated tubules) were highly evident in this group (Figure 1).

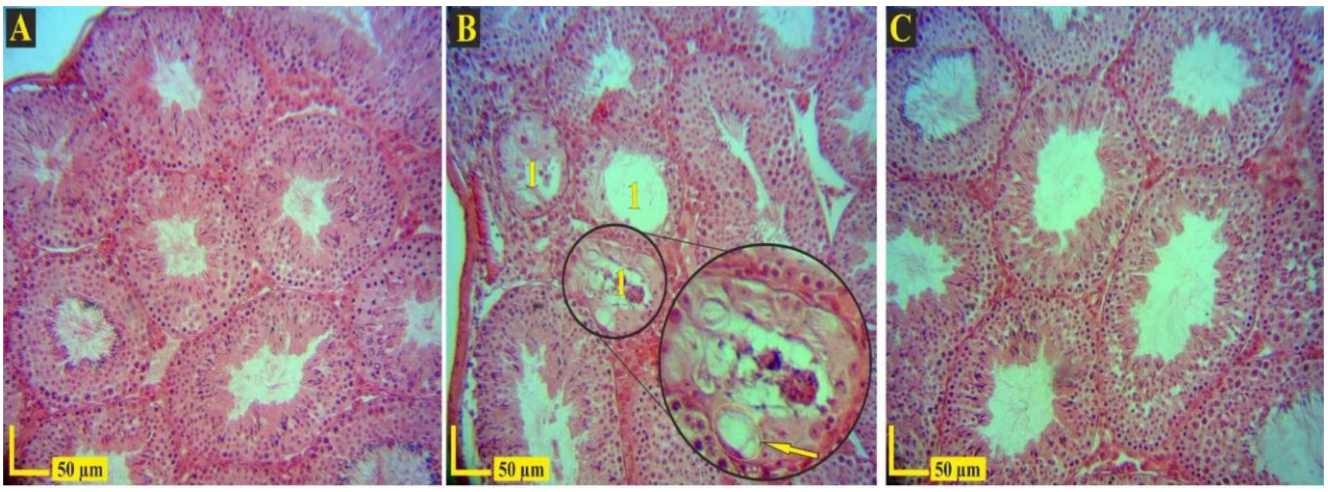

Figure 1. Histological sections of seminiferous tubules of testes H&E staining (400×). A: Control group, B: NaCN group, C: NaCN+EP group.
No. 1, degenerative tubules; Arrow indicates vacuoles appearing on spermatogenesis cell lines.

3.4. Morphometric Study

Spermatogenesis indices, such as TDI, RI, and SI were considerably lower in the NaCN group, in comparison with control and NaCN+EP groups (P<0.05), while there was no significant difference between the latter groups (Figure 2). The diameter of seminiferous tubules was decreased in the NaCN-treated group, compared to the NaCN+EP group (P<0.05); nonetheless, the difference was not significant, compared to the control group (Figure 3).

The height of germinal epithelium was significantly lower in the NaCN group, compared to both other groups (P<0.05); however, the difference was not significant between control and NaCN+EP groups (Figure 3). The diameter of the central lumen in seminiferous tubules displayed no noticeable difference in any group (Figure 3). The quantity of Sertoli cells in the control group was not significantly different from the experimental groups (Figure 4); nonetheless, Leydig cell count was significantly lower in the NaCN group, compared to control and NaCN+EP groups (P<0.05). Moreover, control and NaCN+EP groups did not significantly differ in Leydig cell count (Figure 4).

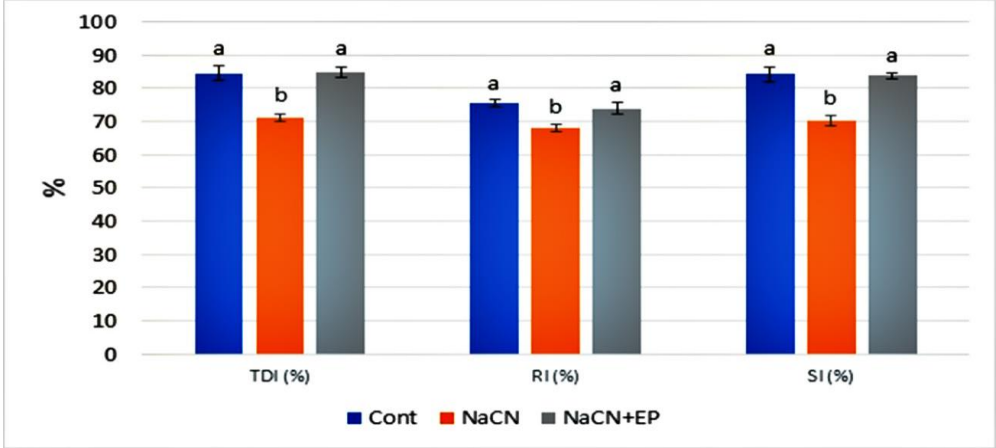

Figure 2. Spermiogenesis index, Repopulation index, and tubular differentiation index in control and treatment groups
Different letters indicate significant differences among groups (P<0.05).

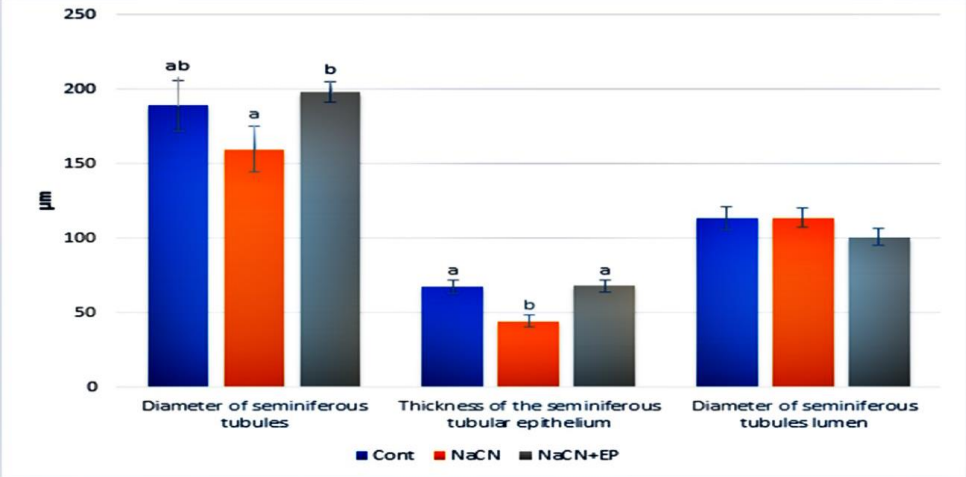

Figure 3. Diameter of seminiferous tubules, the thickness of the seminiferous tubular epithelium, and diameter of seminiferous tubules lumen in control and treatment groups, Different letters indicate significant differences among groups (P<0.05).

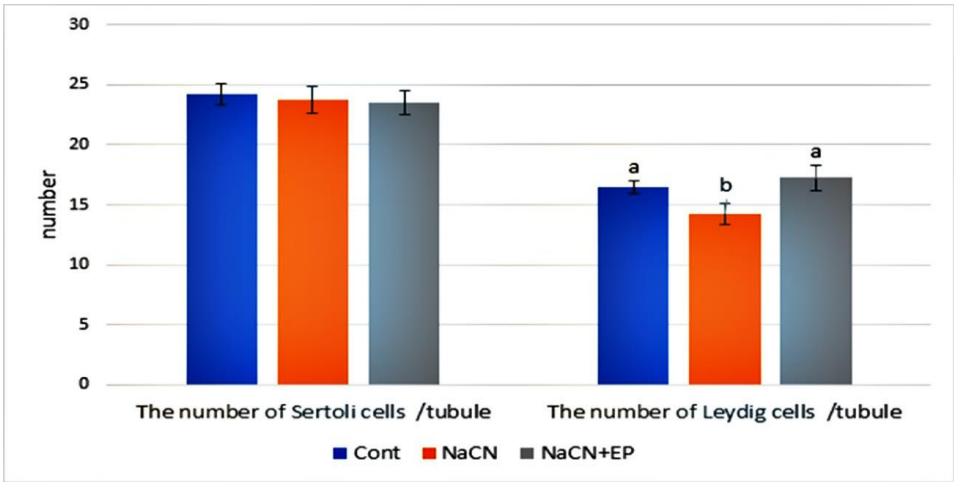

Figure 4. Sertoli and Leydig cell counts (in a section of 50µm radius) in control and treated mice. Different letters indicate significant differences among groups (P<0.05).

3.5. Serum Tests

Ferric reducing ability of plasma (FRAP) assay illustrated a significant decrease in antioxidant capacity of serum in the NaCN-treated group, compared to control and NaCN+EP groups (P<0.05); however, the difference between latter groups was not significant (Table 3).

Similar results were obtained in the investigation of lipid peroxidation; nonetheless, lipid peroxidation was significantly higher in the NaCN group. The control and NaCN+EP groups were not significantly different in the amount of lipid peroxidation (Table 3). Testosterone levels were similar among the three groups (Table 3).

3.6. In vitro Fertilization Results

The IVF results showed significantly lower success rates in Oocyte fertilization for NaCN treated group, compared to both other groups (P<0.05). Counting embryos in early morula and blastocyst stages demonstrated similar results. The NaCN group bore significantly fewer early-stage embryos, compared to both Control and NaCN+EP groups (P<0.05). The assessment of arrested embryonic development also showed a significant difference between the NaCN group and the other groups (P<0.05). The percentage of Type I and II arrested embryos in the NaCN group pointed to a significant increase, compared to control and NaCN+EP groups (P<0.05), while there was no significant difference among groups regarding the amount of Type III arrested embryos. All the results obtained from this experiment are presented in Table 4.

Table 3. Antioxidant activity, malondialdehyde concentration, and testosterone levels of serum in control and treatment groups

	Control	NaCN	NaCN+EP
AOA (µmol/L Absorbance at 532 nm)	0.922 ±0.03^{a}	0.767 ±0.02^{b}	0.975 ±0.02^{c}
MDA (TBARS µmol/ml Absorbance at 535 nm)	0.303 ±0.01^{a}	0.482 ±0.04^{b}	0.295 ±0.05^{a}
Testosterone (ng/ml)	7.92 ±1.07	6.84 ±0.80	7.51 ±0.71

Different letters denote significant differences among groups (P<0.05).

Table 4. Data related to in-vitro fertilization assessment in control and treatment groups

	Fertilization (%)	Bicellular (%)	Blastocyst (%)	Arrested blastocyst (%)			
					Type 1 (%)	Type 2 (%)	Type 3 (%)
Cont	90.65[a]	87.19[a]	62.21[a]	35.78[a]	0[a]	0.05[a]	49.2
NaCN	71.03[b]	43.73[b]	29.85[b]	73.91[b]	19.52[b]	8.11[b]	39.80
NaCN+EP	88.43[a]	77.09[a]	56.22[a]	40.17[a]	2.63[a]	3.18[a]	35.20

Different letters indicate significant differences among groups (P<0.05).

4. Discussion

The current study investigated the effect of NaCN injection as a histotoxic hypoxia inducer on sperm quality parameters and reproduction potential of male mice, as well as the protective role of EP as an antioxidant agent against the deleterious effects of NaCN. The obtained results pointed out that sperm quality factors, reproduction potential, histomorphometric parameters, and total antioxidant capacity of serum were decreased as a result of NaCN treatment, while lipid peroxidation was significantly increased in this group. However, EP managed to considerably diminish the deleterious effects of NaCN.

Tissue hypoxia can be induced by cyanide, and in this form of hypoxia, cells lose their ability to produce ATP. It is reported that industrial and plating wastewater contains free cyanide (from 0.3-216 ppm). The presence of cyanogenic glycosides in many plant species is already verified, and various tissue damages due to cyanide toxicity are reported in humans and animals (Soto-Blanco et al., 2005). It is known that NaCN or potassium cyanide produces hydrogen cyanide in contact with air or gastric hydrochloric acid. This chemical binds to the iron available in cytochrome C oxidase which is found in the mitochondrial membrane of eukaryotic cells. This binding disrupts the electron transfer chain.

The inability of electrons to transfer from cytochrome C to oxygen causes the cells to lose their ability to produce ATP through aerobic processes (Anseeuw et al., 2013). The results of the current study, especially on decreased motility of sperms which is closely related to the mitochondria, are consistent with the aforementioned studies. Nevertheless, another study revealed that oral gavage dosing of male rats with cyanide led to the weight loss of epididymis and testes and a decrease in spermatid count; however, it had no effect on sperm motility (NTP, 1993). The findings of the current study were not in agreement with those obtained in the referred study.

Furthermore, it has been demonstrated that a single dose of 1mg/kg potassium cyanide has no effect on DNA synthesis in mice testes (Friedman and Staub, 1976). In this manner, the current study agrees with previous ones. Despite the fact that chronic administration of NaCN lasted much longer in the present study, no significant differences were observed in the number of sperms with DNA defects. However, some studies have reported increased abnormal sperm count subsequent to chronic exposure to NaCN (Shivanoor and David, 2014). This discrepancy could be attributed to higher dosage (3.2 mg/kg), longer exposure time (90 days), or different rodent species (rat) in their experiment, compared to the present study.

It is reported that exposure to lower doses of NaCN can lead to decreased sperm count and motility in males (NTP, 1993). After investigating the effect of NaCN on some male fertility parameters, Shiddappa

and Munisiwamy argued that chronic exposure to this compound can bring about a decrease in sperm count and motility (Shivanoor and David, 2014). In terms of sperm count and mobility, the results of the present research are in accordance with those reported in the aforementioned studies.

In their study, Ardelt et al. (1994) assessed the increase in lipid peroxidation subsequent to cyanide exposure. They reported that hydrogen peroxide production (as a result of lipid peroxidation) leads to structural and functional changes in membranes, which in turn, damage them. Along the same lines. Daya et al. (2000) indicated that cyanide exposure promotes membrane lipid peroxidation and superoxide anion production. Ardelt et al. (1989) also pointed out that elevated lipid peroxidation and disrupted membrane functionality are led by a decrease in antioxidant compounds.

The results of the current study pertaining to increased lipid peroxidation, decreased antioxidant capacity, and the following changes, such as numerous degenerated seminiferous tubules, lessened germinal epithelium height, and reduced spermatogenesis indices in the NaCN group, were in line with the findings of previous studies. Pyruvate performs a peculiar role in the early stages of embryonic development. It reacts with hydrogen peroxide and acts as a cleanser for free radicals (Leese and Barton, 1985). This could explain the observed results in the current study where fertility rate, morula, blastocyst, and arrested blastocyst counts significantly differed between NaCN+EP and NaCN groups.

Some of the testicular histological complications and impaired reproductive capability in male mice following NaCN prescription may be explained by these free radicals since treatment with a strong antioxidant agent (EP) has successfully diminished these deleterious effects. In accordance with the current study, previous studies have also reported that antioxidant vitamins perform an effective role in neutralizing cyanide effects on body organs (Okolie and Iroanya, 2003). Moreover, it is known that there is a direct relationship between the number of reactive oxygen species (ROS) and the number of abnormal sperms produced (Iwasaki and Gagnon, 1992). It was also demonstrated that antioxidant food additives can result in better sperm quality and improved IVF results (Kalantari Hesari et al., 2015).

Oxidative stress is a result of an imbalance in the production of reactive oxygen species (ROS) and the antioxidant capacity of the body to detoxify them. It can damage the fetus through phospholipid peroxidation and alteration of such molecules as lipids, proteins, and nucleic acids. The consequences of such alterations are changes in mitochondrial structure, embryonic cell arrest, DNA extraction, and eventual apoptosis (Kowaltowski and Vercesi, 1999). In addition, previously conducted studies demonstrated that two-cell embryo arrest is more likely with increased ROS amount (Guerin et al., 2001). Reactive oxygen species are involved in hindered meiotic oocyte division, arrested embryonic cell growth, and cell death (Hashimoto et al., 2000). The results of the present study on the deleterious effects of NaCN prescription on the reproductive potential of male mice are in complete agreement with the findings of previous studies.

The EP has shown protective capabilities against degenerative oxidative stresses in the bovine fetus in vitro (Morales et al., 1999) and protects sperms against reactive oxygen species (ROSs) (Iwasaki and Gagnon, 1992). The application of EP can reduce apoptosis and tissue damage in chronic stresses and enhances testicular function. It seems that their antioxidant properties are the source of this protection (Payabvash et al., 2008). The findings of the current research are in accordance with those reported in previous studies on the protective effects of EP against complications caused by NaCN consumption.

In conclusion, it can be stated that NaCN prescription can induce histotoxic hypoxia which results in reduced reproduction potency and impaired testicular

parameters in mice. The application of EP as an efficacious antioxidant agent can counteract the deleterious effects of NaCN on reproduction potential. Nevertheless, future molecular studies can enhance our knowledge about the routes by which pyruvate acts, especially to impede detrimental effects of NaCN on testicular tissue.

Authors' Contribution

Study concept and design: M. R. N.
Acquisition of data: A. K. H.
Analysis and interpretation of data: A. K. H.
Drafting of the manuscript: A. K. H.
Critical revision of the manuscript for important intellectual content: R. Sh.
Statistical analysis: M. R. A.
Administrative, technical, and material support: M. R. N.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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