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Original Research

Exposure to iron ore attenuates the reproductive potential of adult male Wistar rats

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ABSTRACT

Aim: The aim of the study was to investigate the effects of administration of iron ore on semen parameters and testosterone level. **Materials and Methods:** A total of 20 adult Wistar rat were used for this study and separated into four groups of five rats each. Group A served as control, Group B treated with 1.5 mg/kg iron ore, Group C treated with 3 mg/kg iron ore, Group D treated with 4.5 mg/kg iron ore. After 28 days of treatment, the animals were anesthetized and sacrificed while seminal fluid and blood samples were collected for semen analysis and testosterone assay. ANOVA was used to compare means and $P < 0.05$ was considered statistically significant. **Results:** Iron ore caused a dose-dependent significant ($P < 0.05$) decrease in the sperm count, sperm motility, percentage sperm with normal morphology, and testosterone level. **Conclusion:** Therefore, it was concluded that iron ore could affect male fertility by reducing semen quality and testosterone level.

KEY WORDS: Environmental toxicants, iron ore, male fertility, semen analysis, testosterone

INTRODUCTION

Lead, cadmium, mercury, and arsenic, often referred to as "heavy metals," are toxic to wildlife, experimental animals, and humans [1]. Heavy metals have been used by humans for thousands of years. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries [2]. Iron ore deposits in Nigeria are widespread and have been a backbone to industrial development in the country. Mining besides causing environmental damage, the contaminations resulting from leakage of chemicals also affect the health of the local population [3]. Iron ore is important as it serves as a raw material for the manufacture of iron/steel rods, building materials, metallic plates, poles, and also vital in electroplating and galvanization of metals [4]. Iron ore consists mainly of iron oxides; the primary forms are magnetite (Fe_3O_4), and hematite (Fe_2O_3).

Studies on iron ore toxicity in experimental animal models have shown some level of reduction in the renal and hepatic function [5]. High toxicity of iron also inhibits the thrombin-induced conversion of fibrinogen to fibrin and therefore directly affects coagulation [6]. Excessive presence of elemental iron (toxicity) occurring with acute iron overdose causes the corrosive effects on the gastrointestinal mucosa and the metabolism and hemodynamic effects caused by the exposure to excessive elemental iron has also been reported [7].

Infertility according to World Health Organization is defined as the failure to achieve a clinical pregnancy after 12 months or more or regular unprotected sexual intercourse [8]. Prevalence of primary infertility in Nigeria is put at 22.7% in 15-49 years old male and female subjects [9]. However, 40-45% of most consultations in gynecological clinics are infertility related [10]. Medical research has shown that exposure to pollutants in the environment, lifestyle problem such as excessive alcohol consumption, cigarette smoking and drugs could affect a man's fertility [10]. This study, therefore, aims at establishing the effect of iron ore administration on semen quality (sperm count, sperm morphology, sperm motility) and testosterone level in male albino Wistar rats.

MATERIALS AND METHODS

Iron ore pellets were received from Itakpe, Kogi State, Nigeria. The iron ore which was first in coarse form was then pulverized into fine - smoothed powdered form with mortar and pestle.

Ethical Consideration

All procedures involving animals handling in this study were done in accordance with guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (World Medical Association and American Physiological Society, 2002). The research was approved by the research ethics committee of the college of Health Sciences of Delta State University, Abraka, Nigeria.

Study Design

Twenty adult male albino Wistar adult rats weighing between 180±10 g were obtained from the animal house of the department of Physiology, Delta State University, Abraka. The rats were housed in a cage and fed on rat chow for 2 weeks. After acclimatization, the rats were weighed and divided into four groups ($n = 5$). The animals were randomized into four groups. Group A (Control) were fed with normal rat chow and water, Group B treated with 1.5 mg/kg iron ore, Group C treated with 3 mg/kg iron ore, and Group D treated with 4.5 mg/kg iron ore. These doses were based on a pilot study previously done on this substance. Iron ore solution was administered through oro-gastric cannula once daily. Fresh feed was provided every day and total body weights of the animals were recorded at the beginning and end of the experimental period which lasted for 28 days.

After the experiment, the animals were sacrificed via cervical dislocation and blood samples collected by cardiac puncture and samples put in a plain specimen bottle. Each sample was placed into a dry clean centrifuge tube and centrifuged at 201 × g at 4°C to obtain serum. The experiment and data analysis was done in the year 2013.

Serum Testosterone Assay

The concentrations of serum testosterone were measured according to standard method [11].

Semen Parameters

Sperm motility

Sperm motility was recorded and evaluated immediately after specimen collection. Caudal epididymis was cut into the small pieces and transferred into the petri dishes containing pre-warmed nutrition medium (RPMI). Sperm was allowed to swim out within the 5 min at 37°C. The analysis was carried out under the light microscope magnification of 400 fold. The percentage of sperm motility was calculated using the number of live sperm cells over the total number of sperm cells, both motile and non-motile. The sperm cells that were not moving at all were considered to be non-motile, while the rest, which displayed forward movement were considered to be motile by method of Akdag *et al.* [12].

Sperm count

Epididymal sperm count was done whereby the left testis was decapsulated and the left epididymis was divided into two portions (head and body plus tail). Each part was homogenized for 20 min in 50 mL of saline triton merthiolate solution containing 0.9 NaCl, 0.05% Triton X-100 and 0.01% merthiolate. After that, homogenization sperm were counted in a hemocytometer (Bürker, Germany).

Sperm morphology

A thin-smear was made with the well liquefied semen. It was fixed with 95% (v/v) ethanol for 5 min. Then it was washed off with sodium bicarbonate formalin to remove the mucus present. Thereafter; it was stained with 1:20 (w/v) water solution of *carbolfuchsin* for about 3 min. This solution was then washed off and allowed to drain. It was then counter-stained with coefflers methylene blue for 2 min and then washed off. It was allowed to drain and read microscopically using a ×100 objective lens.

Statistical Analysis

Data collected were statistically analyzed using one-way ANOVA test. All data were expressed as mean ± standard deviation $P < 0.05$ was considered significant.

RESULTS

The effect of iron ore on the body weight [Figure 1], semen parameters (sperm count, progressive sperm motility, normal sperm morphology) [Figures 2-4] and testosterone level [Figure 5] of male Wistar rats were presented. Rats treated with iron ore showed a dose-dependent decrease in body weight with statistical significance ($^*P < 0.05$) in the group treated with 3 mg/kg and 4.5 mg/kg compared to control animals. The percentage change in the body weight with respect to the control is as shown [Figure 1].

The study on the effect of iron ore on sperm parameters showed dose-dependent decrease in sperm count [Figure 2], percentage of sperm with normal morphology [Figure 3], sperm motility [Figure 4], and serum testosterone level after treatment with iron ore. Sperm count and sperm motility decreased with

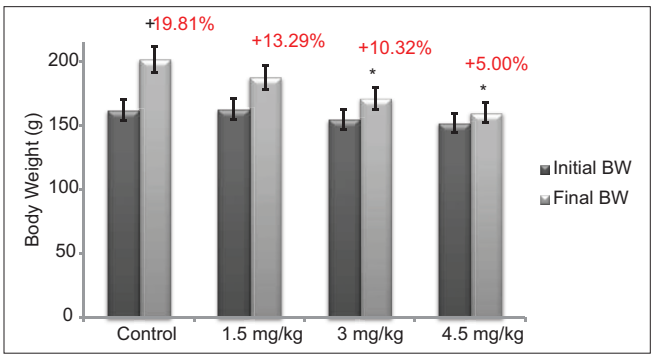

Figure 1: Effect of iron ore on body weight ($n = 5$ in each group); $^*P < 0.05$ compared with initial body weight

increase in iron ore doses when compared to control and was significant ($P < 0.05$) in animals treated with 3 mg/kg and 4.5 mg/kg iron ore only. The dose-dependent decrease in sperm with normal morphology induced by iron ore treatment showed no significant difference when compared to control rats. Iron ore treatment significantly ($P < 0.05$) decreased the serum testosterone level at different doses of 1.5 mg/kg, 3 mg/kg, and 4.5 mg/kg. This decrease was also in a dose-dependent manner. All percentage changes in the values of the parameters as compared to the control are presented on the bar charts.

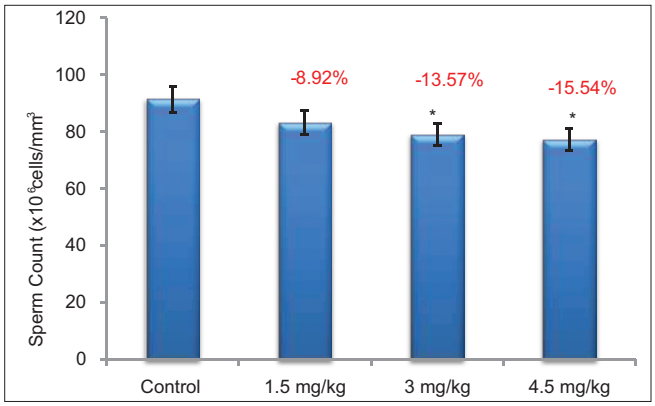

Figure 2: Effect of iron ore on sperm count (n = 5 in each group), *P < 0.05 when compared with normal control group

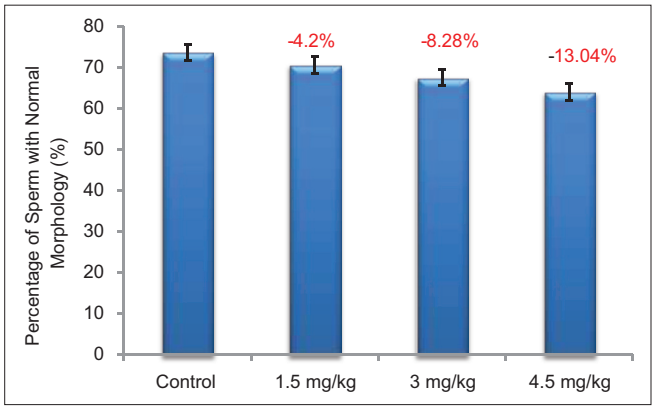

Figure 3: Effect of iron ore on percentage sperm with normal morphology (n = 5 in each group)

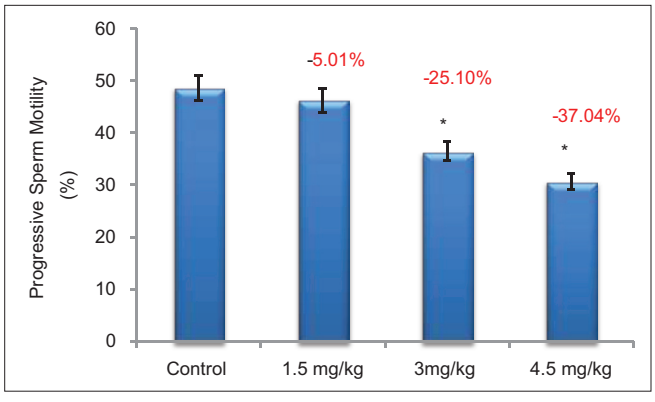

Figure 4: Effect of iron ore on progressive sperm motility (n = 5 in each group), *P < 0.05 when compared with normal control group

DISCUSSION

In recent years, there has been increased interest in the contribution of occupational and environmental exposures to toxic pollutants on declining sperm concentrations and male infertility [13].

The aspect of the study on the effect of iron ore on the body weight of the rats [Figure 1], showed that there was a dose-dependent decrease in percentage final body weights gain of 13.29%, 10.32%, and 5% in the 1.5 mg/kg, 3 mg/kg, and 4.5 mg/kg groups, respectively, as compared to their initial body weight and control group (19.81%).

A deleterious effect of iron ore on semen parameters was observed in this study. This finding could be attributed to decrease level of testosterone as observed in this study [Figure 5], as testosterone play a major role in maturation and maintenance of sperm cells [14]. The decrease in semen parameters and testosterone level could also be attributed to possible distortion of testicular integrity responsible for sperm cell (Sertoli cells) and testosterone (Leydig cells) production [15] which is typical of most environmental pollutant [16].

Several means of exposure to iron ore include food, water, and air, with humans more prone to airborne contamination of the heavy metal with further accumulation in the reproductive organs [17]. According to Bonde *et al.*, in a related study sperm count and chromatin structure correlate with exposure to heavy metals as was also seen with inorganic lead effect in men [18]. Metal or mineral extraction results in problems and issues in balancing ecological, environmental, economic, social advantage factors. Traditional mining and extraction methods have major environmental and health impacts especially to the miners and people living within the neighborhood [19].

Some of the detrimental effects of the iron ore on the sperm count, sperm motility, percentage of sperm with normal morphology, and testosterone level in this study could be attributed to the presence of some metals such as aluminum and manganese, which are known constituents found in iron ore [20]. Spinelli *et al.* reported delay in time to conception,

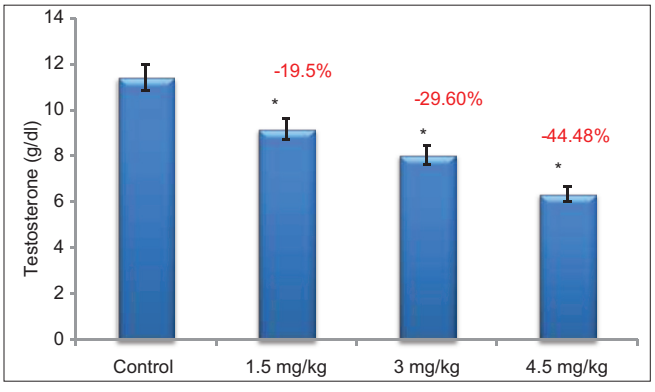

Figure 5: Effect of iron ore on testosterone, *P < 0.05 when compared with normal control group

decreased family size and reduced sperm quality of metal workers in their study [21].

Furthermore, in this present study there was a dose-dependent decrease in the level of testosterone [Figure 5] showing a percentage decrease of 19.5%, 29.60% and 44.48% in the 1.5 mg/kg, 3 mg/kg and 4.5 mg/kg groups, respectively. Guo *et al*. had suggested that the possible mechanism behind antifertility effect of iron ore was due to the reduced testicular acetylcholinesterase (ACE) activity which presumably plays an important role in oxidative damage of iron ore induced testicular toxicity [22]. Furthermore, concentrations of aluminum a major constituent of iron ore in human spermatozoa and seminal fluid are correlated with decreased sperm motility and viability similar to findings of this study [23]. Testicular aluminum accumulation, necrosis of spermatocytes/spermatids and a significant decrease in fertility were found in both male mice and rats [24-26]. A study by Guo *et al*. demonstrated that exposure to aluminum, lowered plasma, and testicular testosterone levels in mice [27].

CONCLUSION

This study has shown that exposure to iron ore, one of the several environmental pollutants may cause anti-fertility by decreasing the sperm count, sperm motility, percentage of normal sperm morphology, and testosterone level. The results obtained, however interesting, are preliminary finding, and therefore further studies may be required. It is therefore suggested that there is a need for proper means of control or avoiding exposures to iron ore.

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