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Effect Of Exposure To Lead And Cadmium In Drinking Water Sources From Nigeria's Niger Delta Region On Fecundity In Albino Wister Rats.

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Abstract: *As Nigeria strives towards attaining the Millennium Development Goal for water, this study was carried out to investigate the effect of exposure to lead (Pd) and cadmium (Cd) in drinking water source in the Niger Delta region on fecundity and reproductive organ weights. Prior to the study, levels of heavy metals in samples were assessed and compared to WHO permissible limit. The levels of Pb and Cd were higher than that of WHO limit in some samples selected and used for the study. WHO permissible limit of lead and cadmium in the drinking water are 0.01 and 0 003mg/L respectively. Forty eight albino Wistar rats (210-240g) of both sexes were assigned into three groups: control (16), Pb-treated (16) and Cd-treated (16). The control group was given distilled water, while the Pd-treated and Cd-treated group were administered drinking water randomly collected from natural source with Pd (0.00-0.142mg/L) and Cd (0.00-0.024mg/L/) .All water was administered in drinking water bottles filled with nozzles for a 28-day period. Male and female rats of each group were then mated as follows: control males versus control females, control males versus Pb-treated females, Pb-treated males versus control females and Pb-treated males versus Pb-treated females. The same procedure was followed for Cd, Gestation day zero was regarded as day spermatozoa were identified in vaginal smear of female rats. Pregnant animals were separated and the pregnancy allowed to come to term. Litter size and morphological appearance of litters from each group and reproductive organ weight were noted. The litter size of group 1 (control) for Pb and Cd was 12; group 11 was 8 and 6 respectively; group 111 was 5 and 2 respectively; while group IV showed no reproductive outcome. The results suggest adverse effect of Pb and Cd in drinking water on fecundity of albino Wistar rats.*

Key Words: *lead, cadmium, drinking water, fecundity, litter size.*

I. Introduction

Lead and cadmium are widely distributed and occur naturally in the earth's crust (Ming-Ho, 2005). Lead is easily solubilized by water and dissolved lead in soils is bioaccumulated by plants and vegetables which might be another major source of the metal. (Rim-Rukeh, 2009). Solubilized lead in water and food is toxic, as lead is stored in bones and the soft tissues, including the liver (Ming-Ho, 2005). The biological fate of lead in human body has physiologic, neurologic, renal, endocrine, reproductive and developmental effects (Ming-Ho, 2005). Studies suggest that long term lead exposure may diminish sperm concentrations, total sperm counts, total sperm motility and impair the stability of sperm chromatin or damage sperm DNA (Alexander et al., 1996; Mangelsdorf et al., 2003). Serum prostate specific antigen (PSA) has also been shown to increase with environmental exposure to cadmium and / or lead (Zeng et al., 2004; Pizent et al., 2012). An increased frequency of miscarriages and still births among women has been reported due to lead exposure (Hu, 2002). Cd is said to enter the food chain when Cd waste from the industrial production is disposed in the sewages and the sewage sludge is used as agricultural fertilizer.

Transgenerational toxicity and adverse effect of Cd contaminated water on reproductive outcome and capacity has been reported by Eteng et al., (2008).The present study was carried out to evaluate the effect of exposure to Pd and Cd in drinking water sourced from Nigeria's Niger Delta region on fecundity of albino Wistar rats

II. Materials And Methods

Collection Of Water Samples And Determination Of Heavy Metals.

Borehole and surface water samples were obtained from selected zones in five states, namely Akwa Ibom, Bayelsa, Cross River, Delta and Rivers. Random collection covered industrial zones waste dump sites, villages, urban and semi-urban centres. Polyethylene terephthalate (PET) sterile bottles of 150ml volume capacity were used for sample collection. The PET bottles were sterilized by soaking them in 10% w/v chlorine solution for 24 hours and pre-numbered for easy sample collection. Water from borehole was allowed to run for a while before filling the pre-coded sterile 150ml sample bottles. The bottles filled with samples were well-stoppered and put in a cooler for easy transportation from the collection site to the laboratory for analysis. Pb and Cd concentration were determined by the method of spectrophotometry as described by Tietz (2008) and compared to WHO permissible limits, and samples with high levels of Cd and lead selected for animal study.

III. Animals, Grouping And Experimental Protocol

Forty eight adult albino Wistar rats of both sexes (201-240g) were obtained from the disease-free stock of the animal house of Biochemistry Department, University of Calabar, Nigeria. The animals were housed in standard cages, placed in a well-ventilated animal room under standard condition of temperature (28 ± 2^{O}C) and relative humidity (46 ± 5%), and were acclimatized for two weeks. The animals were maintained on rat chow (obtained from Pfizer Livestock Feeds, Lagos, Nigeria), and was provided with water *ad libitum* throughout the duration of the work. Permission for use of animals and animal protocols in the present study was obtained from the College of Medical Sciences Animal Ethics Committee, University of Calabar prior to experimentation. The animals were assigned into three study groups of sixteen rats each and exposed directly to the Pb and Cd containing Water samples in drinking water bottles as follows:

Group 1 (Control): Animals were allowed access to distilled water and feed only.

Group 11: Animals were exposed to Pb-contaminated water as obtained from the natural source along with feed.

Group 111: Animals were exposed to Cd –contaminated water as obtained from the natural source along with feed.

The exposure to water lasted for a 28-day period

IV. Mating of the animals

The control and treated animals were mated in the ratio of 1:1 based on the following schedule:

Group A: Control males versus control females
Group B: control males versus Pb –Treated females.
Group C; Pb-treated males versus control females
Group D: Pb-treated males versus Pb-treated females.
The same procedure was followed for cadmium.

V. Detection of pregnancy, Gestation and liter size

Vaginal smear were examined daily for the presence of spermatozoa with the day of sperm detection being considered as gestation day zero, pregnant animals were separated and the pregnancy allowed to come to term as the animals littered. The litter size and morphological appearance of litters from each group were noted. Reproductive organs, testes and ovaries were harvested from sacrificed animals after litter and weighed accordingly.

TABLE 1: Effect of exposure to Pb and Cd in drinking water on fecundity

Group	Number of litters		Morphological appearance	
	Pb-treated	Cd-treated	Ps-treated	Cd-treated
A Control male vs Control female	12	12	No physical abnormality	No physical abnormality
B Control male vs Treated female	8	6	No physical abnormality	No physical abnormality
C Treated male vs Control females	5	2	No physical abnormality	No physical abnormality
D Treated male vs Treated females	-	-	No physical abnormality	No physical abnormality

Table 1.1: Effect of exposure to Pb and Cd in drinking water on reproductive organ weight

Group	Testes (g)	Ovary (g)
Control	2.41 ± 0.03	0.06 ± 0.01
Pb-treated	2.22 ± 0.05	0.04 ± 0.01
Cd-treated	2.16 ± 0.01*	0.02 ± 0.00*

Values are expressed as the mean $\pm$ SEM: n = 3
*****Significantly different from control at P<0.05

Statistics
Values were expressed as mean$\pm$ SEM
Results were subjected to statistical analysis using one way analysis of variance.

The control group A Wistar rats yielded twelve (12) litters: group B eight (8) and six (6) respectively: group C five (5) and two (2) respectively; while group D showed no reproductive outcome. There was no physical abnormality observed in all litters. The number of litters recorded in the cross-breed for control was normal since there was no interference with the reproductive system by any external chemical or endodermic intrusion. The result of Group B may suggest that while the male control still retains their male reproductive integrity, the female may have had their ovaries slightly distorted. The probable reason for the low number of litters (poor pregnancy outcome) compared to the control group may be due to Pb/Cd induce damage to ovary/uterus and reproductive hormonal imbalance (altered endocrine environment). Group C; result may suggest that effect of the Pb/Cd on the male mating pair might have caused damage to the reproductive organ, as reported in the study by Eteng et al. (2008). Free radical generation and their interference with molecular architecture weaken organs (Thompson and Bannigan, 2008), and damage to the sperm membrane can reduce sperm's motility and ability to fuse with the oocyte (Koppers, 2008), hence resulting in fewer litters. Group D recorded no litter at all, and this might be the result of damage on the reproductive organs (testes and ovaries) of the mating pairs, which made it impossible for conception to take place. This outcome was a secondary effect of low hormonal levels (progesterone and testosterone). According to Benoff et al. (2001), Cd^{2+} and Pb^{2+} accumulate in male organs, causing alterations in hormonal concentration, male fertility and sperm parameters. ***Onwuka*** (2006) also showed that Cd poisoning affected reproductive capacity and outcome of albino Wistar rats. The relatively low testes weight of the rats exposed to Cd in drinking water was a general effect and agreed with the report by Aoyagi at al. (2006) that testes weight of rats was markedly reduced by Cd toxicity. There was decrease in ovarian weight, which may be due to receptors bound to the ovarian tissue membrane or the enzymes that synthesize the reproductive hormone. Earlier work by Hensen and Chedrese (2004) also indicated that exposure to cadmium during human pregnancy may lead to reduced birth weights and premature birth.

VI. Conclusion

The concentration of Pb and Cd in drinking water samples randomly collected from various sources in Nigerian's Niger Delta region showed levels above the WHO permissible limits, culminating in the adverse effect of decrease in litter size of albino Wistar rats. The presence of the levels of Pb and Cd in water samples makes them unfit for human consumption and the result in the study showed possible toxicity on the reproductive organs.

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