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Transgenerational Effects of Cadmium Toxicity on Gonadal Steroid Levels and Reproductive Outcome of Wistar Rats

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Abstract: This work was carried out to investigate the transgenerational effects of cadmium toxicity on wistar rats in terms of reproductive outcome. Cadmium was administered subcutaneously to albino wistar rats of both sexes (n=10 for each group) in a single acute dose of 3mg cd/kg body weight. The other male and female groups (n=10 respectively) served as control. The animals were then mated viz: control females vs control males, cadmium treated males versus cadmium treated females, cadmium treated males versus control females, control males versus cadmium treated females, cadmium treated males versus cadmium treated females. Parameters or indices of reproductive outcome assessed were; litter size, morphology and organ weight. The gonadal steroid hormone levels (testosterone (ng/ml) and progesterone (ng/ml)) were also evaluated in offspring after attaining sexual maturity. The same parameters were assessed in control group which were not administered with cadmium. The result revealed a transgenerational effect of cadmium toxicity on the wistar rats reproductive capability as manifested by low sex organ weight, reduced litter size and reduced sex hormone levels of the offspring of the cadmium treated albino wistar rats.

Key words: Cadmium, Transgenerational study, Reproductive outcome

INTRODUCTION

Cadmium is widely distributed in the earth crust as ores of zinc, copper etc, and is washed into surface waters; it percolates into underground water during weathering. It is equally distributed in the environment as products of industrial effluents. Municipal refuse also contains cadmium from discarded cadmium containing waste. Cadmium is used industrially in the following ways, as protective plating on steel, stabilizer for poly vinyl chloride (PVC) products, pigments in plastic and glass, electrode material in nickel - cadmium batteries and as components of various alloys[7].

Cadmium is said to enter the food chain when cadmium waste from the industrial production is disposed in the sewages and the sewage sludge is used as agricultural fertilizer. The crops bioaccumulate cadmium in roots, tubers, leaves and other parts of the plant that are used as food, hence cadmium poisoning in human body is evident. When industrial effluent from factories that use cadmium is discharged into large water bodies, rivers contaminated with cadmium in this manner can contaminate land in turn through irrigation for agricultural purpose or flooding. Again the plants bioaccumulate cadmium and are subsequently utilized by man. Marine food which are contaminated are equally eaten by man[3].

It has been reported that ingested cadmium is stored in the renal and sex organs of experimental animals given single injections of salts corresponding to 2-4mg cd/kg body weight causing renal dysfunction[2] and testicular necrosis[5]. The oil producing areas of Nigeria, Port Harcourt in particular harbours 80% of the petrochemical and other allied industries which discharge cadmium containing waste as Municipal refuse. This eventually percolates into underground water and many of the people drink untreated water from boreholes. There have been increasing incidents of infertility, miscarriages and low sperm counts[4]. Since cadmium induces reproductive and renal disfunction,[2,5] we hypothesized that cadmium which finds its way into drinking water may be linked to the poor reproductive outcomes observed in Port Harcourt Metropolis. The present study therefore investigate whether cadmium toxicity in parent animals has adverse effect on reproductive outcome or is transferred to the offspring, hence a transgenerational effect of cadmium toxicity in wistar rats.

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MATERIALS AND METHODS

Animals: Forty (40) albino rats (20 males and 20 females) of wistar strain weighing between 140-220g were purchased from the disease free stock of the department of Biochemistry animal house, University of Calabar and used for the study. They were acclimatized and reared on a popular commercial stock, diet (Pfizer - Livestock feeds, Aba, Nigeria), for two weeks. Permission and approval for the animal studies were obtained from the College of Medical Science Animal Ethics Committee, University of Calabar. The rats were weighed and randomly assigned on the basis of their weight into four study groups of ten rats each. The animals were housed in plastic cages with wire screen tops. They were kept under adequate ventilation at room temperature and relative humidity of 28 ± 2^0C and 46% respectively. Food and water provided *ad libitum*.

Experimental Design: The design consisted of four study groups viz: a_1, b_1, b_2, of ten rats per group. Animals in group a_1, (10 male rats) and those in group a_2, (10 female rats) were fed normal diet and served as male and female control.

Group b_1 consisted of 10 male rats placed on normal diet and cadmium, while group b_2 was composed of 10 female rats fed normal rat chow with subcutaneous dose of cadmium.

Cadmium Administration and Mating: Cadmium chloride was given subcutaneously at dose 3mg/kg body weight and administered to all cadmium treated groups in a single acute dose. 24 hours after the cadmium treatment, the rats were co-habited to achieve mating in the following design:

- Cd treated males Vs Cd treated females
- Cd treated males Vs control females
- Cd treated females Vs control males
- Control males Vs control females

After the mating period, the parent female rats gave birth to offspring from various mating groups and were allowed to be sexually matured, weighing up to 120g. The number of births was recorded from each mating group, and also at sexual maturity, animals were sacrificed by suffocation in chloroform vapour and dissected. Blood collected by cardiac puncture using sterile syringe/needle from which serum was prepared and used for assay of testosterone and progesterone levels. The sex organ weights (g) were also estimated.

Biochemical Assays:

Estimation of Testosterone and Progesterone Levels: The testoterone and progesterone levels of male and female wistar rats were carried out in duplicates using the method employed in micro well Enzyme liked Immuno Sorbent Assay (ELISA) testoterone and progesterone Enzyme immunoassay test kits. The sensitivity of the assay was reported as 0.85ng/ml.

RESULTS AND DISCUSSION

Results: The effect of cadmium administration at the dose 3mg cd/kg body weight on the first generation of parent albino rats (offsprings), is summarized in Table 1. There were no births from cadmium treated and cohabited males and females. However, there were four (4) birth from mating of cadmium treated females and control males, while cadmium exposed males versus control females had two (2) births. The control animals (Cd-free male and female) had twelve (12) births.

Table 2 shows the sex organ weights (g) and hormonal level of the offspring of the various parents mating groups. Group III (Cd – treated females vs control males), had 2.12 ± 0.02 and 1.50 ± 0.02 for testes and ovary weight respectively, while the control group had 2.50 ± 0.01 and 1.98 ± 0.03 values for testes and ovary weights respectively.

The result showed a statistically significant ($p < 0.05$) decrease in sex organ weight compared to control values.

The sex hormone assay levels (Testosterone and progesterone) for group III was 0.98 ± 0.01 and 0.86 ± 0.04 respectively. The value for the control group was 1.26 ± 0.03 and 1.08 ± 0.07 for testosterone and progesterone respectively. This shows a significant ($p<0.05$) decrease in hormone levels compared to the control values.

Discussion: The number of births achieved through mating of different groups of parent rats and the morphology of the offspring showed that group iv (Control rats) had twelve (12) numbers of offspring from a mating between control males and female parents. This was expected because there was no impediment to their conception by any external chemical. Group I (Cd treated male and female rats), had no offspring. This is likely due to damage on the reproduction organs (tests and ovaries), of the parent rats making it impossible for conception to occur. Group III (Control males + Cd treated females) recorded four (4) numbers of births. This result was also expected because the control males still retain

Table 1: Effect of cadmium treatment of parent rats on reproductive outcome (number of births) and morphology of offspring.

Treatment group	Mating of parents rats	No. of births	Morphology
i.	Cadmium treated males Vs Cadmium treated females	NIL	NIL
ii.	Cadmium treated males Vs Control females	2	No physical abnormality
iii.	Cadmium treated females Vs Control males	4	No physical abnormality
iv.	Control males Vs Control females	12	No physical abnormality

Table 2: Sex organ weights, testosterone and progesterone levels of offspring

Treatment group	Testes weight (g)	Ovaries weight (g)	Testosterone (ng/ml)	Progesterone (ng/ml)
i. (Cadmium treated males) Vs (Cadmium treated female)	NIL	NIL	NIL	NIL
ii. (Cadmium treated males) Vs (Control female)	*2.52 ± 0.02	NIL	*1.29 ± 0.06	NIL
iii. (Cadmium treated females) Vs (Control males)	*2.12 ± 0.02	*1.50 ± 0.2	*0.98 ± 0.04	*0.86 ± 0.0
iv. (Control males) Vs (Control female)	2.5 ± 0.01	1.98 ± 0.034	1.26 ± 0.03	1.08 ± 0.01

Values are mean ± SD, *P < 0.05 significantly different compared to control.

their reproductive integrity, while the cd-treated females have their ovaries slightly distorted. The reason for a reduced number of births compared to the control could arise from either miscarriages due to weak organs caused by cadmium, or loss of some eggs that could be fertilized by normal sperm cells. Group II (cd-treated males + control females) recorded only two (2) births. This observation showed that the acute dosage of cadmium (3mg cd/kg) to the male parents caused partial destruction of male reproductive system, hence, few births was achieved.

The mean weights of the testes and ovary of the rats in various groups assessed revealed that the weight of the testes and ovaries in group III showed a significant decrease in comparison to the control. This result is attributed to a carry over of reproductive malformation trait of the parent rats caused by toxic distortion of the female and male reproductive organ by cadmium. This result was supported by report of Popharm and Webster[6], who observed a significant decrease in the fecundity of nematodes exposed to 3.26 X 10^{-7} moles of cadmium for 6 hours. The report showed that those exposed to 4 X 10^{-8} moles of cadmium never grow to the same length as the control. Van Kessel *et. al.*[8] exposed juvenile nematodes to various concentrations of cadmium chloride and found that growth and subsequent reproductive abilities were significantly reduced even at concentrations of 1 mmol/L. In a study by Benolt *et. al.*[1], three generations of grook trout were exposed to various concentrations of cadmium and a number of 1st and 2nd generation adult males died. Concentrations of 6.4 ng/I was found to significantly retard the growth of juvenile 2nd and 3rd generation offspring. The testosterone and progesterone levels were reduced significantly in cadmium treated group in comparison to the control values. This reduced hormone levels in the offsprings may be due to malformation of the reproductive system caused by cadmium toxicity on the parent rats which manifested in offspring reproductive functions.

Conclusion: Generally, the whole result showed that cadmium toxicity on the parent animals, affected the 1st generation in terms of reproductive outcome. Therefore, cadmium toxicity may not be unconnected with high incidence of infertility in oil producing communities.

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