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RESEARCH ARTICLE

STUDY ON EFFECT OF HEAVY METAL LEAD ON GERMINAL CELLS & BEHAVIOURAL PATTERN OF MALE *MUS MUSCULUS*

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ABSTRACT

Numerous heavy metals show significant mutagenic, teratogenic, and clastogenic. Most of the heavy metals such as lead, arsenic, mercury, and cadmium affect on male reproductive function, including sperm head morphology and spermatogenesis. Several studies have reported that heavy metals caused testicular oxidative stress, which has been linked to infertility. The aim of the present research his to investigate the cytotoxic effect of lead acetate on production of sperm cells and behavioural pattern during dosing period of two doses (20 and 40 mg/kg body weight/ per day) of lead acetate for 40 days in male Swiss albino mice (*Mus musculus*). For the research Adult Male *Mus musculus* of same age and average body weight 25 -30 gm taken and were divided into three groups one group was considered control group and other two were considered treated groups. Sperm counting and behaviour pattern parameter were included in this research to investigate the cytotoxic effect of lead acetate. After 40 days of treatment, the experimental animals were sacrificed and epididymal part were taken for study of sperm counting. The result shows that in both lower dose and higher dose of lead acetate were significantly decline the sperm counting ($p < 0.05$). The number of sperms was 150.4 ± 3.12 (control), 87.08 ± 2.21 (lower dose), and 48.1 ± 1.4 (higher dose) million cells/ml. In behavioural pattern feeding habits were not changed. However, aggressiveness, sleeping time, and loss of body hair increased in all treated groups as compared to the control group. In the present research, lead acetate administration shows significant on behavioural dysfunction and cytotoxic effect in germinal cells by disturbing sperm production.

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INTRODUCTION

In recent years many researchers have focused on the potentially toxic effect of various chemicals on human fertility. The deleterious health effect of heavy metals in the environment are now a serious concern and a global issue (Masindi & Muedi, 2018). Many researchers and authors have reported the detrimental effect of environmental pollutants on reproductive functions such as pesticides and heavy metals like lead, arsenic, cadmium, mercury, or nickel. Among these pollutants, lead is the most abundant toxic metal in the environment (Okereafor *et al.,* 2020). Lead does not have any detectable beneficial biological role however its detrimental effect on cardiovascular system (Vaziri 2008), nervous system (Al-khafaf *et al.,* 2021), gastrointestinal tract (Begovic 2008), kidney (Rastogi, 2008), lungs (Kaczynska *et al.,* 2013).biochemical, and on behavioural pattern have been documented in animals by many authors (Silbergeld 1974, Cherian 1979, John 2008, Patra *et al*., 2011). Lead toxicity is manifested in male reproductive function by deposition of lead in testis, caput epididymis, cauda epididymis, vas deference, seminal vesicles, etc (Batra 2001). The mechanism through which lead causes gonadotoxicity are Reactive oxygen species, a decrease in testicular antioxidants, and oxidative stress (Turk *et al.,* 2008).

Upon a permanent increase of reactive oxygen species level, it disturbs the antioxidant defense system and can promote apoptosis, cell damage, or alternation in the hypothalamic – pituitary – gonads axis. (Gandhi *et al.,* 2017). The aim of this research is to investigate the cytotoxic effect of lead acetate on sperm cells and behavioural pattern in Swiss albino mice (*M. musculus*).

MATERIAL AND METHOD

Experimental animals: For the research experiment, 15 adult Male Mice (*M. musculus)* of same age and average weight of 25 -30 gm body weight were taken from the animal house of the University Department of Zoology, T.M. Bhagalpur University, Bhagalpur. All the *M.musculus* were kept in a polypropylene cage under hygienic conditions in a well-ventilated room with 6 – 8 hrs photoperiod at an ambient temperature of 25 ± 2 Celsius under animal husbandry conditions. And were divided into equal number of mice in three groups one group was considered the control (A) group and the other two were considered treated (B, & C) groups.

Methodology: Control group of *M. musculus* was fed 1 ml glass distilled water (G.D.W.) and one treated group were fed 1 ml (20

mg/kg body wt. /per day) of lead acetate. Another treated group fed 1 ml (40 mg/kg body wt./per day) of lead acetate orally with the help of gastric gavage orally. After the completion of 40 days of treatment, the control and treated group mice were sacrificed by cervical dislocation and epididymal parts were exposed into watch glass. Each epididymal part minced with forceps and needle in 1 ml of normal saline and were sieved by a metallic filter to avoid the tissue debris in seminal content. Sperm count was done with a Neubar haemocytometer (Eliasson, 1975) at 40 x under compound microscope.

Statistical analysis: Data were analyzed using excel 2019 and SPSS (Statistical package for Social Sciences) software. In each experimental variant obtained data from the control and treated groups are expressed as Mean ± SEM and for the comparison of data between the control and treated groups ANOVA was used to determine at significant level $p < 0.05$.

RESULT AND DISCUSSION

In the present research oral feeding of lead acetate cause significant decline in sperm counting. The number of sperms was 150.4 ± 3.12 (control), 87.08 ± 2.21* (lower dose), and 48.1 ± 1.4** (higher dose) million cells/ml in groups A, B, and C respectively (Table– 1, Figure - 1).

The higher and lower dose of lead can reduce sperm count significantly. Behavioural patterns were also observed during dosing period. Parameters including feeding habits, aggressiveness, sleep time, and body hair were recorded. Feeding habit were not changed. However, aggressiveness, sleeping time and loss of body hair increased in all treated groups as compared to the control group. (Table–2., Figure 2.). Most of the heavy metals such as lead, arsenic, mercury, cadmium affect the male reproductive function, including sperm head morphology (Meekaer *et al*., 2008) and spermatogenesis (Chowdhury 2009). Several studies have reported that heavy metals caused testicular oxidative stress, which has been linked to infertility (Takeshima, 2021). Lead is a cytotoxic that can increase the production of ROS (Reactive oxygen species) and can lower the antioxidant reserves in response to cell damage. The mechanism of lead cytotoxicity increases the production of reactive oxygen species and lowers the reserve antioxidant (Patrick.,2006). An increase of Reactive oxygen species will cause DNA damage and lipid peroxidation (Patocka & Cerny 2003). Accumulation of ROS in germ cells especially superoxide radical (O_2^-) and Hydrogen peroxide radicals (H_2O_2) may lead to apoptosis and cell death (Ercal *et al.,* 2001). Apoptosis and cell death lead to decreases production of sperms. In the present study we observe that lead acetate administration caused cytotoxicity by disturbing sperm production. The production of Sperm could be altered either because of change in the germinal cell or epididymal storage.

Table 1. Effect of lead acetate at different concentration on sperm production of *Mus musculus*

Sl.NO.	Groups	Dose	Sperm count ($\times 10^4$sperm/ml
1	Control (5)	Distilled water	150.4 ± 3.12
2	Lower dose (5)	20mg/kg B.wt	87.08 ± 2.21*
3	Higher dose (5)	40mg/kg B.wt	48.1 ± 1.4**

Data presented as Mean ± SEM, show significant (* -Shows significant, ** - Highly significant).

Table 2. Behavioural pattern during dosing period at different concentrations of lead acetate

Behaviour Parameter	Control group	During dose period	
		20 mg/kg bwt.	40 mg/kg bwt.
Feeding habit	0	0	0
Aggressiveness	0	+	++
Sleep time	0	+	++
Stiffness of body hair	0	++	+++
Loss of body hair	0	+	++

0-No change,+-increase

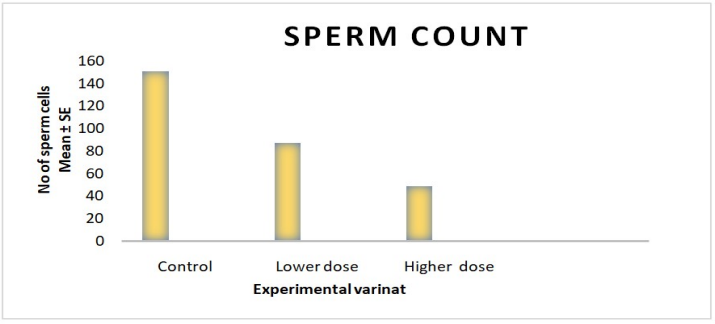

Figure 1. Graph showing No. of sperm cells at different concentration variant

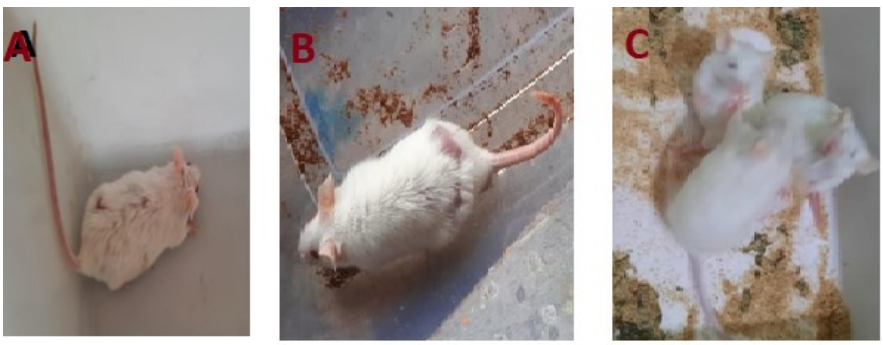

Figure 2. Behavioural pattern displayed by *Mus musculus* during dosing period (A)- Stiffness of body hair, (B)- Loss of body hair (C)- Aggressive behaviour

Any agent that interferes with meiotic cell division is also known to reduce sperm count (Aarnoud *et al.,* 2002). Decrease in sperm production was also observed by other authors after lead exposure in mice and which is in agreement of our research (Wadi & Ahmad., 1999, Acharya *et al.,* 2003; Graca *et al.,* 2004, Wang *et al.,* 2008, Bairy*etal.,* 2009).

CONCLUSION

From the obtained result data it is concluded that lead acetate produces a cytotoxic effect on germinal cells and reduces the number of sperm cells, it is very effective on behavioural patterns also in male Swiss albino mice (*Mus musculus*).

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