International Journal of Health Sciences
March 2016, Vol. 4, No. 1, pp. 82-85
ISSN: 2372-5060 (Print), 2372-5079 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/ijhs.v4n1a10
URL: http://dx.doi.org/10.15640/ijhs.v4n1a10

Evaluation the Effects of Aluminium Phosphid on Testicular Histomorphometric in Adult Rat

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Abstract

Aluminium phosphide (ALP) is available in the form of tablets or pellets. Tablets are dark brown or grayish in color and ALP poisoning was found to be the most common cause of acute poisoning in India**.** The aim of this study was to evaluate the effect of ALP poisoning on spermatogenic cells**.** This study was performed on 14 male wistar that rats divided into 2 groups of 7 rats per group. For experimental groups was regularly fed ALP for 24hour in 2 mg/kg concentration. Treatment was carried out for 24 hours. Histological evaluation on testis section was performed using tissue processing and hematoxylineosin stainig and parameters of seminiferous tubules in testes. The result indicated that there were no significant differences between groups for spermatogonia, spermatocytes, spermatid, sertoli, leydig cells number and also in diameter, circumference and area of seminiferous tubules in experimental group when compared with control group. We canclued that ALP have not significant differences in low dose on reproductive system, it may have histopathological changes in upper dose on reproductive system, more experimental investigation are necessary to do.

Keywords: Aluminiumphosphide , Spermatocyte, Spermatogonia, Spermatid, Leydig, Sertoli

Introduction

Testis is one of the pair of male gonads that produces sperm and testosterone. The adult testes are suspended in the scrotum by the spermatic cords; the coverings of the testes are the skin and the dartos tunic of the scrotum, the external spermatic fascia, the cremasteric layer, the internal spermatic fascia, and the tunica vaginalis. Each testis containing several hundred lobules consists of seminiferous tubules, in which spermatozoa develop (1).

Aluminium phosphide (AlP) is a solid fumigant which has been in extensive use since the 1940s (2). ALP poisoning was found to be the most common cause of acute poisoning in India (3).ALP is available in the form of tablets or pellets. Tablets are dark brown orgrayish in colour and contain two compounds: ALP and aluminium carbonate. It is freely available in the markets with the major virtues of being cheap and not leaving toxic residues (4).The exact mechanism of toxicity of ALP is still unknown however After ingestion of ALP, phosphine gas is released in the stomach which after absorption into the circulation affects the most organs and signs and symptoms appear in patients. Early symptoms include nausea, vomiting, retrosternal and epigastric pain, dyspnea, anxious, agitation and smell of garlic (5-8). However, some of the ALP is absorbed and metabolised in the liver (9). and also Phosphine is excreted through the breath and urine (10, 11) ingestion caused high superoxide dismutase activity and low catalase levels that result in increased formation of free radicals and accelerated lipid peroxidation (12).

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Lipid peroxidation damaged cellular membrane and caused disruption of ionic barrier, nucleic acid damage and cell death (4). The aim of this study was to evaluate the effect of ALP poisoning on spermatogenic cells**.**

Method

Animals

This study was performed on 14 male wistar rat that delivered by pastor center in karaj. Animals were kept under standard conditions (12-hour light/dark cycle at 22-24°Co, with free access to water and food) and weighting between 180-250 g then rats divided into 2 groups of 7 rats per group. For experimental groups was regularly fed ALP for 24hours in 2mg/kg concentration. The control group was fed on schedule with normal saline. Experimental and control groups were without access to water and food one night. Treatment was carried out for 24 hours. At the end of the treatment, animals were killed, right Testes were removed and fixed in a 10% formalin solution.

Histological investigation

Right testes were fixed in a 10% formalin solution and embedded in paraffin (13). The paraffin blocks were cut in slices (5μm) and mounted on silanized microscope slides. Sections (3 per animal) were stained with hematoxylin and eosin, and examined under light microscopy (14). For each animal 25 tubules with visible circular cross section were randomly chosen and spermatogonia, spermatocyte , spermatid, lydig, sertoli cells were counted. In addition, for each tubule the following parameters were determined under the light microscope equipped with motic camera 2.0m pixel: area, circumference and diameter (14).

Statistical analysis

Statistical analysis was performed with SPSS: 18. Statistical Evaluation was performed by Mann Whitney U test and The level of statistical significance was <0.05 .

Result

Testicular histomorphometry

The result indicated that there were no significant differences between groups for spermatogonia, spermatocytes, spermatid, sertoli, leydig cells number when compared with control group (Table 1).

Parameters of seminiferous tubules

Our result did not observed significant difference in diameter, circumference and area of seminiferous tubules in experimental group when compared with control group (table 2).

Discussion

In this study, treatment of rats with ALP induced histopathological changes in testis organ of rats. ALP poisoning is common in the rural belt of Northern India (3). ALP is a fumigant pesticide which generates phosphine and it can inhibit the activity of cytochrome oxidase (11), the terminal enzyme of electron transport chain in mitochondria and when there is a defect in cytochrome oxidase, it can cause excessive generation of reactive oxygen species which result in producing oxidative stress (15). Interference with the electron transport chain may also stimulate production of hydrogen peroxide from mitochondria, leading to amplitude of oxidant-induced cellular damage, resulting from attack by products of hydrogen peroxide reduction such as very reactive hydroxyl radicals (16). This may facilitate peroxidation of polyunsaturated fatty acids with loss of membrane integrity(17).Several studies indicated that ALP poisoning had histopathological changes in various organs of the body ie, lungs, liver, kidneys, heart, brain, stomach and adrenals.

The histopathological changes revealed varying degrees of congestion, edema and leucocytic infiltration, changes suggestive of cellular hypoxia. The most dramatic effects were produced in lungs, kidneys and adrenals (18). Studies of Aluminium Chloridedone on testes histological in rat and it shown that the histological observations revealed seminiferous tubules that attained different shapes, vacuolar cytoplasm with loss of normal distribution of the epithelial linning in treated groups when compared with control group (19).

So far, studies of ALP has not done on testes, we done it on dose of 2mg/kg but our result no showed significant differences, in spite of ALP generate phosphin which cause production of ROS which damaged cell DNA and result to cell apoptosis, it may have significant differences in upper dose 2mg/kg, therefore, it necessary to investigate more. ALP is very poison and it has histopathological changes in various organs of the body, According to it may have these changes in upper dose on reproductive system, more experimental investigation are necessary to do.

Acknowledgement

The authors are very grateful Dr. hajilui for his help in use of his lab for some staining.

Result

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Results:

Table 1: The effects of ALP on testicular histomorphomertic

	control		2 mg/kg		Mann-Whitney Test	
Feature	**Mean**	**SE**	**Mean**	**SE**	**Statistic (Z)**	**P-Value**
Spermatogonia	43.14	0.35	42.77	0.33	-0.72	0.47
Spermatocytes	121.20	0.54	120.84	0.54	-0.69	0.48
Spermatid	106.46	0.63	106.26	0.63	-0.26	0.79
Sertoli	11.68	0.19	11.55	0.19	-0.46	0.64
Leydig	13.15	0.22	12.52	0.20	-1.93	0.053

There was no significant different in 2mg/kg when compared with control group.

Table 2: The effects of ALP on seminiferous tubule parameters in testes

	control		2 mg/kg		Mann-Whitney Test	
Feature	**Mean**	**SE**	**Mean**	**SE**	**Statistic (Z)**	**P-Value**
Area ($\times 10^{-8} m^2$)	10.58	0.20	10.36	0.20	-0.81	0.415
Circumference ($\times 10^{-3} m$)	1.19	0.5	1.12	0.01	-0.79	0.429
Diameter mean ($\times 10^{-3} m$)	0.35	0.003	0.34	0.003	-0.96	0.33

There was no significant different in 2mg/kg when compared with control group.