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Research article

EFFECT OF α-TOCOPHEROL PRETREATMENT ON EPIDIDYMAL WEIGHT, SPERM COUNT & SPERM MORPHOLOGY ON CADMIUM INDUCED TESTICULAR DAMAGE IN MALE ALBINO RATS

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ABSTRACT
Cadmium is one of environmental pollutantsarising from electroplating, fertilizers, pigment and plastic manufactures. Clinical and animalstudies indicate that abnormalities of spermatogenesis result from exposure to this toxic metal. The purpose of present study was to evaluate the protective role of α-tocopherol on cadmium chloride induced testicular damage.
Material & Methods: Adult male wistar rats (n=6/group) were divided in four groups, normal control(0.9% saline treated), Vitamin E treated(100mg/kg bw) orally for 30 days, cadmium chloride treated(0.5mg/kg bw) & vitamin E for 30 days+ cadmium treated. Animals were sacrificed 15 days after cadmium administration or saline administration.Epididymal weight & sperm count ,sperm morphology were estimated in each group.Results: Exposure of rats to cadmium chloride showed a decrease in the epididymal weight ($P<0.001$) and, sperm count($P<0.001$), increase in the incidence of abnormal sperms($P<0.001$). Pre-treatment with α-tocopherol showed a significant increase in the epididymal weight ($P<0.001$) and, sperm count($P<0.001$), decrease in the incidence of abnormal sperms($P<0.001$) compared to on cadmium treated rats. The present study show that cadmium at the dose of 1mg/ kg b w administered is responsible for testicular damage and that α-tocopherol protects the testis from cadmium induced damage
Key words: cadmium, Epididymal weight, sperm count, sperm morphology

INTRODUCTION

Cadmium is one of environmental pollutants arising from electroplating, fertilizers, pigment and plastic manufactures. Therefore, itwill easily contaminate the soil, plants, air and water [1].Humans and animals can easily expose to cadmium toxicity by consuming plants, water and air.Cadmium is a recognized reproductive toxicant and has been reported to reduce male fertility and altered sexual behavior in both humans and rodents [2]. Testes are included among the most targetorgans for cadmium intoxication [3]. Exposure to cadmium can negatively affect the male reproductive system via degenerative changes in testes, epididymis, and seminal vesicles[4]. Recently, azoospermic persons were found to have higher serum and seminal plasma cadmium level compared with oligospermic ones [5]. Also positive relationship was found between cadmium exposure and asthenozoospermia in a rat model [6]. Cadmium is a potent human carcinogen and occasional exposure to it has been associated with cancer of lungs, the pancreas, the prostate and the kidneys [7].
Cadmium is considered as ubiquitous toxic metal that induces oxidative damage by disturbing the peroxidant – antioxidant balance in the tissue[1]. Exposure to cadmium metal is known to induce the formation of reactive oxygen species (ROS) like superoxide radical, hydroxyl ion and hydrogen peroxide [8]. To counteract the damaging ROS, aerobic cells are provided with extensive antioxidant defense mechanisms.

These consist mainly of antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase etc.) and small molecule antioxidant (e.g., glutathione, N-acetyl-cystein, vitamin E and vitamin C) [9].Vitamin E or α-tocopherol is a lipid phase antioxidant because of its lipophilic property.It terminates chain reaction of lipid peroxidation in membranes [9].It is well accepted as nature's most effective lipid- soluble, chain- breaking antioxidant, protecting cell membrane from peroxidative damage [10]. Hence, the present study was undertakento study the protective role of α-tocopherolon sperm count, sperm morphology&epididymal weight on cadmium induced testicular damage.

MATERIAL AND METHODS

The present study was conducted following approval from Institutional Bioethical Committee and strict internationally accepted guidelines, for the usage of animals in experimental study were followed. Inbred adult male albino rats of wistar strain weighing 200-300g (with an average weight of 230g) were used in the present study. Animals were housed in polypropylene cages (4-5 rats per cage) under standard laboratory conditions and fed ad libitum with commercial rodent chow (Hindusthan lever limited) and water**.**Rats were segregated into four groups

Group I: Normal control group. Administrated with a single dose of 0.9% normal saline intraperitoneally& sacrificed after 15 days.

Group II (pre-treated control) received vitamin E (100mg/kg bw orally) for 30 daysorally& sacrificed after the treatment

Group III (Cadmium treated) received a single dose of 1mg/kg bw cadmium chloride dissolved in 0.9% saline intraperitoneally& animals were sacrificed 15 days after cadmium administration

Group IV (vitamin E+ cadmium group) pre-treated with Vitamin E (100mg/kg bw)for 30days & then administered cadmium chloride & animals were sacrificed 15 days after cadmium administration

Following the completion of the experimental protocol, animals in each group were anaesthetized by injecting sodium pentabaritone (40mg/kg bw) intraperitoneally under aseptic condition. Laparotomy was performed and the reproductive organs were exposed. Epididymis was carefully separated from the testis. Both the epididymis were removed and cleaned of fat tissue and blood and kept in cold buffered saline (0.9%).The epididymiswere blotted dry and weighed.

Sperm count

The epididymis was minced in 1ml of phosphate buffered saline (pH 7.2) to obtain a suspension. The suspension was filtered through a nylon mesh. The sperm count was conducted in the filtrate as per the standard method in Neubauer's chamber [11,12]. Briefly, an aliquot from the suspension (up to 0.5ml) was taken in leukocyte hemocytometer and diluted with phosphate buffered saline up to the mark 11. The suspension was well-mixed and charged into Neubauer's counting chamber. The total sperm count in 8 squares (except the central erythrocyte area) of $1mm^2$ each was determined and multiplied by 5×10^4 to express the number of spermatozoa/epididymis.

Sperm morphology test

For the evaluation of the sperm morphology the filtrate obtained was stained with 1% eosin Y or periodic acid-Schiff's reaction and morphological defects were analyzed as explained elsewhere[13]. Briefly, the sperms in the smears were visualized under oil immersion objectives and any abnormalities of either heads or tails were noted. The microcephaly, which was also a type of head abnormality, and cephalo-caudal junction defects (CC), which were a type of tail defects have been classified separately. Two hundred sperms were screened for each animal and total abnormality was expressed as incidence/200 sperms/animal.

Statistical analysis:

The data was expressed as Mean + SD. The difference between groups was compared for statistical significance by student t test with the level of significance set at $P<0.05$.

RESULTS

In the present study administration of 1mg/kg bw cadmium chloride showed a significant decrease in the epididymal weight as well as sperm count. Epididymal weight & Sperm count (P<0.001) was significantly low in cadmium treated group compared to normal control group (Table 1). A significant increase in total sperm shape abnormality was recorded in animals exposed cadmium chloride over control groups (P<0.001) (Table 2). Pre-treatment with alpha-tocopherol showed a significant increase in the epididymal weight as well as sperm count compared to cadmium treated rats. A significant decrease in sperm shape abnormality was seen in rats pretreated withalpha-tocopherol prior to cadmium administration compared cadmium treated rats.

Table 1: Effect of pre-treatment with alpha-tocopherol on cadmium chloride induced damage on epididymal weight(gm)& sperm count (x 10^6) in wistar male rats; Mean ± SD, n=6 in each group.***P<0.001 compared control group & alpha tocopherol treated. ♦♦♦P<0.001 compared cadmium treated group

Parameters	Control group	Alpha-tocopherol treated	Cadmium treated	Alpha-tocopherol& cadmium treated
Epididymal weight	0.471± 0.09	0. 468±0.07	0.289±0.09***	0.456±0.07♦♦♦
sperm count	919.83±8.90	912.74± 8.14	250.83±9.32***	806±6.15♦♦♦

Table 2: Effect of vitamin E pretreatment prior to cadmium chloride administration on sperm morphology in rats; Mean ± SD, n=6 in each group.***p<0.001 ,**P< 0.01,*P<0.05 compared to normal control & vitamin E treated. ♦♦♦P<0.001, ♦♦P<0.01, ♦P<0.05 compared cadmium treated rats

Group	Normal	HA	TA	MC	CC	Total abnormality
Normal Control	172.83 ± 2.48	12.67 ± 0.52	11.50 + 0.55	0.66 + 0.52	2.33 + 0.02	26.50 ± 1.20
Vitmin E treated	171.67 ± 1.34	12.12 ± 0.42	12.01 ± 0.38	0.75 ± 0.64	2.84 ± 0.04	27.72 ± 5.99
Cadmium treated	120.33 ± 1.25***	33.67 + 2.66 **	32.17 + 1.28 *	3.50 ± 0.34*	4.17 + 0.75***	74.17 ± 5.46***
Vit E + Cadmium treated	163. 74 ± 1.36***	18.36 + 0.89**	12.36 + 1.03*	1.23 + 1.03*	2.84 + 1.36***	34.79 ± 8.10***

HA – Head abnormality,TA – Tail abnormality, MC – Microcephaly ,CC – Cephalocaudal junction

DISCUSSION

Cadmium is an environmental toxic pollutant affecting various tissues and organ including testes[14]. In the present study, exposure cadmium chloride in rats resulted in decreased sperm count and increased sperm abnormalities. These toxic effects induced by cadmium are in agreement with the effects of many other toxic metal[15]. The testicular &epididymal weights were significantly decreased in the cadmium exposed rat compared to control group. In the cadmium treated rats, most of the germ cells might have been destroyed either due the membranous damage or macromolecular degradation incurred by ROS leading to significant decline in the sperm count and ultimately testicular &epididymal weight loss.

Statistically significant increase in the percentage of sperm abnormalities in the cadmium treated rats emphasizes the possibility of gene alteration in germ cells induced by ROS generated through cadmium toxicity. Cadmium compounds do not appear to damage DNA directly, but through generating ROS which apparently causes DNA breaks. Moreover available literature during past have revealed the causation gene mutation induced by heavy metals [16-18]. On the contrary, testicular germ cells carrying minor gene mutations are not eliminated but are manifested as morphologically deformed sperm. It is also documented that certain metals, including cadmium are germ cell mutagens affecting specific gene loci in spermatogonial cells thereby increasing the percentage of sperm abnormality [19, 20]. It is further stated that, sperm cell morphology is genetically controlled by numerous autosomal and sex-linked genes [21]. Hence, formation of abnormal sperm population in the present study is very likely due to mutagenic effects of cadmium induced ROS on specific gene loci of germ cell chromosomes involved in the maintenance of normal sperm structure. The testicular germ cells might have been destroyed either due to membrane damage or macromolecular degradation incurred by ROS leading to a significant decline in sperm count , increased the incidence of abnormal sperms and ultimately testicular weight loss.

The evolutionary survival process has provided aerobic organisms with protective systems to neutralize the oxidative effect of oxygen and its reactive metabolites. These self sustained protective components are called as “antioxidant protective systems [22-23]. Antioxidants do their function either by quenching of electron mobility, scavenging free radicals or by breaking free radical chain reaction. Vitamin E or α-tocopherolhas been recognized since the 1940s thatitis a powerful lipophilic antioxidant that is absolutely vital for the maintenance of mammalian spermatogenesis [24]. In the present study, pretreatment with α-tocopherol prior to cadmium administration showed a significant increase in testicular &epididymal weight compared cadmium treated rats. Rats which we repre-treated with α-tocopherolprior to cadmium administration also showed the normal sperm morphology.

In summary, it is concluded from present study that cadmium at the dose of 1mg/ kg b w administered is responsible for testicular damage & that α-tocopherol protects the testis from cadmium induced damage.

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