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Full Length Research Paper

Cadmium toxicity exposure – Induced oxidative stress in postnatal development of wistar rats

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Effect of cadmium on ovary increased reactive oxygen species (ROS) was studied in rat. Lipid peroxidation and the reactive oxygen species, hydrogen peroxide were increased in the ovary of Wistar rat administered with cadmium (50 and 200 ppm) in drinking water on 45 and 65 days of post natal development. Elevation of reactive oxygen species and lipid peroxidation is suggestive of tissue damage in the rat ovary due to cadmium poisoning.

Key words: Cadmium, lipid peroxidation, reactive oxygen species, hydrogen peroxide ovary.

INTRODUCTION

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 (Wilson, 1988). Cadmium was found to result in oxidative stress (Hendy et al., 1992; Somashekaraiah et al., 1992) by inducing oxygen free radical production (Balaknina et al., 2005; Demirevska-Kepava et al., 2006).

Reactive oxygen species (ROS) are short lived reactive molecules that can modify cellular components including nucleic acids, proteins and lipids (Etienne and Hall, 2002). Involvement of ROS is implicated in neurodegenerative and other disorders, e.g Alzheimer's disease, Parkinson's disease, multiple sclerosis, Down's syndrome, inflammation, viral infection, autoimmune pathology, and digestive ulcers (Aruoma, 2003; Repetto and Llesuy, 2002; Surh and Ferguson, 2003). Numerous studies support the role of ROS in male infertility (Aitken et al., 2003). Recently reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of reproductive system and in the pathogenesis of infertility in females (Ashok et al., 2004).

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The present study was designed to test the effect of cadmium exposure through drinking water that could induce an oxidative damage in ovary of developing female Wistar rats.

MATERIALS AND METHODS

Thiobarbituruc acid, dithio-bis-nitrobenzoic acid, sodium azide, and bovine serum albumin were purchased from Sigma-Aldrich Co, St. Louis, USA. Sulphuric acid, hydrogen peroxide, chloroform and sodium hydroxide were procured from Qualigens Fine Chemicals, Mumbai, India. Pyrogallol and petroleum ether were purchased from E-Merck (India) Limited, Mumbai, India. All other chemicals were purchased from Sisco research Laboratories Private Limited, Mumbai, India.

For toxicity evaluations, cadmium (Cadmium in the form of Cadmium chloride) was selected. After preliminary toxicity evaluation, two test doses 50 and 200 ppm were selected.

EXPERIMENTAL DESIGN

Animals

90 day-old female albino rats of Wistar strain (*Rattus norvegicus*) obtained from National Institute of Nutrition, Hyderabad, weighing 140 ± 10 g was used for the present investigation. Rats were maintained in a temperature controlled animals quarter with 12 h dark: 12 h light schedule and were fed standard rat pellet diet (Broke Bond Lipton India Ltd., India) and drinking water *ad libitum.* The animals were dewormed with albendazole (Bendex - 400,

Protec Cipala Ltd., India) (10 mg/kg body weight, orally), before the initiation of the experiment. The females were mated with males at a ratio of 2: 1. Cohabitation began at approximately 16.30 h on each mating day. The following morning, the females were removed from the mating cages and individually, smeared for the presence of sperm in the vaginal lavage. The presence of sperm in the vaginal lavage is indicative of the females that had mated and they were selected for further studies. The pregnant animals were allowed to give birth.

The mother animals with female pups were divided into the following groups: Group I: Control; Group II: 50 ppm; Group III: 200 ppm.

The minimum (50 ppm) and maximum effective doses (200 ppm) were selected (Samuel, 2001), and the mother rats along with female pups were treated with cadmium in the form of cadmium chloride through drinking water from 0 to 65 day pp. Sub group I: 45 days puberty occurred. Sub group II: 65 days full growth of ovary.

At the end of the experimental period, animals were killed by cervical decapitation, and uterus was dissected out and washed with ice-cold saline. A 10% homogenate (100 mg in 1 ml buffer) of washed tissue was prepared in 0.1 M Tris-HCl buffer, pH 7.4 and used for the assay of the following biochemical parameters.

Protein

The protein content was determined by the method of Lowry et al. (1985). The total protein content was expressed as g/100 g tissue.

Lipid peroxidation

Tissue lipid peroxidation was measured by the method of Devasagayam and Tarachand (1987). The malondialdehyde content of the samples was expressed as nmoles of MDA formed/mg protein.

Reactive oxygen species

Hydrogen peroxide

Hydrogen peroxide production was assessed by the spectrophotometric method of Holland and Storey (1981). The malondialdehyde content of the sample was expressed as µ moles of MDA formed/mg protein.

Statistical analysis

All data were presented as means ± standard error of the mean (SEM). Statistical significance was calculated using student's't' test to test the significance of individual variations. Where n_1 and n_2 are the number of observations in the two classes being compared (Ostle, 1966). The value of probability was obtained from the degree of freedom by using standard table value, given by Fisher and Yates (1948). If the calculated value was more than the table value, it was significant at the probability level. The level of significance was assessed at $P< 0.05$.

RESULTS

Lipid peroxidation (LPO)

LPO levels in the ovary of cadmium exposed rat and control group were shown in Figure 1. A gradual dose dependent significant increase was observed in treated groups when compared to control. The peak level was observed in the 65 days of postnatal development (PND) rat ovary.

Reactive oxygen species

Hydrogen peroxide

Figure 2 shows the hydrogen peroxide levels in the ovary of control and the exposed groups. The production of ROS (hydrogen peroxide) significantly increased with age and dosage of cadmium when compared to control.

DISCUSSION

Lipid peroxidation is one of the main manifestations of oxidative damage, which plays an important role in the toxicity of many xenobiotics (Stohs and Bagchi, 1995; Anane and Creppy, 2001). Lipid peroxyl radicals react with other lipids, proteins and nucleic acids, propagating thereby the transfer of electrons and bringing about the oxidation of substrates. Cell membranes, which are structurally made up of large amounts of poly unsaturated fatty acids are highly susceptible to oxidative attack and consequently changes in membrane fluidity, permeability and cellular metabolic functions result (Bandyopaddhyay et al., 1999). Moreover, the end products of lipid peroxidation like melondialdehyde (MDA) can also cause tissue injury by interacting with bio macromolecules (Freeman and Crapo, 1982; Valentine et al., 1998; Mylonas and Kouretas, 1999). Cadmium, arsenic and mercury toxicity all involve similar pathways of cellular damage, inhibition of mitochondrial enzymes, and suppression of protein synthesis and production of free radicals (Fowler, 1978). Ognianovic et al. (2003) reported that Cd stimulated reactive oxygen species (ROS) thus causing oxidative damage in various tissues. Cadmium causes a significant increase of LP concentration in liver and kidney of rats, since it causes lipid peroxidation in numerous tissues both *in vivo* and *in vitro* (Kostic et al., 1993; Sarkar et al., 1998; Ognijanovic et al., 2003; El-Demerdash et al., 2004). The present study revealed an oxidative stress in rats exposed to 50 and 200 ppm cadmium through drinking water during postnatal development of rats. The dose dependent increase in lipid peroxidation (LPO) was observed in both the treatment groups (Figure 1).

Cadmium may be inducing oxidative stress by producing hydroxyl radicals (O'Brien and Salasinski, 1998), superoxide anions, nitric oxide and hydrogen peroxide (Kozzumi et al., 1996, Waisberg et al., 2003). Watanabe et al (2003), showed generation of non-radical hydrogen peroxide which by itself became a significant source of free radicals via the Fenton chemistry. Hydrogen peroxide at micromolar levels also appears

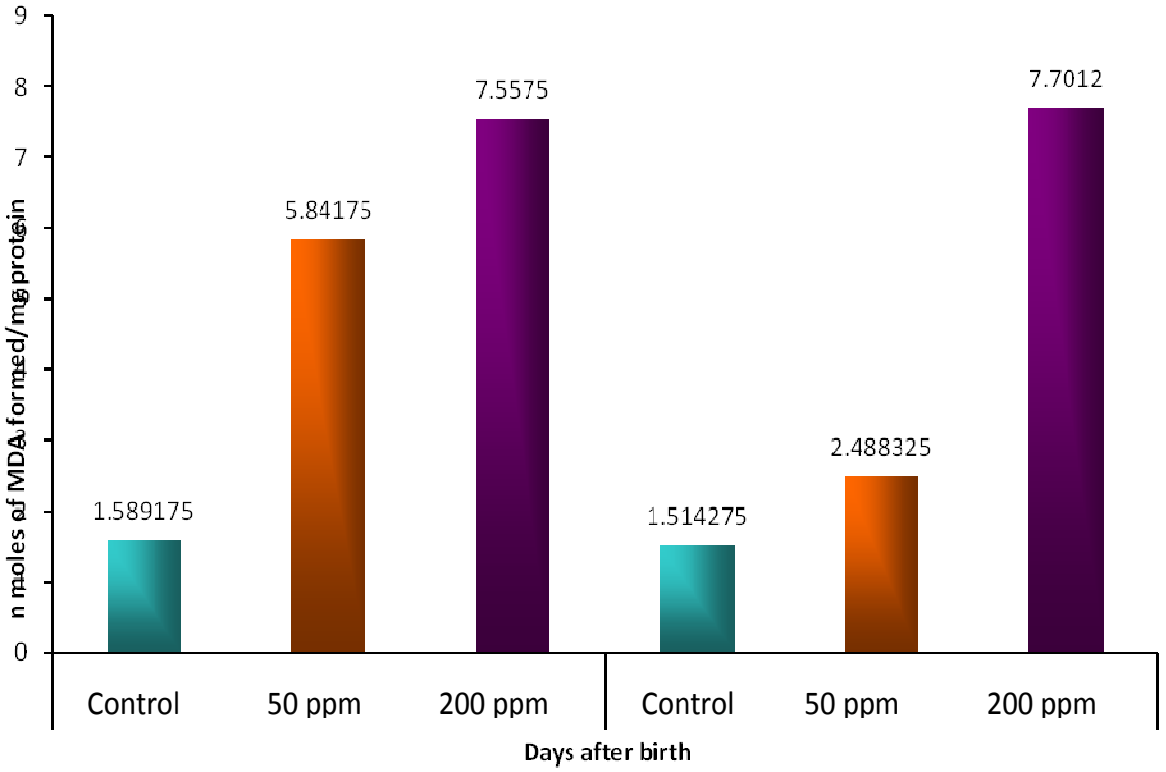

Figure 1. Effect of Cd exposure on ovarian lipid peroxidation in developing female rats.Each bar represents the mean and SEM (n=4). Statistical significance of difference among groups at $p < 0.05$; Control versus experiment: 50 versus 200 ppm.

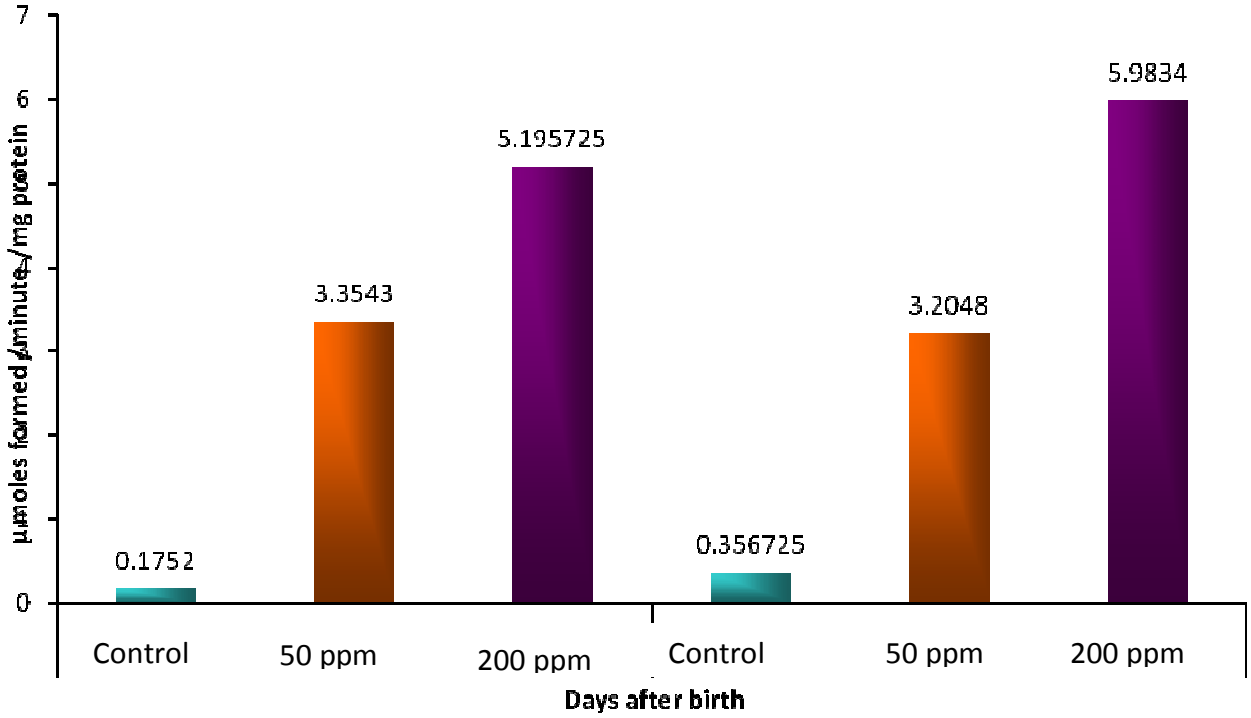

Figure 2. Effect of Cd exposure on hydrogen peroxide production in ovaries of developing female rats. Each bar represents the mean and SEM (n=4) Statistical significance of difference among groups at $p < 0.05$; Control versus experiment; 50 versus 200 ppm.

poorly reactive, but higher levels of H_2O_2 can attack certain cellular targets (Chance et al., 1979; Meler et al., 1990). The increase in hydrogen peroxide observed in the ovary of 65 day of PND was higher than 45 day PND rat ovary (Figure 2). This may be due to the formation of radicals by metal dependent reactions.

The reaction stating the formation of ROS, via the sequential univalent process of O_2 reduction forming several intermediates should be supplied.

The observed uterus LPO and H_2O_2 could have resulted in an elevated oxidative stress to the developing organ like ovary thereby altering ovary development and processing. Though low concentration of ROS is essential for normal reproductive process in the female reproductive tract, high in appropriate production of ROS is known to cause opposite effects (Man'kovs'ka and Serebrovs'ka, 1998). Therefore, the increase in ovarian LPO observed in the present study could be due to the concomitant increase in the generation of free radicals like hydrogen peroxide in the ovary of cadmium exposed rats.

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