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Effect of diethanolamine on testicular steroidogenesis and its amelioration by curcumin

Sneha Panchal, Ramtej J. Verma*

Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad 380009, India

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ABSTRACT

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Keywords: Diethanolamine Curcumin Testosterone Steroidogenesis **Objective:** To determine the toxic effect of diethanolamine on testicular steroidogenesis and its possible amelioration by curcumin in mice.

Methods: Adult Swiss strain male albino mice were orally administered with diethanolamine (110, 165 and 330 mg/kg body weight) for 45 days. In another set of experiment, male albino mice were orally administered with curcumin (10, 25 and 50 mg/ kg body weight) along with diethanolamine (330 mg/kg body weight) for 45 days. On completion of treatment, animals were humanely sacrificed, testis dissected out, blotted free of blood and used for biochemical analysis. The obtained serum samples were analyzed for serum testosterone level.

Results: Diethanolamine caused significant (P < 0.05), dose-dependent reduction in total lipid and cholesterol contents, activities of 3 β - and 17 β -hydroxysteroid dehydrogenases in testis as well as significant reduction in serum testosterone level. However, co-treatment of curcumin along with diethanolamine caused significant (P < 0.05) dose-dependent amelioration in diethanolamine-induced changes in total lipid and cholesterol contents, activities of 3 β - and 17 β -hydroxysteroid dehydrogenases and serum testosterone levels.

Conclusions: The results of the present study showed that curcumin significantly ameliorates diethanolamine reduced testicular steroidogenesis.

1. Introduction

In recent decades, the industrial world has become inundated with an ever-increasing number of chemical and physical agents whose toxicity on male reproduction is very little known [1]. About 104000 chemical and physical agents existing in workplaces and the toxicity of most of these agents is not known or has been partially studied [2]. The male reproductive system is vulnerable to the effects of these physical and chemical agents which could adversely affect male reproductive system by either disrupting the gonadal endocrine axis or the spermatogenesis process which may results in poor sperm quality and creates infertility in humans [3]. It is undeniable that good sperm quality is an essential for reproductive success. So it is necessary that the effect of occupational chemical agents on male reproductive health

*Corresponding author: R.J. Verma, Zoology Department, University School of Sciences, Gujarat University, Ahmedabad 380009, India. Tel: +91 9825323077 (mobile), +91 79 26302362 (office)

E-mail: ramteiverma2000@vahoo.com

need to be studied in great detail. Diethanolamine (DEA) is one of these occupational chemical agents.

Diethanolamine (DEA) is an alkanolamine which is produced by reacting 2 moles of ethylene oxide with 1 mole of ammonia and combining both the properties of alcohols and amines. Diethanolamine is widely used as an industrial and agricultural chemicals, metal working fluids and personal care products like cosmetics, shampoos and hair conditioners [4]. Aqueous DEA solutions are also used as solvents for numerous drugs that are administered intravenously [5]. It is widely used in preparation of DEA salts of long chain fatty acids that are formulated into soaps and surfactants used in liquid laundry and dishwashing detergents. Diethanolamine production and its wide use in industrial and consumer products may results in its release to the environment and large, unaltered amount of the chemical being discharged into water and sewage [6,7].

General populations are exposed to DEA via dermal exposure through consumer products such as soaps, shampoos and cosmetics [7]. Occupational exposure to DEA may occur by inhalation of vapors and aerosols and also by skin contact during the use of DEA in many industries [7]. At workplace DEA exposure could occur in a manufacturing facility or a

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facility which makes products using DEA as a raw material. The potential exposure may occur during process sampling, filter changes and material loading. Diethanolamine also has been detected in workplace air in the metal manufacturing industry. It was present in bulk cutting fluids at concentrations ranging from 4 to 5% [8]. National Occupational Exposure Survey estimated that about 800000 metal workers and others in United States were potentially exposed to DEA [9].

Diethanolamine is metabolized by biosynthetic routes common to endogenous alkanolamines (ethanolamine and choline) and incorporated into phospholipids in liver, kidney, spleen and brain of mice and rats [6]. It interacts with lipid metabolism probably by the enzyme-catalyzed in vivo transphosphatidylation of phosphatidylcholine by phospholipase D [10]. Diethanolamine can be incorporated into phospholipids and inhibit the in vitro and in vivo synthesis of phospholipid derivatives of choline and ethanolamine [11]. It is excreted predominantly unchanged with a half-life of approximately one week in urine [12]. Diethanolamine is an irritant to skin and eyes and cause systemic toxicity mainly in liver, kidney, red blood cells and the nervous system following oral and/or dermal exposure in laboratory animals [13]. It also creates choline deficiency which includes increased generation of free radicals and increased susceptibility to oxidative damage which may induce DNA damage and alter gene expression, increased cell death and increased cellular proliferation [11,14].

Herbal medicine is the oldest form of healthcare known to mankind because of the general belief that herbal medicines have no side-effects besides being cheap and locally available [15]. Curcumin is a major active chemical constituent isolated as yellow pigment of turmeric powder produced from the rhizome of the plant *Curcuma longa* which is commonly used as dietary spice and colouring agent in food [16]. Scientists have reported that curcumin possesses wide variety of desirable preventive or putative biological and medicinal properties such as anti-inflammatory, anti-platelet, antioxidant, antidiabetic, antimutagenic, anticancer, anti-infection and antibacterial [17].

Male infertility is a major clinical worldwide problem which affects the people medically and psychologically. Hence main aim of this study was to evaluate the toxic effect of diethanolamine on testicular function in Swiss albino mice and its possible amelioration by curcumin, an herbal remedy.

2. Materials and methods

2.1. Experimental animals

For this study animals were approved by Institutional Animal Ethics Committee of Gujarat University, Ahmedabad and also approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg-167/1999/CPCSEA), New Delhi, India. Healthy young inbred Swiss-strain male albino mice (*Mus musculus*), weighing approximately 30–35 g were obtained from Cadila Pharmaceuticals, Ahmedabad and were maintained under laboratory conditions in a 12-h light/dark cycle at (25 ± 2) °C and relative humidity of 50–55%. Animals were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and potable water *ad libitum*. Animals were handled according to the guidelines published by the Indian National Science Academy, New Delhi, India (1991).

2.2. Chemicals

Diethanolamine was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India and was of analytical grade. Curcumin was purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India. All the other chemicals used in the present study were of analytical grade and purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India, Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Sigma Aldrich, St. Louis, MO, USA. Olive oil was obtained from Figaro, Madrid, Spain.

2.3. Experimental design

Ninety animals were randomly divided in nine groups and caged separately. Group 1 (untreated control) animals were maintained without any treatment. Animals of group 2 (vehicle control) received olive oil (0.2 mL/animal/day) for 45 days as olive oil was used as vehicle to dissolve curcumin. Animals of group 3 (antidote control) received 50 mg/kg bw/day of curcumin. Animals of group 4, 5 and 6 were orally administered with low dose (110 mg/kg bw/day), mid dose (165 mg/kg bw/day) and high dose (330 mg/kg bw/day) of DEA respectively. Animals of group 7, 8 and 9 were orally administered with 10, 25 and 50 mg/kg bw/day of curcumin respectively along with high dose of DEA (330 mg/kg bw/day).

2.4. Determination of biochemical parameters

After completion of treatment animals were humanly sacrificed by cervical dislocation. Then the testes were quickly dissected out, blotted free of blood and used for biochemical analysis such as total lipid content [18], cholesterol content [19] and activities of 3β and 17β -hydroxysteroid dehydrogenases [20].

2.5. Serum testosterone level

Blood samples were collected by cardiac puncture in nonanticoagulant added tubes, allowed to clot for 20–30 min and centrifuged at $1000 \times g$ for 10 min at 4 °C. The non-haemolysed serum samples obtained were stored at -4 °C and used for analysis of serum testosterone level. Serum testosterone was estimated by Chemiluminescence Immunoassay (CLIA) method using Advia Centaur XP (Siemens) Immunoassay system.

2.6. Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) followed by Tukey's test using GraphPad Instant software version 5.03. Data are expressed as the means \pm S.E.M. The level of significance was accepted with P < 0.05. Pearson's correlation analysis was used to determine the correlation between control and treated.

3. Results

Oral administration of DEA for 45 days (Groups 4–6) caused significant (P < 0.05), dose-dependent decrease in total lipid (r = -0.9893) and cholesterol content (r = -0.9962) as compared to untreated control (Groups 1). Diethanolamine also caused significant (P < 0.05), dose-dependent reduction in activities of 3β -hydroxysteroid dehydrogenase (r = -0.9983) and 17β -

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