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Ameliorative effect of selenium on carbendazim induced oral sub-chronic testicular toxicity in bucks

Bibhuti Ranjan*, P.S. Daundkar, Satyavan Rampal

Department of Pharmacology and Toxicology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India

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ABSTRACT

In the present investigation, ameliorative effect of selenium (Se), an antioxidant, on oral sub chronic toxicity of carbendazim (CBZ) was assessed by studying various indices of antioxidant defense system. Goat bucks were randomly divided into four groups of four animals each. Group I served as control, Group II was orally administered carbendazim at the dose rate of 50 mg/kg, Group III was orally administered selenium in the form of sodium selenite at the dose rate of 0.05 mg/kg and Group IV was orally administered carbendazim along with selenium at same dose rates for 90 consecutive days. Prolonged administration of carbendazim produced oxidative stress as evidenced by increased lipid peroxidation (LPO) and significant decrease in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The co-administration of selenium with carbendazim produced less marked alterations in the indices of antioxidant status. Carbendazim resulted in significant alteration in sperm count, live sperm percent and also produced sperm abnormalities. Carbendazim co-administered with selenium lead to partial restoration of sperm count and live sperm per cent and resulted in diminished sperm abnormalities. Carbendazim produced anti-androgenic effect as depicted by significant decrease in testosterone level. On the contrary, bucks co-administered with carbendazim and selenium resulted in partial restoration of testosterone level. Thus, from the findings of present investigation it can be concluded that carbendazim leads to testicular oxidative stress as well as antiandrogenic effect and selenium supplementation partially protect from carbendazim induced testicular toxicity.

1. Introduction

Pesticides are being used as an integral part of modern agriculture practices. However, use of these pesticides is not highly regulated in developing countries. Thus, pesticide use has been accompanied with unintended environmental consequences and harmful effects on non target species (Xavier et al., 2004). Benzimidazoles have been reported to produce reproductive toxicity in rodents. Carbendazim (methyl-2-benzimidazole carbamate) is a broad spectrum benzimidazole fungicide extensively used on fruits, vegetables, field crops and ornamental plants for control of molds, rots, and blights (Vettorazzi, 1976). Carbendazim (CBZ) was found to be two times more potent than its parent compound benomyl (Lim and Miller, 1997). Carbendazim alters antioxidant defense system

* Corresponding author at: Department of Pharmacology and Toxicology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana 141004, India.
Tel.: +91 8884091555; fax: +91 1612400822.
E-mail addresses: bibvet@gmail.com (B. Ranjan), prashant1985gadvasu@gmail.com (P.S. Daundkar), satyavan1@sify.com (S. Rampal).

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and antioxidants have protective effect against the carbendazim induced testicular toxicity (Rajeswary et al., 2007b). Carbendazim induces testicular oxidative stress leading to generation of free radicals and alterations in the antioxidants or oxygen free radical scavenging enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase (Rajeswary et al., 2007a) which could be responsible for sperm abnormalities (Metwally et al., 2011).

Selenium (Se), an essential nutrient, is an integral component of the cellular antioxidant defense system. Glutathione peroxidase (GPx), a selenoenzyme, scavenges free radicals and is a key regulator of lipid peroxidation. Moreover normal spermatogenesis of mammals requires selenium and is mainly mediated by two selenoproteins, namely phospholipid hydroperoxide glutathione peroxidase (PHGPx/GPx4) and selenoprotein P (Boitani and Puglisi, 2008). Daily oral administration of linseed oil with concomitant exposure to carbendazim for 48 days to rats results in significant reduction in sperm morphology defect and improves the sperm count (Metwally et al., 2011).

Although some work on the toxicity of carbendazim has been done in different species but there is limited information available regarding its testicular toxicity in goat bucks. Therefore, the current study was designed to investigate the ability of carbendazim and selenium to modulate the activities or concentrations of endogenous antioxidants in bucks.

2. Materials and methods

2.1. Experimental animals and treatment group

The experiment was conducted from 12.01.2011 to 15.4.2011 in the animal house of the Department of Veterinary Pharmacology & Toxicology, GADVASU, Ludhiana, Punjab located in the normal trans Gangetic plains in north India. All the bucks were of beetle breed aging around one and half year. All the four groups were statistically (Levene's test, significance > 0.05) homogenous with the average body weight of 51.0 ± 3.00 kg. All bucks were healthy having shiny body coat and having a scrotal circumference of 20.0 ± 1.00 cm. All the experimental animals were examined for general health and were also screened for any gross scrotal/testicular abnormalities. The animal house fulfills all measures of husbandry to ensure healthy and homogenous ambient conditions throughout experiment. Animals were fed with Berseem (4–5 kg) mixed with chopped wheat straw (1.5–2 kg) along with 500 g concentrate per animal per day. Water was provided ad libitum throughout the experiment. Goat bucks were sexually rested throughout the study period and they were divided into four groups of four animals each. Animals were acclimatized in departmental shed for 3 weeks and deworming was done prior to the experiment.

Group I served as control, Bavistin contains 50% (w/w) of carbendazim. Carbendazim (BavistinR) was in powder form weighed, mixed daily in the distilled water to make 40% solution and then drenched to Group II orally through drenching tube at the dose rate of 50 mg/kg for 90 consecutive days. Selenium (sodium selenite, Loba Chemicals, Mumbai) was also available as powder, weighed daily, dissolved in distilled water so as to make 0.4 mg/ml selenium solution and then administered orally to Group III through drenching tube at the dose rate of 0.05 mg/kg for the same period. Group IV was orally administered carbendazim along with selenium in the similar manner throughout experiment. All the animals were weighed weekly to make necessary changes in dosage. Dose of carbendazim was calculated on the basis of data available from Punjab Agriculture University, Ludhiana (daily fodder and water intake of a buck on body weight basis and carbendazim residues present in it). It was the least toxic dose when compared to the previous studies in laboratory animals (Markelewicz et al., 2004; Lu et al., 2006; Gawande et al., 2010; Metwally et al., 2011).

2.2. Testicular oxidative analysis

After the completion of experiment, one testicle from each animal was removed by surgical resection and was stored at −80 °C for further analysis. 10% testicular homogenate was prepared in ice cold 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 4000 rpm for 15 min to harvest supernatant. Supernatant was used for estimation of activity of superoxide dismutase (Marklund and Marklund, 1974), catalase (Aebi, 1983) and glutathione peroxidase (Hafeman et al., 1974). Lipid peroxidation (Stocks and Dormandy, 1971) was estimated using 10% testes homogenates in PBS, pH 7.4.

2.3. Sperm count, sperm viability and sperm abnormalities

In brief, seminal fluid was diluted with 0.2% of eosin solution and was observed under the microscope for sperm count. Sperm viability and sperm abnormalities were carried out by mixing the drop of seminal fluid with the drop of eosin and nigrosin stain and observed under the microscope (Bearden and Fuquay, 1997).

2.4. Hormone analysis

Testosterone is secreted in discrete peaks and chances are that the serum concentrations are biased in a given moment so for estimation of hormone, blood samples were collected at 20 min interval for 6 h to calculate mean hormone level. All the blood samples were collected from the jugular vein in heparinized and sterile vials for separation of plasma on 90th day of treatment. Testosterone was assayed by using Lumax™ Model 4101 Chemiluminiscence Immuno Assay (CLIA) Strip Reader (Monobind, Inc., USA), using Acculite CLIA microwells (Monobind, Inc., USA) according to the standard manufacturer's protocol. The assay was having sensitivity 0.04 ng/ml sample concentration. Coefficients of variation (within and between assays) were 4.85% and 5.73% respectively.