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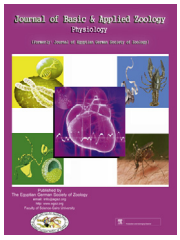

Effect of CoO nanoparticles on the carbohydrate metabolism of the brain of mice "*Mus musculus*"

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Abstract The effect of CoO nanoparticles (NPs) on the brain of mice administered through gastrointestinal tract for a period of 30 days was studied. AAS analysis revealed that NPs administered orally were retained by cerebellum, cerebral cortex, medulla oblongata and olfactory bulb. This retention of nanoparticles by the brain promoted a significant increase in glucose, pyruvate, lactate and glycogen levels along with the concomitant increase in hexokinase, glucose 6 phosphatase, and lactate dehydrogense activities. However, a decrease in glucose 6 phosphate dehydrogenase activity was observed in the brain regions indicating a deterioration of the pentose phosphate pathway. Thus, the present study suggests that the CoO NPs affect the carbohydrate metabolism of the brain.

Introduction

Cobalt is an important metal, which is used as a binder in the metal industry and as a constituent of most alloys (Jensen and Tüchsen, 1990). Cobalt-based NPs, particularly cobalt oxide NPs are attracting enormous interest currently due to their unique shape and size-dependent properties and are used in different applications like catalysis, magnetism, sensors, electrochemistry, pigments, and energy storage (Liu et al., 2005; Papis et al., 2009). They are one of the interesting chemical compounds used in biomedical applications as a starting material for the construction of dextran coating and magnetic polymer microspheres. In medicine, cobalt is known as an MRI contrast agent in combination with iron, gold, graphite and platinum (Rebello et al., 2010; Magaye et al., 2012).

Cobalt is also used for anaerobic waste water treatment and cancer therapy. Exposure of cobalt to humans occurs mainly from the environment, industry or after joint replacement in implants from the cobalt-chrome alloy. These exposures may lead to numerous lung diseases, including fibrosis, interstitial pneumonitis and asthma (Magaye et al., 2012). The potential of cobalt and its compounds as carcinogenic agent was evaluated by IARC in 1991.

Currently, the application and use of cobalt NPs range from industry to medicine, but research data on its bioeffects are limited. In addition, very little is known about the toxicity of cobalt nanoparticles and it is assumed that its biological activity is mediated by ionic form and can be determined by evaluating its soluble compound as for other metals but evidences showed that the biological activity of cobalt is not exclusively mediated by ionic form dissolved in biological media (Magaye et al., 2012).

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Cobalt nanoparticles are known to be genotoxic *in vitro*. These NPs are internalized by leukocytes, further they interact with DNA, leading to genotoxic effects which are seen on the reticuloendothelial system (Magaye et al., 2012). Further it is reported that cobalt oxide NPs enter the cells, remains confined to vesicles causing production of ROS in human cell lines (Papis et al., 2009). The *in vivo* study carried out in rats revealed that cobalt nanoparticles induced malignant mesenchymal tumors (Magaye et al., 2012). The inhalation study exposed to CoO aerosols with hamsters remained non-positive (Jensen and Tüchsen, 1990) wherein, Syrian golden hamsters exposed to cobalt oxide dusts showed no increase in tumors, however, the study was faulted for poor survival (IARC, 1991). Doses given to the mixed population of rats over 2 years showed bronchio-alveolar proliferation at high rates showing benign lung tumor, bronchio-alveolar carcinoma, adenocarcinomas and bronchioalveolar adenomas (Bucher et al., 1999). These studies indicate that cobalt nanoparticles cause genotoxic and carcinogenic activity *in vitro* and *in vivo* (Magaye et al., 2012). However, a few reports indicate that NPs can reach the brain and are associated with neurodegeneration leading to Parkinson's disease, Huntington's disease, Alzheimer's disease and primary brain tumors (Win-Shwe and Fujimaki, 2011). The exact cause of these diseases is still unknown, but environmental factors or pollutants, including NPs, may be a potential risk factor. NPs can enter the body via different routes like inhalation, dermal penetration, and ingestion, and then are distributed to various tissues including the brain by means of systemic circulation, so it becomes necessary to evaluate the toxic effects of these NPs on the brain (Win-Shwe and Fujimaki, 2011). Thus the review of literature indicates that most of the studies of cobalt effects are with reference to *in vitro* effects showing pseudo-tumor formation, genotoxicity on mammalian cells, peripheral leuckocytes, BALB/3T3 mouse fibroblast cells and macrophages whereas, the *in vivo* studies are only on malignant mesenchymal tumors. However, there is hardly any report of CoO NPs action on the brain as well as their retention and influence on carbohydrate metabolism of the brain. Therefore, here an attempt is made to investigate the action of CoO NPs on carbohydrate metabolism of the brain of mice.

Materials and methods

Nanoparticles

Cobalt oxide nanoparticles measuring average size 50 nm, spherical in shape, black in color, 99% pure, powder in form, without any odor, having specific surface area $\geqslant 10\ m^2/g$, density 6.1 g/cm^3 and with Zeta potential −20.4 mV were obtained from Nanoshel, Wilmington, USA (Product code NS6130-03-380).

Preparation of nanoparticles suspensions

The NPs were suspended directly in mammalian saline (0.9% NaCl prepared with deionized water (DI water)) in order to coat the NPS with saline for their stabilzation and then dispersed by using a sonicator. A stock concentration of 100 mg/10 ml was prepared, further the dilutions were made as per the concentration and body weight of mice. Three concentrations were used as 5 mg, 10 mg and 20 mg/kg body weight (bwt) of mice. The stock and diluted suspensions of CoO NPs were monitored for any agglomeration through microscopic observations of sample drops of stock and suspensions. The shaker bath set at 30 °C helped to maintain suspension of NPs in mammalian saline.

Maintenance of animals

The healthy Mice (*Mus musculus*), weighing 22–32 g were housed in polypropylene cages. The animals were maintained at ambient laboratory conditions, with a dark and light cycle of 12 h having free access to water and standard pellet diet (Hindustan Lever, Bangalore, India). Ethical approval (Ref No. 105/C-2013) was obtained from the Institutional Animal Ethics Committee, based on the CPCSEA guidelines (CPCSEA, 2003) and were followed throughout the study period. The animals were maintained at animal house facility in Department of Zoology, Goa University.

Chronic treatment (30 days)

Mice were divided into 4 groups (5 mice/group) such as Controls, Exp 1(5 mg/kg), Exp 2(10 mg/kg) and Exp 3 (20 mg/kg). Based on the Ld50 values the sub lethal doses were selected for exposure (Shaikh et al., 2015). The animals were fed orally with nanoparticles (suspended in 0.5 ml mammalian saline (0.9% NaCl)) for 30 days (respective dose referred above at 10 am every day). The controls received 0.5 ml of mammalian saline. After thirty days, the animals were anaesthetized using avertin and perfused by mammalian saline. For perfusion a small cut was given at the right atrium and the butterfly needle containing 20 ml of saline was slowly flushed through the left atrium at the rate of 2 ml/min. Further the mice were decapitated and the brain was immediately harvested and transferred to an ice cold mammalian saline (0.9% NaCl). All the brain regions viz., cerebellum, cerebral hemisphere, olfactory bulb, medulla oblongata, hippocampus, cingulate gyrus, pons were quickly isolated aseptically in a cold chamber set at −4 °C. Each isolated region was further cut into tiny pieces and thoroughly and repeatedly rinsed till all traces of blood were removed. Further the tiny pieces were viewed under stereo zoom microscope to ensure that there were no blood traces. Such pieces were once again rinsed thoroughly with cold saline and then immediately stored at −20 °C until use. Assessment of post mortem induced changes to the mice brain was performed as described by Hunsucker et al. (2008) as well as by viewing fresh frozen sections under the microscope.

Deposition of nanoparticles in the brain

After perfusion, the mice were sacrificed by decapitation method, the brain was removed, rinsed with mammalian saline, and its cerebral cortex, cerebellum, medulla oblongata, olfactory bulb, hippocampus, cingulate gyrus, pons were separated carefully as mentioned above. The remaining brain tissue was also carefully rinsed with mammalian saline and stored for detection of CoO NPs at −4 °C until use (hippocampus, cingulate gyrus, pons and remaining brain tissue when separated had CoO NPs below detectable limit, so they were pooled together for detecting CoO NPs). These brain parts along with