Histological Changes in the Duodenum of Mice Treated with Cobalt

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Abstract :

The present study was conducted using 16 adult male Swiss albino mice (weighing 40-50g). They were equally divided into four experimental groups. The first group was only given tap water as control during experimentation period (40 days). Group II, group III and group IV of mice were daily given a subcutaneous injection of cobalt as cobalt chloride ($CoCl_2$) at a dose of 20mg, 40mg, and 60mg /Kg body weight respectively. The examination of the histological sections of the second experimental group of mice (low dose group) showed no particular changes in the columnar epithelial cells of the duodenum of mice. The examination of the histological sections of the third experimental group of mice (moderate dose group) showed abundant degenerative changes in the columnar epithelial cells of the duodenum of mice such as increased size, presence of spaces, and dark appearance of the nucleus while these changes were more abundant in the columnar epithelial cells of the duodenum of the fourth experimental group of mice (high dose group) in comparison with the third experimental group such as increased size, presence of spaces, and dark appearance of the nucleus.

Key words: Cobalt, Carcinogenicity, Duodenum, Mice.

التغيرات النسجية في الأثني عشر للفئران المعاملة بالكوبالت

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الخلاصة :

أجريت هذه الدراسة على ستة عشر فأراً بالغاً من الذكور تراوحت أوزانها بين 40−50غم. قسمت الحيوانات بالتساوي الى أربعة مجاميع تجريبية. المجموعة الأولى كانت بمثابة مجموعة السيطرة حيث اعطيت الماء العادي طوال فترة الدراسة البالغة (40) يوم. اعطيت فئران المجموعة الثانية والثالثة والرابعة حقنة يومية من الكوبالت (Co) تحت الجلد على شكل كلوريد الكوبالت $Co\ Cl_2$ بجرعة مقدارها 20، 40، و60ملغم/ كغم من وزن الجسم على التوالي. اظهرت نتائج فحص المقاطع النسجيه لفئران المجموعة الثانية عدم وجود تغيرات ملحوظة في الخلايا الطلائية العمودية للاثني عشر(مجموعة الجرعة الواطئة).اظهرت نتائج فحص المقاطع النسجية لفئران المجموعة الثالثة تغيرات نسجية انحلالية واضحة في الخلايا الطلائية العمودية للأثني عشر (مجموعة الجرعة المعتدلة) كزيادة حجم الخلايا، وجود فراغات، والمظهر الغامق للنواة بينما كانت هذه التغيرات أكثر وضوحاً في الخلايا الطلائية العمودية للأثني عشر لفئران المجموعة الرابعة (مجموعة الجرعة العالية) مقارنة بفئران المجموعة الثالثة كزيادة حجم الخلايا، وجود فراغات، والمظهر الغامق للنواة.

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Introduction :

Cobalt (Co) is a metallic element occurring in the most common compounds in the +2 or +3 oxidation states. Cobalt salts have been commonly used for many years in animal nutrition. Six cobalt salts are currently authorised in the European Union (E.U) as feed additives (cobalt acetate tetrahydrate, basic cobalt carbonate monohydrate, cobalt chloride hexahydrate, cobalt nitrate hexahydrate, cobalt sulphate monohydrate and cobalt sulphate heptahydrate) for all animal species with a total maximum content of 2mg Co/Kg complete feedingstuffs [1].

The apparent absorption of cobalt from cobalt chloride measured in laboratory animals was in the range of 13-34%. Cobalt is predominantly excreted via the faecal route. Absorbed cobalt follows aqueous excretion routes, via kidney but also via the milk. About 43% of body cobalt is stored in muscle; however, kidney and liver are the edible tissues containing the highest cobalt concentrations and are most susceptible to increase such concentrations as a response to increased cobalt concentrations in feed [1].

In an unspecified strain of rabbits administered 0.25 mg/kg cobalt sulfate per day orally or by injection for 2 months, some accumulation of cobalt occurred in the liver, small intestine, lung, blood, kidney, and stomach [2]. Occupational exposure to cobalt occurs principally in refining processes, in the production of alloys, and in the tungsten carbide hard metal industry [3]. Exposure under these conditions is primarily dermal or via inhalation of cobalt metal dusts or fumes, often in combination with other elements such as nickel, arsenic, or tungsten; adverse respiratory effects (pneumoconiosis) have been reported at cobalt concentrations between 0.1 and 2mg/m^3 [4]. Cobalt promotes aberrant microtubule assembly [5] and can alter the activity of metaloenzymes such as carboxypeptidase [6]. Cobalt also inhibits the activity of DNA polymerase I from *Micrococcus luteus* [7]. Occupational exposure of humans to cobalt- containing dust, either as cobalt metal or as hard metal, has been shown to result in cardiomyopathy, characterized by functional effects on the ventricles [8] and/or enlargement of the heart [9], [10]. Necrosis of the thymus was reported in rats exposed to 19mg cobalt/m^3 as cobalt sulfate over 16 days, and hyperplasia of the mediastinal lymph nodes was found in mice exposed to 11.4 mg cobalt/m^3 for 13 weeks [11]. Occupational exposure to cobalt in humans has been reported to cause several effects on the nervous system, including memory loss (Wechsler Memory Scale-Revised), nerve deafness, and a decreased visual acuity [12], [13]. Several studies have evaluated the effects of inhalation of cobalt-containing compounds on possible carcinogenicity in humans. The mortality of a cohort of 1,143 workers in a plant that refined and processed cobalt and sodium was analyzed [14]; the French national population mortality data were used as a reference population. Cobalt plays a critical role in the synthesis of vitamin B12. In contrast, excessive exposure to cobalt is associated with several conditions, including asthma, pneumonia, and hematological abnormalities [15]. In addition, nickel, cobalt, cadmium, and other metals are known or suspected carcinogens [16]. Studies in various systems have shown that exposure to certain metals, such as cobalt, promotes a response similar to hypoxia. Hypoxia is defined as a state when oxygen tension drops below normal limits and it plays a central role in development and several pathological conditions including stroke, cardiovascular disease, and tumorigenesis [17]. A single injection of 35 mg/Kg cobalt chloride caused degranulation and disintegration of the α cells of the pancreatic islets of rabbits [18]. This was followed by degranulation of the β cells. Shabaan *et al*.[19] observed fibrosarcomas in 14/40 male Wistar rats 8 months to 1 year after administration of 40 mg/Kg cobalt chloride by subcutaneous injection once per day for 10 days. Four of these neoplasms were not at the site of injection. Cobalt has been shown to cross the placenta; cobalt chloride and nitrite salt solutions induced fetal cleft palates when injected alone into mouse dams but inhibited cleft formation caused by cortisone or phenytoin [20], [21]. Sprague Dawley rats maintained on diets containing 265ppm cobalt for 98 days showed degenerative changes in the testis; these changes were considered secondary to hypoxia [22]. The objectives of this study were to examine the possible effects of cobalt on mice duodenum and to determine whether or not cobalt can cause histological alterations which could affect duodenum functions.

Materials and Methods:

In the present study, 16 adult male mice were used (weighing 40-50g) which were divided into four equal experimental groups. Mice were maintained in plastic cages and allowed free access to standard laboratory food and tap water

ad libitum. The first group of mice which considered as control group drank only tap water during the entire period of experimentation (40 days). The second group of mice was daily given a subcutaneous injection of cobalt as cobalt chloride ($CoCl_2$) at a dose of 20 mg /Kg body weight (Considered as low dose group), the third group was daily given a subcutaneous injection of cobalt as cobalt chloride ($CoCl_2$) at a dose of 40 mg /Kg body weight (Considered as moderate dose group) while the fourth group was daily given a subcutaneous injection of cobalt as cobalt chloride ($CoCl_2$) at a dose of 60 mg /Kg body weight (Considered as high dose group). Twenty- four hours after injection of last dose, two animals from each group were sacrificed (the other two mice from each group were kept in case of necessity). Duodenums were excised and subsequently fixed in 10% formalin overnight. After fixation, the duodenums were processed, wax block and slides were prepared then stained in haematoxylin and eosin for histological studies [23]. Histological sections were examined by light microscope and photographed at a magnification of 10 & 40X.

Results:

In the present work, the examination of the histological sections of the first group of mice (control) showed no histological changes in the duodenum of mice Figure 1-. The examination of the histological sections of the second experimental group of mice given a subcutaneous injection of cobalt at a dose of 20 mg Co/Kg (low dose group) showed no particular changes in the duodenum of mice Figure 2. On the other hand, the examination of the histological sections of the third experimental group of mice given a subcutaneous injection of cobalt at a dose of 40 mg Co/Kg (moderate dose group) showed abundant degenerative changes in the columnar epithelial cells of the duodenum of mice such as increased size, presence of spaces, and dark appearance of the nucleus, figure 3, while these degenerative changes were more abundant in the columnar epithelial cells of the duodenum of mice of high dose group given a subcutaneous injection of cobalt at a dose of 60 mg Co/Kg, figure 4 in comparison with the changes in the columnar epithelial cells of the duodenum of mice of moderate dose group such as increased size, presence of spaces, and dark appearance of the nucleus.

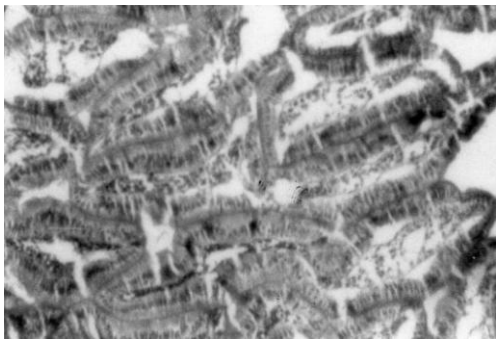

Figure 1- Transverse section in the duodenum of control group of mice showing normal columnar epithelial cells.
Stain: Haematoxylin & Eosin 10X.

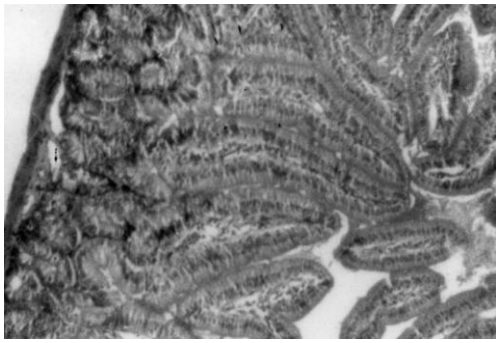

Figure 2- Transverse section in the duodenum of mice treated with 20 mg Co /Kg (low dose group) showing no particular changes in the columnar epithelial cells.
Stain: Haematoxylin & Eosin 10X.

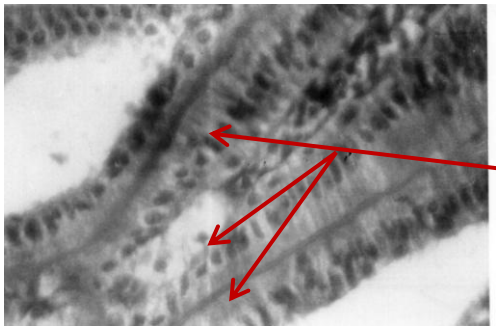

Figure 3- Transverse section in the duodenum of mice treated with 40 mg Co /Kg (moderate dose group) showing abundant degenerative changes in the columnar epithelial cells such as increased size, presence of spaces, and dark appearance of the nucleus.
Stain: Haematoxylin & Eosin 40X.

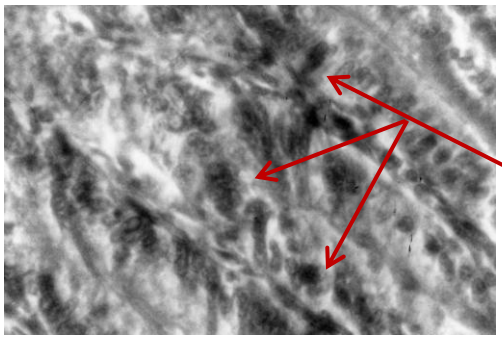

Figure 4- Transverse section in the duodenum of mice treated with 60 mg Co /Kg (high dose group) showing more abundant degenerative changes in the columnar epithelial cells such as increased size, presence of spaces, and dark appearance of the nucleus.
Stain: Haematoxylin & Eosin 40X.

Discussion :

The results of the present study confirmed that cobalt affected the duodenum of the mice and caused degenerations in the columnar epithelial cells of the duodenum. These results are consistent with the opinion expressed by Gál *et al*. [24] that excessive Co intake may cause adverse effects in target species. The present study suggests that under certain doses of cobalt could cause damages to the columnar epithelial cells of the duodenum of mice. That coincides largely with the notion of Carson *et al*. [25] that case reports have suggested that acute intakes following ingestion of >30 mg Co/day may cause gastrointestinal upset. Several experiments performed in laboratory animals support the *in vivo* carcinogenicity of Co salts when administered by different routes, and namely local tumours (sarcomas) at injection sites and lung tumours after intratracheal instillation [26]. Smith and Carson [27] also reported that cobalt is distributed to all tissues after administration by the oral or inhalation routes or by injection.
Lastly, exposure to cobalt results in a wide spectrum of toxicities in mammals [6]. In conclusion, it seems reasonable to consider that the present study provided evidences regarding degenerative and harmful effects of cobalt in the duodenum of mice.

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