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" Protective Roles of Chitosan Nanoparticles and Ascorbic Acid Against Chromium Induced Thyroid Toxicity in Albino Rats"

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ABSTRACT:

Because of the genotoxic, carcinogenic, neurotoxic, nephrotoxic, and immunotoxic properties of hexavalent chromium (Cr VI) which is a heavy metal that is used extensively in many industries, it is considered dangerous for both humans and animals and extremely toxic to all living things. Through oxidative stress, it causes thyroid gland toxicity.

Chitosan nanoparticles are naturally occurring materials with exceptional biological, physicochemical, and antimicrobial qualities. Their bioactivity is safe for humans and they are an excellent environmentally friendly material .Chitosan is a proven antioxidant agent.

Ascorbic acid, also known as vitamin C, is a necessary micronutrient that helps humans carry out critical metabolic processes. This powerful water-soluble vitamin can be found in some foods naturally as well as a dietary supplement. Because of its antioxidant properties, it has generated a lot of discussion.

Therefore, Chitosan nanoparticles and Ascorbic acid are known to have antioxidant effects, so they are considered important substances for humans and animals.

Aim of the work: This study aims to evaluate the protective effect of chitosan nanoparticles and ascorbic acid against thyroid toxicity induced by potassium dichromate in adult male albino rats.

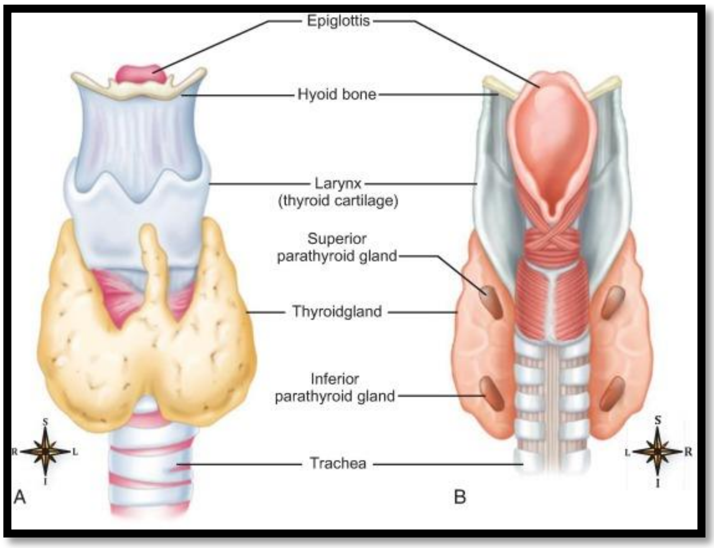
Keywords: Thyroid gland, toxicity, ascorbic acid, chitosan.

INTRODUCTION

Structure and function of the thyroid gland

A- Anatomical structure of the thyroid gland:

The anterior region of the neck contains the thyroid gland, a single bilobed endocrine gland that is connected to the upper trachea, the sides of the larynx, and the lower portion of the larynx (**Fig. 1**) (**Standring**, **2021**; **Nair**, **2024**).



(Fig. 1): The anatomical structure of the thyroid gland. (**Das** et al., **2022**)

The two large lateral lobes of the thyroid gland are joined by a narrow isthmus. This shape, resembling an ancient shield, gave the gland its name which is derived from the Greek word (thyros), meaning a shield. Each lobe, which is located on either side of the larynx and upper trachea, is roughly 5 cm long, 2.5 cm wide, and 20 to 30 gm in weight. The anterior surface of the second and third tracheal cartilage are crossed by the isthmus, a narrow band of glandular tissue (**Khan and Farhana**, **2019**).

From the left side of the isthmus, an extra pyramidal lobe frequently protrudes upward. Approximately 40% of the population has it (**Mohebati and Shaha**, **2012**; **Nair, 2024**). Normally, each lateral lobe's posterior aspect contains two parathyroid glands (**Gartner and Lee**, **2021**; **Nair, 2024**).

<u>In rats:</u> The thyroid gland is positioned differently. It is located in the upper neck region, and the esophagus is closely related to the two lateral lobes that extend posterolaterally from it. It typically extends along the first four or five tracheal rings (**Hussein** et al., **2023**).

B- Histological structure of the thyroid gland:

The thyroid gland consists of stroma and parenchyma. The deep cervical fascia that encircles the thyroid gland gives rise to a slender, dense, irregular collagenous connective tissue capsule that makes up the gland's stroma, thin irregular septa sent from the capsule into the glandular tissue subdividing the gland parenchyma into irregular lobes and lobules, and interfollicular connective tissue which contains a moderate amount of collagen fibers, a network of reticular fibers, and numerous microfibrils, and provides a conduit for blood vessels, lymphatic vessels, and nerve fibers (**Gartner and Lee**, **2021**).

The follicles are surrounded by a vast network of fenestrated capillaries that are derived from the superior and inferior thyroid arteries, which guarantees a steady and uninterrupted blood supply to the gland. Thus, they represent the main route for hormone release. Additionally, the interfollicular connective tissue contains blind-ended lymphatic capillaries, which could offer a second pathway for the hormones to leave the gland (**Gartner and Lee**, **2021**).

The structural and functional units of the thyroid gland are called thyroid follicles, and they make up the parenchyma of the gland. About three million follicles have been reported to be present in an adult thyroid gland.In contrast to the majority of endocrine glands, which keep their secretory materials in parenchymal cells, the thyroid gland stores its secretory materials extracellularly in follicular lumina (**Gartner and Lee**, **2021**).

Each follicle has a diameter of 0.2 to 1.0 mm and resembles a roughly spherical cyst. It consists of a central lumen enclosed by a wall. The follicular wall consists of follicular epithelium, whereas colloid, a substance that resembles gel, is found in the follicular lumen. A dense network of reticular fibers and a rich capillary plexus surround the follicles (**Gartner and Lee**, **2021**).

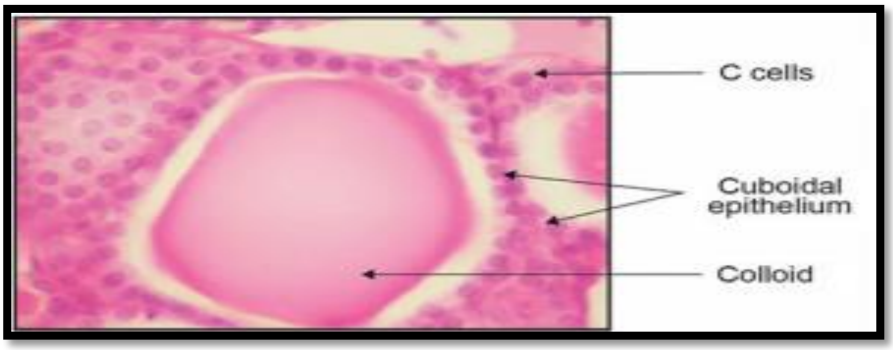
In rats: Thyroid follicles come in different sizes, with the larger follicles located on the gland's periphery. (**Mense** et al., **2018**; **Hussein** et al., **2023**).

Follicular epithelium

There are two different cell types found in the follicular epithelium: **follicular cells** and **parafollicular cells**.

A. Follicular cells (principal cells):

The major cells that make up the follicular wall are called follicular cells; thus, they also go under the name of principal cells. These cells are characterized as polarized, simple cubical to low columnar, with their basal surfaces resting on the basal laminae and their apical surfaces facing the colloid as shown in figure 2. On the other hand, their size and form differ depending on how well the gland functions. Tall columnar cells surround hyperfunctioning follicles, whereas flattened or even squamous cells surround hypofunctioning follicles (**Gartner and Lee**, **2021**).



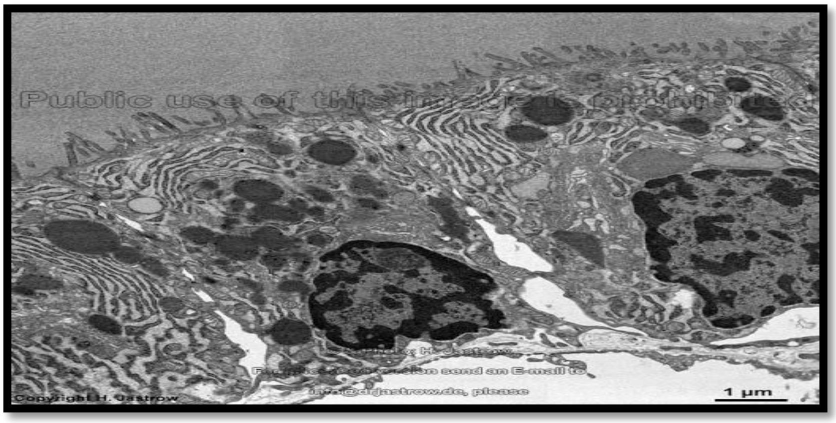
(Fig. 2): Cross-section showing the thyroid follicles with an epithelium comprised of cuboidal cells with a basal cytoplasm and circular nuclei. It can be seen that the follicular lumen contains a colloidal substance and C cells can be seen at a distance from the cavity H&E magnification X 100. (**Al-Suhaimi** et al., **2022**)

In standard hematoxylin and eosin (H&E) preparations:

Follicular cells contain round to ovoid nuclei with homogenous chromatin and one or more prominent nucleoli, and a basal cytoplasm that is slightly basophilic. Lipid droplets and PAS-positive droplets in the cytoplasm can be identified with the appropriate staining (**Pawlina and Ross**, **2018**).

At the electron microscope level:

The cytoplasm displays organelles that are frequently involved in secretory and absorptive processes, such as the supranuclear Golgi complex and the rod-shaped mitochondria that are found throughout the cytoplasm. The basal region contains roughsurfaced endoplasmic reticulum (rER), which has ribosome-free zones. There are tiny secretory vesicles in the apical cytoplasm that resemble vesicles connected to the Golgi apparatus morphologically. The apical cytoplasm also contains a large number of endocytotic vesicles, known as colloid resorption droplets, and lysosomes. The follicular cells have many short microvilli extending into the colloid on their free apical surface. Additionally, in hyperfunctioning follicles, pseudopods can be observed extending from the apical surface into the colloid (**Fig. 3**) (**Pawlina and Ross**, **2018**).



(Fig. 3): Electron micrograph of follicular cells, showing a single layer of epithelium containing low columnar follicular cells. The apical surfaces with microvilli are in contact with the colloid, whereas the basal surfaces rest on the basal lamina. Extracellular connective tissue space separates the follicular cells from the lumen of the capillary. The fenestrated endothelial cells lining capillary lumen rest on the basal lamina. Accumulation of lysosomes and colloid resorption droplets, Golgi apparatus, rough endoplasmic reticulum, and presence of enlarged intercellular spaces are indicative of intensive activity of follicular cells.**(Ross and Pawlina, 2018).**

B. Parafollicular cells (clear cells; C-cells):

Parafollicular cells are located at the edge of the follicular epithelium within the follicular basal lamina. They are present as solitary cells or small clusters of cells withing the follicular cells. Since they make up only 0.1% of the follicular epithelium, they are less common. These cells do not come into contact with the colloid or the follicle lumen (**Gartner and Lee**, **2021**).

In standard H&E preparations:

Large pale staining cells that are two to three times larger than follicular cells are known as parafollicular cells. However, light microscopy has difficulty identifying human parafollicular cells (**Pawlina and Ross**, **2018**).

At the electron microscope level:

A single, rounded, eccentric vesicular nucleus with a prominent nucleolus, a moderate rER, elongated mitochondria, a well-developed Golgi complex, and numerous, small, dense secretory granules with diameters ranging from 60 to 550 nm are visible in each parafollicular cell. These granules are located in the basal cytoplasm and have electron-dense cores that are separated from their limiting membranes by clear halos (**Pawlina and Ross**, **2018**; **Kierszenbaum and Tres**, **2019**).

<u>In rats:</u> Not only are parafollicular cells present singly or in groups in the interfollicular connective tissue, but they are also dispersed widely throughout the gland in between the follicular cells (**De Felice and Di Lauro**, **2015**; **Hussein** et al., **2023**).

Colloid

Colloid, a clear, uniform, gelatinous substance that is strongly PAS-positive and stained with both basic and acidic dyes, fills the follicular lumina. Thyroglobulin, a large iodinated glycoprotein weighing 660 kDa and containing approximately 120 tyrosine residues, is the main constituent of colloid. Other constituents include various enzymes and other glycoproteins. Rather than being a hormone, thyroglobulin is an inactive form of storage of the thyroid hormones. When the hormones are ready to be released, follicular cells endocytose the hormone-bound thyroglobulin, and lysosomal proteases separate the hormones from it. Only after a sequence of cellular processing steps is active thyroid hormone freed from thyroglobulin and released into fenestrated blood capillaries surrounding the follicles (**Pawlina and Ross**, **2018; Kierszenbaum and Tres**, **2019**).

C- Physiology of thyroid gland:

The thyroid gland secretes both **thyroid hormones** and **calcitonin.**

I. Thyroid hormones:

The two main hormones produced and secreted by the thyroid follicular cells are thyroxine (tetraiodothyronine, T4) and triiodothyronine (T3). These are referred to as the "thyroid hormones" (**Pirahanchi** et al., **2023**)

Effects of thyroid hormones:

Sexual maturation and growth and development are regulated by thyroid hormones. Additional effects include increased heart rate, heart contraction, protein synthesis, metabolism of carbohydrates and fats, and an overall rise in the body's functional activity (**Shahid** et al., **2018**; **Campbell and Jialal**, **2019**).

II. Calcitonin:

Calcitonin is the main hormone that thyroid parafollicular cells (C-cells) produce and secrete (**Shahid** et al., **2018**).

Effects of calcitonin:

In addition to acting in opposition to parathyroid hormone (PTH), calcitonin plays a significant role in controlling the levels of calcium and phosphorus in the blood. The main way that calcitonin lowers blood calcium concentration is by inhibiting osteoclasts' ability to reabsorb calcium, which reduces the amount of calcium that is released from bone into the blood. Calculitonin's second, longer-lasting effect is to inhibit the growth of new osteoclasts. Moreover, it accelerates the rate of osteoid calcification, which allows calcium to be deposited in the bones. The renal tubules' ability to absorb phosphate is also reduced by calcitonin (**Xie** et al., **2020**).

Calcitonin plays a more significant role in calcium homeostasis in young humans and a number of animal species, including rats. The primary function of calcitonin in adult humans is to shield the skeleton during times of calcitonin stress, such as growth, pregnancy, and lactation (**Campbell and Jialal**, **2019**).

Chromium (Hexavalent chromium)

In the crust of the earth, Cr is the seventeenth most abundant element. It can be found in soils, rocks, plants, animals, and volcanic dust and gases. When combined with other elements that exhibit a broad range of colors, Cr gives emerald green and ruby red hues. This vital trace element is crucial for the metabolism of fat, cholesterol, and glucose (**Anwer and Hassanin**, **2023**).

Chromium's physical and chemical characteristics:

Trivalent Cr (Cr III) and hexavalent Cr (Cr VI) are the most prevalent forms in the workplace. While Cr (VI) is water soluble and can pass through membranes, Cr (III) is not soluble in water and is not well transported across cellular membranes. Additionally, compared to Cr (III), Cr (VI) compounds are about 100 times more mutagenic and cytotoxic (**Fedala** et al., **2021**).

Principal uses for Cr (VI):

Cr (VI) is primarily used in industry. It considers usage in over 50 industries. The metallurgical, chemical, and refractory (heat-resistant) industries are the main users of its compounds. Stainless steel, cast iron, non-ferrous alloys, and chrome plating are among the products produced in these industries. Additional industrial uses for Cr (VI) include the production of safety matches, metal finishing, glassware cleaning solutions, tanning leather, welding, and corrosion inhibition in boilers and cooking systems (**Verger** et al., **2018**).

Exposure of humans to Cr (VI):

It can happen either directly or indirectly. Workers in greenhouses, agriculture, and industry are subjected to direct exposure. Inhalation is the primary method through which Cr (VI) enters the body. Direct skin contact with products containing Cr (VI) is an additional pathway (**Pavesi and Moreira**, **2020**; **Monga** et al., **2022**).

When Cr (VI) is consumed orally through food or drink, it results in indirect exposure. Food grains, vegetables, cereals, grass, and other plants cultivated in soil contaminated with Cr (VI) are major sources of this environmental contaminant. Stainless-steel container use may also lead to food contamination with Cr (VI). Additionally, drinking water and public water systems contain Cr (VI). In addition, it is released into hazardous wastes, car emissions, and cigarette smoke. Increased use of Cr (VI) and inappropriate disposal cause pollution of the environment and a number of health risks (**Hossini** et al., **2022**).

Cr (VI) Toxicodynamics:

The lungs and stomach of the body have defense mechanisms that convert Cr (VI) into less toxic Cr (III). Therefore, the body may be able to absorb less Cr (VI) at low exposure levels before it interacts with DNA. Nevertheless, toxicity happens at high concentrations (**Hossini** et al., **2022**; **Murthy** et al., **2023**).

As Cr (VI) is metabolically reduced to Cr (III) through a series of oxidation states, there may be an excess of reactive oxygen species (ROS) and free hydroxyl radicals produced. This can lead to oxidative stress, tissue damage, organ dysfunction, and a series of cellular events that include damage to cellular proteins, lipids, and DNA, damage to cell membranes, decreased cell viability, and necrotic and apoptotic cell death.Moreover, Cr (VI) results in DNA-protein cross-links and single-strand breaks in DNA. There are numerous mutagenic and genotoxic effects of Cr (VI) that are caused by these toxic compounds (**Qin and Wang, 2017**).

Toxic Effects of Cr (VI) on Thyroid gland :

The endocrine system, particularly the thyroid gland, can be severely disrupted by heavy metals such as Cr (VI) salts. Thyroid underactivity brought on by Cr (VI) exposure results in a thyroid hormone shortage below normal. Oxidative stress, apoptosis, and inflammation lead to changes in hormone levels and thyroid gland dysfunction. Thyroid gland normal architecture is disrupted by Cr (VI). Thyroid tissue damage and hypofunction have been observed in a number of recent rat studies after exposure to potassium dichromate (K2Cr2O7), which is a form of Cr (VI) (**Fedala** et al., **2021**; **Belal** et al., **2023**).

Chitosan Nanoparticles

Chitosan is a deacetylated type of chitin which is a polysaccharide found in exoskeleton of shellfish, for example, shrimp, lobster or crabs and cell mass of parasites and so on. Chitin is also found in insects, algae, bacteria and fungi (**Frigaard** et al., **2022**).

Sources of Chitosan nanoparticles:

The main biomass source used in the industrial synthesis of chitin and chitosan is leftover shell from shrimp and crabs. The recycling of seafood industry waste produces biomaterials that promote environmental sustainability. Protein, inorganic salts, chitin, and lipids make up a crustacean's shell. To begin with, the shells are ground into smaller pieces, and the minerals (mainly calcium carbonate) are extracted using diluted hydrochloric acid and then stirred at room temperature (**Bakshi** et al., **2020**).

Applications of Chitosan Nanoparticles:

Chitosan has many uses in food, cosmetics, fabrics, water treatment, and biomedical applications due to its qualities like mucoadhesion, biocompatibility, and biodegradability (**Frigaard** et al., **2022**). Chitosan nanoparticles are naturally occurring materials with exceptional biological, physicochemical, and antimicrobial qualities. Their bioactivity is safe for humans and they are an excellent environmentally friendly material (**Confederat** et al., **2021**). Due to these unique properties, chitosan nanoparticles find a wide array of applications.

Antioxidant activity

Chitosan is a proven antioxidant agent. By contributing a hydrogen atom or a single electron, it can scavenge free radicals and chelate metal ions (**Ivanova and Yaneva**, **2020**; **Muñoz-Tebar** et al., **2023**). In order to initiate various processes like adsorption, chelation, and ion exchange, the amino and hydroxyl functional groups of chitosan interact with metal ions (**Al-Salman** et al., **2023**; **Liu X.** et al., **2023**). It is impossible for chitosan to separate from the metal ions due to its semicrystalline structure and strong hydrogen bonding (**Thambiliyagodage** et al., **2023**). Chitosan/fucoidan nanoparticles proved their ability to scavenge reactive oxygen species (ROS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (**Liu M.** et al., **2022**; **Caro-León** et al., **2023**). Chitosan showed the capacity to chelate iron and scavenge hydroxyl radicals (**Liu T.** et al., **2022**; **Sreeja** et al., **2024**).

Ascorbic Acid

Ascorbic acid, also known as vitamin C, is a necessary micronutrient that helps humans carry out critical metabolic processes. This powerful water-soluble vitamin can be found in some foods naturally as well as as a dietary supplement. Because of its antioxidant properties, it has generated a lot of discussion (**Gęgotek and Skrzydlewska**, **2023**).

It is well known that ascorbic acid strengthens immunity. Moreover, it is believed that it plays a role in a number of cell processes, such as glycolysis, oxidative metabolism, cell signaling pathways, and epigenetic processes. It does this by stabilizing the sites for copper and iron hydroxylases, which draws attention to the need to maintain a sufficient concentration of ascorbic acid in bodily fluids for cell metabolism and gene expression control, in addition to binding to specific sites (**Yin** et al., **2022**).

Sources of Ascorbic acid:

While foods derived from plants and animals also contain ascorbic acid, fresh fruits and vegetables are the best sources. Broccoli, cabbage, potatoes, peas, red peppers, brussel sprouts, cauliflower, cantaloupe, strawberries, mangoes, tangerines, orange, grapefruit, lemons, and limes are among the foods that naturally contain ascorbic acid. However, losses during storage and cooking may be significant due to ascorbic acid's instability when exposed to an alkaline environment, oxygen, light, and heat. Fruit juices, fruit juice drinks, other similar beverages, milk, dairy products, and some breakfast cereals frequently contain ascorbic acid added as a fortifier (**Berretta** et al., **2020**; **Yin** et al., **2022**).

Anti-oxidant role of Ascorbic acid:

In addition to being a potent radical scavenger and significant physiological antioxidant, ascorbic acid can also help prevent or postpone the onset of some diseases where oxidative stress is at play. By efficiently metabolizing heavy metals like copper, cadmium, and Cr (VI), ascorbic acid works to protect body tissues from the harmful effects of oxidative stress and prevent damage to the liver, kidneys, and brain (**Bo** et al., **2024**).

The function and application of natural antioxidants in diabetes mellitus (DM) with elevated oxidative stress in averting oxidative damage has garnered significant attention. When rats with diabetes caused by streptozotocin (STZ) are given ascorbic acid orally, their blood sugar levels drop and their glucose tolerance test gets better (**Mousa**, **2021**).

It has been reported that intraperitoneal ascorbic acid administration in STZ-induced diabetic rats improves both enzymic and nonenzymic antioxidants and reduces the levels of thiobarbituric acid reactive substances (TBARS) (**Baloglu** et al., **2023**).

According to a different study, ascorbic acid lowers triglycerides and plasma cholesterol in rats with diabetes caused by alloxan (**Aldalou**, **2021**; **Rauf** et al., **2021**).

Thus, the aim of this study is to clarify how potassium dichromate (K2Cr2O7), a form of hexavalent chromium (Cr [VI]), affects thyroid gland structure and whether chitosan nanoparticles or ascorbic acid can prevent the thyroid toxicity caused by potassium dichromate in adult male albino rats through histological and immunohistochemical analyses.

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