EFFECT OF SODIUM FLUORIDE ON THE LIVER OF ADULT ALBINO RATS AND THE POSSIBLE PROTECTIVE ROLE OF VITAMIN C

Light and Electron Microscopic Study

Thesis

Submitted in partial fulfillment of M.Sc Degree in human anatomy

By

Amany Elsayed Mohammed Hamoud

(M.B.B.ch.).Cairo University

Supervised by

Prof. Dr. Fayza Abdel Raouf Abdel Gawad

Professor of anatomy, Faculty of Medicine

Cairo University

Prof. Dr. Maha Hussein Ashmawy

Professor of anatomy, Faculty of Medicine

Cairo University

Dr. Medhat Mohammed Morsy

Assistant Professor of anatomy, Faculty of Medicine

Cairo University

Anatomy department

Faculty of Medicine, Cairo University

2010

Acknowledgement

I would like to express my deepest gratitude to **Prof .Dr. Nabila Yousef Abdel-Halim,** Head of anatomy department, Faculty of Medicine, Cairo University for her continuous support, advice and encouragement.

I would like to thank **Prof.Dr. Fayza Abd Elraouf Abd Elgawad** professor of anatomy, Faculty of medicine, Cairo University for her kind support and for giving me a valuable part of her time and her patience.

I would like to thank **Prof .Dr. Maha Hussein Ashmawy** professor of anatomy, Faculty of medicine, Cairo University, for her encouragement, faithful discussion which has helped in achieving this thesis.

I am also like to thank **Dr. Medhat Mohammed Morsy** Assistant Professor of anatomy, Faculty of medicine Cairo University for his assistance during the progress of this work.

I would like to thank all members in the anatomy department who helped this work to be completed.

ABSTRACT

The present work demonstrated that the effect of sodium fluoride injection for three successive weeks produced variable histological changes in the liver of rats. These changes appeared as loss of hepatic architecture, pyknosis of the nuclei and extensive vacuolation of the hepatocytes. Widespread congestion and dilatation of the hepatic sinusoids and branches of the portal vein were noticed. Inflammatory cellular infiltration was also seen and was mainly in the periportal areas.

KAY WORDS

Effect

Liver

possible

Table of contents:

INTRODUCTION

Prevalent in many parts of the world, chronic fluorosis is caused by excessive ingestion of fluoride over a prolonged period and endangers the health of humans as well as animals. Sodium fluoride is used in various pesticide formulations, including insecticides and food preservatives, it is used also in oral caries prophylaxis in the form of fluoridated drinking water .As well, it is found that it can be deposited into soil from several sources, both directly through phosphate fertilizers and indirectly through atmospheric pollution from industrial activities **(Arnesen, 1998).**

As a very active site of metabolism, the liver was found to be especially susceptible to fluoride intoxication **(Akdogan et al., 2002).** Previously **(kour et al., 1981)** showed that fluoride might produce pathological changes in the liver including dilatation of the sinusoids, inflammation and degeneration of the hepatic cells but these findings contradicting the results of **Manocha et al., (1975) and Decamargo and merzel , (1980)** who denied the presence of any changes in the liver of fluoridated animals as compared to the control.

However , **Aydin et al ., (2003) and Cicek et al ., (2005**) mentioned that excessive fluoride ingestion over a prolonged period might adversely influence many organs as liver , lung , kidneys and ovary and was characterized by a vast array of symptoms and histopathological changes , in addition to the well known effect of fluoride on bone and teeth

Recently**, Nair et al., (2004)** found that the hepatotoxic effect induced by fluoride could be prevented by coadministration of vitamin C thus confirming the suggestion of **Sharma and Chinoy , (1998)** who reported that fluoride-induced toxicity in the liver of mice was reversible.

On reviewing such conflicting literature, it was decided to throw more light on the effect of sodium fluoride on the liver of adult albino rats. Moroever, an evaluation of the possible protective role of vitamin C (as an antioxidant) in inhibiting hepatotoxicity which might be induced by fluoride was done in this work.

The structure of the liver :

The liver is essential for maintenance of vertebrate animal life. It has two blood supplies, the hepatic artery and the portal vein. The hepatic artery provides nutrition, while the portal vein delivers substances absorbed by the gastrointestinal tract for removal and metabolic conversion **(Popp and Cattley, 1991).**

Histologically, the liver has a lobular architecture that is similar in all species. Despite the frequent over simplification of the liver as a cluster of hepatocytes, each of several cell types in the liver is important for normal function and participates in toxic responses **(Popp and Cattley, 1991).**

The parenchymal cell of the liver is the hetocyte which represents approximately 60% of the cells in the liver, and 80 % of the liver volume; they are polygonal cells with round nuclei with one or two prominent nucleoli. The nuclei vary somewhat in size, with 40-60% being polypoid with abundant cytoplasm **(Fawcett, 1994).**

The hepatocytes are radially disposed in the liver lobule. They form a layer of one or two cell thick, arranged like the bricks of a wall. These cellular plates are directed from the periphery of the lobule to its center and anastomose freely, forming a labyrinthine and sponge like structure. The plates are separated from vascular sinusoids so; the cell membrane of the hepatocytes has three domains: perisinusoidal, intercellular and pericanalicular **(Junqueira et al., 1992).**

Blood flows through the sinusoids from portal to central venous regions, resulting in metabolic zonation due to delivery of varying nutrients and oxygen tension, based on the location along sinusoids. Distribution of hepatocytes along sinusoids has thus been described in both anatomic and functional terms. Anatomically,

hepatocytes are distributed in three indistinctly separate areas of a lobule, periportal, mid zonal and central lobular. Functionally, hepatocytes are considered to reside in acini composed of three metabolic zones, the first zone 1 reflecting greatest proximity to vascular supply of substances and oxygen; this roughly approximates the periportal area. Zone 2 is an intermediate region that receives second-class blood as far as the central vein; its hepatocytes have to depend on blood that is relatively low in nutrients and oxygen **(Cormack, 1997).**

The difference in anatomic distribution and metabolic function result in subtle morphologic variations in hepatocytes. For example, central lobular hepatocytes tend to be larger than periportal hepatocytes and contain smoother endoplasmic reticulum and less cytoplasmic lipid when estimated by ultra structural morphology **(Popp and Cattley, 1991).**

The importance of liver in numerous homeostatic activities is attributed to the extreme diversity of hepatocyte functions. Many of these functions are related to the intermediary role of hepatocyte metabolism between dietary sources of energy and extrahepatic tissue demands for energy. Most of these hepatocytes functions represent variation in magnitude of activity relative to other cells in the body. However, some functions are qualitatively specific for hepatocytes including: amino acid deamination, resulting in the production of urea. This compound is transported in the blood to the kidney and excreted by that organ, also bile formation in the sense that the hepatocytes promote the uptake, transformation and excretion of blood components in to bile canaliculi **(Junqueira et al., 1992).**

The life span of the hepatocytes is estimated to be 200 days in rats, although highly differentiated hepatocytes undergo cell division to replace those which are lost either through normal attrition or due to injury. Under normal conditions, he-

patocytes division occurs mainly in the periportal region; the aging hepatocytes move from the periportal to the central lobular region, while hepatocytes may divide to produce additional hepatocytes, stem cells and oval cells also have been proposed as hepatocytes progenitor cells in rodents. Regenerations in other species are roughly in inverse relation to the size of animal. Although the human liver has considerable regenerative capacity, it falls far short of that seen in laboratory animals **(Fawcett, 1994).**

The non-parenchymal cells consist of the sinusoidal, perisinusoidal and biliary cells. The biliary epithelial cells form a system of ducts that conduct the bile out of the liver. Bile ducts, along with hepatic arterioles and portal venules are found in portal areas which are normally separated from the sinuoidal hepatocytes by a so-called "limiting plate" of hepatocytes. Bile ducts are lined by cuboidal or columnar epithelium and have a distinct connective tissue sheath. These epithelial cells have fewer mitochondria than hepatocytes; the ducts are connected with the canaliculi by short intermediate canals (canals of Hering) or ductules, these are composed of cuboidal cells with a clear cytoplasm and scant cytoplasmic organelles and small dark nuclei **(Junquerira, et al., 1992).**

The sinusoids are separated from the perisinusoidal space (space of Disse) by fenestrated flat endothelial cells which are devoid of basement membrane. The liver has a permanent population of sinusoid-associated macrophages known as kupffer cells. These stellate monocyte-derived macrophages engulf debris and remove any bacteria that may arrive in the portal blood **(Cormack, 1997),** these are sparse populations of two cell types in the perisinusoidal space; the first type is the fat storing cells (lipocytes or stellate cells) which contain multiple lipid droplets in their cytoplasm. They are more common near the center of the classical lobule than they are at its periphery, they store vitamin A. The second type is the pit cells,

which are found in small numbers in the liver of rodents, but they have not yet been reported in the human liver. They are small cells with short pseudopodia, but they are not phagocytes, their cytoplasm contains small dense granules and vesicles containing a rod like inclusions **(Fawcett, 1994).**

Sodium Fluoride :

Sodium fluoride is still used in oral caries prophylaxis in the form of fluoridated drinking water, fluoride tablets and fluoridated salts or milk and because of its good solubility in water, easy absorption from the alimentary tract as well as for an economic reason, sodium fluoride is the most commonly used compound in oral caries prophylaxis. During oral exposure, it can positively affect the oral environment. However, when consumed with food via the alimentary tract it can change, depending on the dose and exposure time, cell and tissue metabolism **(Dabrowska et al., 2006).**

Fluoride intake in low concentrations during tooth development results in the formation of dental enamel that is more resistant to caries. It has been established that a concentration of 0.7 mg /l fluoride reduces caries by 40-49% in primary teeth and 50-59% in permnant teeth, with no clinical appearance of adverse effects **(WHO, 1994).**

Endemic fluorosis is caused by excessive fluoride ingestion over a prolonged period. In addition to the well-known effects on the skeleton and teeth, fluorosis can adversely affect many tissues and organs as exhibited by a broad array of symptoms and pathological changes. Endemic fluorosis is thus a severe hazard to human health and often a serious health problem in a number of developing countries. However, the manner in which the whole body effects are produced is still

unclear, and efforts to prevent and treat fluorosis by therapeutic measures have had only limited success. Epidemiological investigations and animal experiments indicate that the histological structure and function of liver are altered in animals and humans with fluorosis, but the mechanisms are not fully understood (**Editorial review, 1978).**

In a differing view, consumption of drinking water containing elevated but non-toxic levels of fluoride has been proposed for the prevention of Alzheimer's disease as aluminium is one of the contributing factors in the occurrence of this disease. **(Kraus and Forbes, 1992).**

It is now well established that ingestion of fluoride not only affects the teeth and bones but also other organs. Structural and biochemical changes of several soft tissues have been reported in male and female rats and mice with different doses of fluoride**. (Chinoy, 1991).**

The most obvious early toxic effects of fluoride in humans are dental and skeletal fluorosis, which are endemic in areas with elevated exposure to fluoride. Fluoride is also known to cross the cell membranes and to enter soft tissues. Impairment of soft-tissue function has been demonstrated in fluoride-intoxicated animals **(Waldbott et al., 1978).**

Effect of Sodium Fluoride on The Liver:

Muehlberger ., (1930) observed that the liver of animals drinking excessive amount of sodium fluoride (0.05 to 0.2 gm /kg /day) in their water exhibited focal areas of hyperplasia of the hepatic cells with congestion of the central vein and dilatation of the sinusoids.

Mello et al ., (1963) also found degenerative changes in the liver of rats , even with only 1 ppm (part per million) fluoride in their drinking water .In contradiction **, Decamargo and Merzel (1980)** reported that intake of sodium fluoride in different concentrations 1,10,100 ppm (part per million) in the drinking water of rats for a period of 180 days , produced no changes neither in the body weight nor in the histological pattern of the liver .

Kour et al ., (1981) studied the effect of sodium fluoride administration at doses of 10, 500, and 1000 part per milion in their drinking water for 1-2 months in guinea pigs , the authors recorded focal areas of hepatic cell hyperplasia accompanied by areas of liver cell necrosis.

Humiczewska et al ., (1994) studied the histological and histochemical effect of fluoride on the lungs & liver of rats as well the possible beneficial effect of two pollen extracts Quercitin and Cernitin, known as detoxicating agents. The authors found that the liver of the rats exposed to ammonium fluoride for 3 months appeared brighter due to excessive accumulation of glycogen, the blood vessels were dilated and fibrosis was seen. They added that following 6 months of exposure to fluoride the laminar structure of lobules was obliterated, particularly at their peripheral parts and the liver cells seemed to be diffused with no distinct borders between them and the connective tissue strands were more extensive. The authors

found that the liver of rats receiving pollen extract and ammonium fluoride for three and six months showed no histological changes as compared to the control.

Bogdanff et al., (1995) found that rats and mice receiving up to 2500 ppm (part per million) vinyl fluoride in their drinking water suffered from sinusoidal dilatation , hepatocellular adenoma , hepatic hemangiosarcoma and carcinoma.

Kolodziejczyk et al., (2000) studied the oxidative activity of sodium fluoride given in a daily dose of 20 mg /kg bw for three months on the histological structure of the liver of male rats. The authors found degeneration and necrosis of the hepatocytes as well as disintegration and swelling of mitochondria .They also found decreased activity of the enzyme α-glycerophsphate dehydrogenase and succinate dehydrogenase enzyme needed in krebs cycle in the liver.

Shashi and Thapar (2000) studied the hepatic damage in young albino male and female rabbits induced by subcutaneous injection of sodium fluoride at doses of 5, 10, 20, and 50 mg/kg body weight/day for fifteen weeks . The authors observed that the histological examination of the liver revealed congestion and dilatation of the sinusoids with areas of hemorrhages, extensive vacuolization of the hepatocytes and hepatocellular necrosis.

Shivarajashankara et al., (2001) studied the effect of fluoride intoxication on lipid peroxidation and antioxidant systems in the blood, brain, and liver of rats. These rats were administared 100-part per milion fluoride (as sodium fluoride) in their drinking water for four months. In the red blood cells the levels of malondialdehyde (MDA) and glutathione (GSH) increased, along with the activity of glutathione peroxidase (GSH-Px). In the brain and liver, MDA and GSH levels increased, as did the activities of GSH-Px and glutathione S-transferase (GST). These results suggested that fluoride enhanced lipid peroxidation in the red blood

corpuscles, brain and liver of rats and caused increased or decreased enzyme activity associated with free radical metabolism.

Machalinska et al ., (2002) examined the early response of hematopoietic organs of adult mice to sodium fluoride given as three injections in their tail vein on alternate day with a total dose of 10 , 50 mg / kg bw for 3 weeks . They noticed morphological abnormalities in the spleen as increased lymphocyte nodules, decreased red pulp, increased white pulp and lymphocytes infiltration. On the other hand the authors found that there was no difference in the histological structure of the liver of both the control and the experimental groups.

Czerny et al ., (2003) found that sodium fluoride given to rats in a dose of 20 mg/kg bw , five days a week , for six months resulted in significant adverse changes in the hepatic enzymes as increased alanin and aspartate aminotransferase , reduced the liver content of cytochrome p-450 and the activity of hepatic NADPH-cytochrome C reductase .

Inkielewicz and Krechniak , (2003) studied the fluoride content in the serum , liver , kidney and testis of rats receiving sodium fluoride in their water at concentrations of 5 and 25 mg fluoride / liter for 12 weeks . The authors determined the fluoride content at the beginning of the experiment and after 12 weeks of exposure and they found that it was about seven-folds in the liver and kidney, twelve folds in the testis and two folds in the serum .The significant increase of fluoride in these organs as a result of higher exposure to fluoride might explain the negative impact of fluoride on cellular structure and activity of many enzymes in the investigated organs.

Working on rabbits **, Shashi (2003)** studied the toxic effects of sodium fluoride on the hepatic functions of these animals when given doses of 5 ,10 ,20 or

50 mg /kg bw /day for fifteen weeks .The parameters of hepatotoxicity were proteins , DNA , RNA , free amino acids and cholesterol . The authors found marked reduction in all these parameters in the liver, except hepatic free amino acids which were highly elevated and he thought that this was due to reduced incorporation of amino acids into proteins synthesis.

Xiao-ying et al., (2003) studied the role of oxidative stress of fluoride-induced hepatotoxicity in 4-week-old wistar rats . The authors found that rats given 50 ,100, and 150 mg sodium fluoride daily in their water for three months exhibited significant increase of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT) activities thus suggesting hepatic damage. They also believed that alterations of the oxidative and the antioxidant systems in the liver were due to the significant increase in malondialdehyde (MDA) together with enhanced superoxide dismutase (SOD) activity in that organ.

Dabrowska et al ., (2004) and Dabrowska et al ., (2006) studied the effect of sodium fluoride on the liver of rats given sodium fluoride in a dose of 10 mg /kg and 32 mg / kg daily for 90 days using the electron microscope . They found that pronounced changes in the mitochondrial size and shape occured in overall any organelles examined as the nucleus, endoplasmic reticulum and Golgi apparatus. In the group receiving 10 mg sodium fluoride slight changes occured in the mitochondria in the form of swelling and vacuolar changes, while in the group receiving 32 mg sodium fluoride the mitochondria became polymorphic with blurred internal structure .These changes in the mitochondria persisted when the sodium fluoride was withdrawn and the rats survived without any treatment.

Wang et al ., (2004) evaluated the toxicity of fluoride and the antagonistic effect of selenium on the normal human primary hepatocytes in vitro , as the hepatocytes were incubated with sodium fluoride (80 μg/mL) and/or sodium selenite