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RESEARCH ARTICLE

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Histopathological characteristics of submandibular salivary glands in experimental animal albino rat induced hypervitaminosis A

ABSTRACT:

The present work was designed to investigate the histopathological alterations in the submandibular salivary glands of the albino rat after induction high dose of vitamin A (hypervitaminosis A). In this study, a total of 40male albino rats (120 - 150 grams body weight) were utilized. They were randomly divided into three groups. Group I, 10 animals were reserved as control group and was given sesame oil additionally to their food. While, Group II and III (15 animals each) were administered vitamin A interperitoneally (500 IU/kg body weight/day/rat dissolve in sesame oil for 2and 3 weeks). Salivary glands were excised, weighed and fixed in 10% phosphate buffered formalin (pH7.4) and stained with H & E stain. Histological analysis of salivary gland was done using light microscopy. Group I showed normal acini, striated ducts and granular ducts. Whereas, group II and III showed atrophic changes in serous acini, loss of acinar architecture as well as congestion of inter-acinar blood capillaries with more predominant alterations in group III as a result of increasing induction period. The excessive intake of vitamin A more than recommended dosages as supplements or medications cause toxic effect and salivary gland alterations as a reflection of stress and injury.

KEY WORDS:

Hypervitaminosis A, Albino Rats, Submandibular Salivary glands, Histopathology.

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INTRODUCTION:

Vitamin A (retinoids) are fat-soluble vitamins, essential nutrient for human and are involved in a wide variety of biochemical functions including, vision, reproduction, skeletal development, neurodevelopment, growth maintenance of epithelial tissues (Bendich and Langseth, 1989; Weiss *et al.,* 2010), as well as control of cell proliferation& differentiation process, and embryonic development (Novák *et al.*, 2008). They also act as a protective agent against infections, heart disease and some types of cancer (Love and Gudas, 1994).

They are absorbed in the intestine and stored mainly in the liver, kidney, and adipose tissues. Hypervitaminosis A (high doses of vitamin A) is occurred as a result of long term of medication, with the ease of its accumulation in the body and the difficulty of excreting excess amounts (Rutkowski and Grzegorczyk, 2012).

There are more symptoms of hypervitaminosis A such as nausea, jaundice, irritability, anorexia, vomiting, blurry vision, headaches, hair loss, muscle, and abdominal pain, weakness, and an altered mental state (Ramanathan *et al*., 2009), and increased susceptibility to infections, which is due to a

weakened immune system (Chandra, 1993), as well as fibrosis and hepatocellular dysfunction (Choudhary and Swami, 2012). In pregnant female rats, mice, and humans, elevated intake of vitamin A can induce a wide spectrum of embryonic defects (Freytag *et al.*, 2003).

Meanwhile, Salivary gland has an important role in the preparation of food for chewing and swallowing owing to its lubrication feature by secretion saliva (Tucker, 2007).

Furthermore, this secretion also helps the remineralization of teeth with supplying inorganic ions (calcium, phosphate), neutralization of oral acid and simplification of speech (Wolff *et al*., 2008; Aliko *et al*., 2015).

MATERIAL AND METHODS:

Animals:

Forty male-albino rats with a weigh of 120 - 150 were obtained from the Animal House of the Faculty of Veterinary Medicine in Moshtohor city, Benha University, Benha, Egypt. Animals were housed in a 12 hrs light / 12 hrs night cycle and had free access to food and water *ad libitum*. A balanced standard diet composed of dried skim milk (5%), fish meal (10%), soybean meal (12%), alfalfa meal (4%), corn gluten meal (3%), ground corn (24.5%), ground hard winter wheat (23%), wheat middling (10%), Brewer's yeast (2%), molasses (1.5%), soybean oil (2.5%), plus minerals and vitamins prepared at Ministry of Agriculture, Egypt.

Forty rats were divided into three groups:

Group I: served as control group (10 animals) and was given sesame oil additionally to their food.

Group II: Animals of this group (15 rats) were injected with vitamin A at a dose level of 500 I.U. interperitoneally daily for two weeks.

Group III: Animals of this group (15 rats) were injected with vitamin A at a dose level of 500 I.U. interperitoneally daily for three weeks.

Histological Investigation:

Animals of different groups were dissected out after two and three weeks. The submandibular salivary glands were carefully separated and washed in normal saline. Specimens were fixed in 10% phosphate buffered formalin (pH7.4). Fixed materials were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in paraffin wax. Sections of 5 micrometres thickness were cut using rotary microtome and mounted on clean slides. After that, slides were stained with haematoxylin and eosin (H & E) for histological examination (Bernet *et al.,* 1999).

RESULTS:

The submandibular salivary glands are muco-serous, tubulo-acinar glands, with secretory acini and the initial part of the duct system also participates in the secretory process. Secretions from glands close to the oral cavity are mainly mucous. Structurally, each salivary gland is divided by connective tissue septa into lobes, which are in turn subdivided into lobules. Examination of control group sections showed normal architecture as the parenchyma was formed of secretory end-pieces, excretory ducts and granular convoluted tubules. The secretory end-pieces or acini were found to be consisted of pyramidal shaped serous cells with round deeply basophilic nuclei in the basal half of the acinar cells. Few mucous acini and serous Demilune were present. The striated duct was formed of low columnar cells with centrally placed nuclei (Fig. 1 a& b).

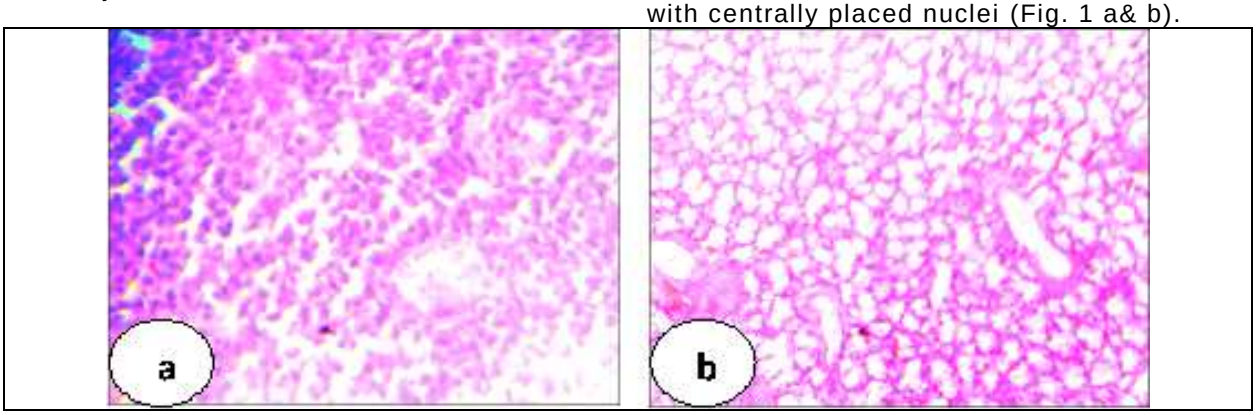

Fig. 1. Photomicrograph of submandibular salivary glands of control albino rats showing normal acini, striated duct and granular duct (H&E stain). a (x 100) b (x 400)

The histological alterations in salivary glands of rats induced high dose of vitamin A (500 IU) daily for 2 weeks showed congestion of inter-acinar blood capillaries, loss of acinar architecture (amalgamation), hyperchromatic and polymorphic nuclei. Also, presence of numerous cytoplasmic vacuoles, few atrophied acini and nuclei of variable size and density (Fig. 2. A-f).

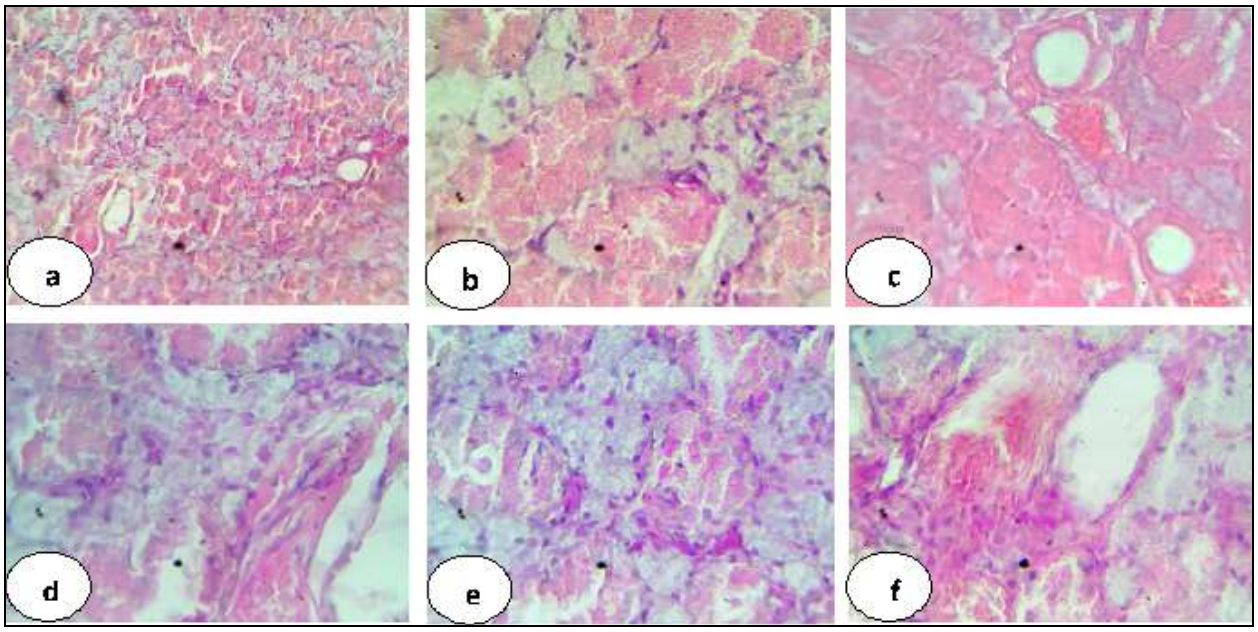

Fig. 2. Effect of high dose of vitamin A 500 IU daily for2 weeks showing: congestion of inter-acinar blood capillaries, loss of acinar architecture (amalgamation), hyperchromatic and polymorphic nuclei and showing areas of hemorrhage surround the striated ducts. (H&E stain). a (X 100); b-f (X 400)

Inducing animals with high dose of vitamin A (500 IU) daily for 3 weeks showing: degenerative and oedematous changes and vacuolization in the acini, degenerative changes in striated duct and granular tubule, dilatation and congestion of inter-acinar blood capillaries with loss of normal architecture (Fig. 3. G-I). the histopathological alterations increase with the increase induction time.

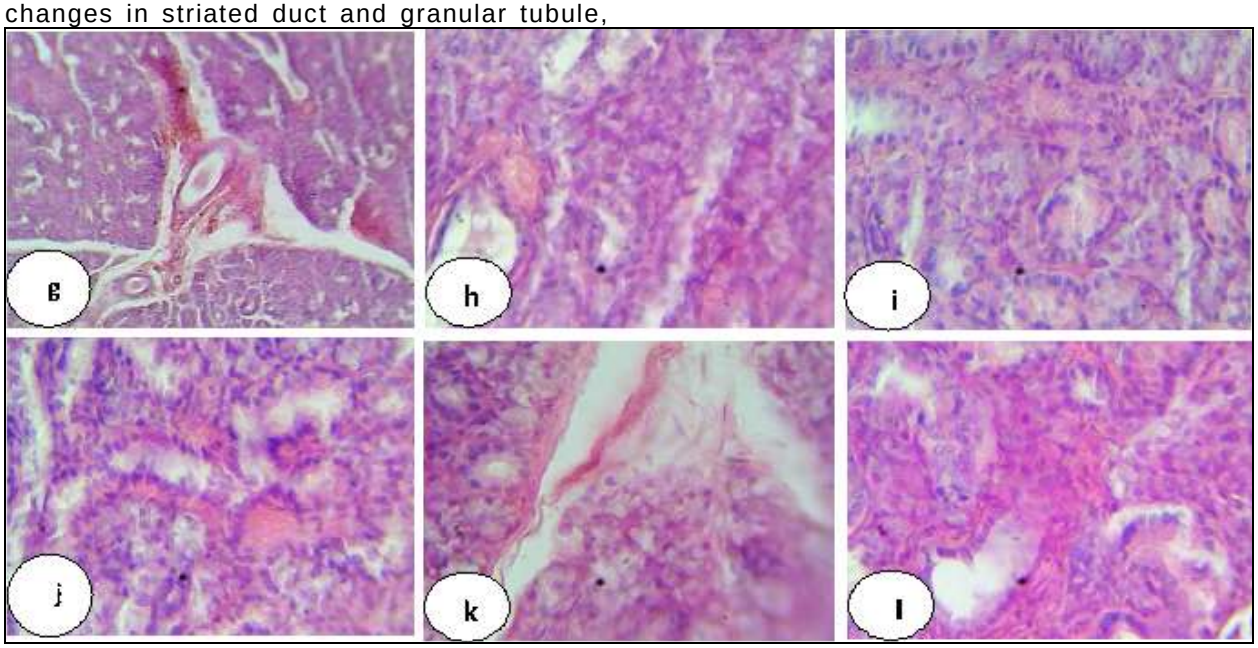

Fig. 3. Effect of hypervitaminosis A 500 IU daily for 3 weeks showing: degenerative changes and vacuolization in the acini, degenerative changes in striated duct and granular tubule, dilatation and congestion of inter-acinar blood capillaries. (H&E stain). g (X 100), h-l (X 400)

DISCUSSION:

The salivary glands are exocrine glands that secrete saliva through a system of ducts. There are three paired major salivary glands; parotid, submandibular, and sublingual as well as hundreds of minor salivary glands (Edgar *et al.,* 2012). These types have a similar structural framework of acinar and ductal cells organized as bunch of grapes on a stem (Greenburg *et al.,* 2008). The principle function of acinar cells is the secretion of initial isotonic salivary fluid which is later modified by exchange of ions through the ductal cells and finally secreted into the oral cavity (Greenburg *et al.,* 2008). Also, the importance of saliva is not limited to the mouth only, but it supports the oesophageal clearance and buffer of gastric acid (Shafik *et al*., 2005). On the hand, xerostomia (the symptom of dry mouth) or salivary gland hypofunction (reduced production of saliva) are the result of salivary gland dysfunction which associated with significant impairment

of quality of life *(*Riley *et al*., 2017).

In the present investigation, the acinar cells and ductal epithelial cells of salivary gland of rats with high dose of vitamin A revealed degenerative changes and vacuolization of the acini. In addition, the connective tissue septa showed increased in thickness with enlargement and congestion in blood vessels that agreed with El-Sakhawy *et al.* (2017). Also, Similar-appearing cells have been reported by Recezi and Rowe (1972) in the submandibular gland of rats induced high dose of vitamin A.

The histopathological alterations in the acini may cause loss of salivary gland function as recorded by Polihronis *et al*. (1998) who concluded that salivary gland apoptosis may be one of the underlying pathogenic causes impairing gland function. Hammoud *et al*. (2014) recorded elevation in the level of calcium, sodium and protein in saliva of albino rats induced high dose of vitamin A. Vitamin A excess induces mucous metaplasia and mucous gland formation in keratinized adult epithelium (Reynold, 1963; Fox, 1989; Feng *et al*., 2005).

Vitamin A excess has been demonstrated to exert an inhibitory or protective effect (Hitchcock, 1954; Chu and Malmgren, 1965; Davis, 1967; Saffiotti *et al*., 1967).

The loss of normal architecture of acini may attributed to the high dietary supplementation of vitamin A which accumulate and alter the metabolic process in the cell. This agreed with Hough *et al.* (1988) who showed that the histological picture was derangement in the serous cells of salivary glands that may be found as the adverse effect of hypervitaminosis A by imbalance in the enzymatic content of saliva.

CONCLUSION:

The excessive intake of vitamin A more than recommended dosages as supplements or medications cause toxic effect and salivary gland alterations as a reflection of stress and injury.

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التغيرات النسيجية في الغدد اللعابية تحت الفكية في الفئران التجارب الناجم عن زيادة فيتامين أ

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الهدف من هذا العمل التحقيق في التغيرات المرضية في الغدد اللعابية تحت الفكية في الفئران البيضاء بعد التعريض لجرعة عالية من فيتامين (أ). تم في هذه الدراسة استخدام مجموعه من أربعين ذكر الفئران البيضاء (وزن الجسم 120-150 غرام). تم تقسيمهم بشكل عشوائي إلى ثلاث مجموعات؛ المجموعة الأولى، تم حجز 10 حيوانات كمجموعة ضابطة وأعطيت زيت السمسم بالإضافة إلى طعامهم. بينما تم إعطاء المجموعة الثانية والثالثة (15 حيوانًا لكل منهما) فيتامين (أ) بالحقن بالغشاء البريتوني (500 وحدة دولية / كجم من وزن الجسم / يوم / فأر مذاب في زيت السمسم لمدة اسبوعان وثلاث أسابيع). تم استئصال الغدد اللعابية وتم ثبيتها في 10٪ فورمالين المتعادل (pH7.4) وعمل قطاعات وتم صبغ القطاعات بصبغة H&E والفحص النسيجي للغدة اللعابية باستخدام المجهر الضوئي. أظهرت المجموعة الأولى الضابطة فصيص وعنيب طبيعي وقنوات مخططة وقنوات حبيبية. في حين أظهرت المجموعة الثانية والثالثة تغيرات ضارة في مصل العنيب وتركيب غير منتظم، فصيصات وعنيبات مفصولة وفقدان في الشكل الطبيعي للعينات مع احتقان الشعيرات الدموية بين العنيبية مع تغييرات أكثر في المجموعة الثالثة نتيجة لزيادة فترة الحث ووجود مناطق ضمور في الخلايا اللعابية. وقد لوحظ وجود فجوات في الخلايا اللعابية. وتمدد واتساع في الاوعية الدموية. وتخلص الدراسة إلى أن الإفراط في تناول فيتامين (أ) أكثر من الجرعات الموصي بها كمكملات أو أدوية تسبب تأثيرًا ضارا وسامًا على التركيب النسيجي للغدة اللعابية تحت الفكية للفئران البيضاء كانعكاس للتوتر والإصابة.