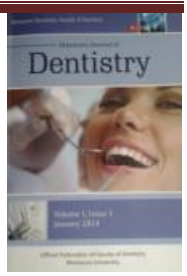
Biological effects on the tongue intrinsic musculature after pentylenetetrazole (PTZ) injection in Albino Rats



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Introduction

The tongue is a muscular organ in the mouth which covered with mucosa. [1] Tiny papillae gives the tongue its rough texture. Thousands of taste buds cover the surfaces of the papillae. Taste buds are collections of nerve-like cells that connect to nerves running into the brain. Tongue is the most accessible organ of the oral cavity. It participates in a wide variety of oromotor behaviors which include mastication, swallowing, and respiration. It lacks any internal bony skeleton and composed almost entirely of muscle. [2]

Tongue musculature comprises intrinsic muscles (originate and terminate within the tongue) and extrinsic muscles (an external bony origin and insertion into the tongue base). Extrinsic tongue muscles alter the position of the tongue, whereas the intrinsic tongue muscles alter tongue shape. [3] The easy clinical accessibility of tongue makes it a good indicator in oral and general clinical examinations. Due to its strategic location, various oral as well as systemic diseases often affect tongue and consequently can change its structure and functions so that it is a diagnostic indicator of various systemic diseases and truly mirrors the body. [4]

The involvement of tongue in various disorders and diseases poses a diagnostic and therapeutic challenge to the dentist for example sudden increase in the size of tongue may indicate neoplasm or endocrine disorder or metabolic disease, abnormal tongue movements are suggestive of motor neuron disease, depapillation with redness is a common feature of nutritional deficiencies, erythematous tongue may be a first indicator of candidiasis. [5, 6]

Neuromuscular disorders affect the peripheral nervous system and muscle. The principle effect of neuromuscular disorders is therefore on the ability to perform voluntary movements. They cause significant incapacity, including, at the most extreme, almost complete paralysis. They have onset any time from in utero until old age. [7]

As the tongue is a muscular organ in the craniofacial region and plays fundamental roles in almost all oral motor functions. A number of neuromuscular diseases, such as epilepsy, multiple sclerosis, cerebral palsy, muscular dystrophy, Parkinson's, significantly affect tongue motor functions. These negative effects include reduced or complete loss of control in moving the tongue (tongue displacement) and/or changing the shape of the tongue (tongue deformation), tongue spasm or convulsion, muscle dystonia, and ankyloglossia. Several sensational disorders may also occur due to these neuromuscular diseases, including burning tongue, loss of taste function (ageusia), decreased ability to taste (hypogeusia), and changes in taste (dysgeusia). [8]

Pentylenetetrazole (PTZ) is a drug formerly used as a circulatory and respiratory stimulant. [9] It is also known to be a convulsant drug at higher doses. [9] It has been used in convulsive therapy and was found to be effective primarily for depression but side-effects such as uncontrolled seizures were difficult to avoid. Its administration is preferred approach used for studying brain excitability. Chemically induced seizures with PTZ are myoclonic, generalized tonic-clonic (primary generalized) seizure models. [10]

Kindling is a phenomenon resulted with progressive intensity of convulsion activity due to repetitive administration of electrical or chemical sub-convulsive stimulators so that PTZ can be used for initiating acute as well as chronic (sub-convulsive doses) animal models of convulsions. [11]
According to the above mentioned review, PTZ may have some biological effects on the tongue musculature after the drug injection and through this approach, we will investigate these changes.

Aim of the study

This study will be done to evaluate the biological effects on the tongue intrinsic musculature after pentylenetetrazole (PTZ) injection in Albino Rats.

Materials and methods

Twenty four pathogen-free male albino Spraug Dawly rats, weighing 180-200 g, were selected. All experimental procedures were performed under the protocol approved by the Ethical Committee of Faculty of Dentistry, Mansoura University, and the animal house unit of Nile Center for Experimental research, Mansoura city, Egypt. The rats received water ad libitum and a standard pelleted diet and were kept in a 12 hr. light/dark cycle.
Rats will be randomly divided into 2 equal groups:

Group I (control group)

12 healthy rats received 0.2 mL saline via intraperitoneal injection (i.p.) once/ 48 hours, (12 dose).

Group II

12 rats received a single dose of 35 mg/kg PTZ (sub-convulsive dose) dissolved in 0.2 ml of normal saline i.p. every 48 hrs, for 24 days i.e. 12 doses were given to every rat. The convulsions observed for 30 min after each PTZ injection.

Rats from both groups were sacrificed 2 weeks later from the last injection. Scarification occurred by overdose of intraperitoneal sodium thiopental injection (120 mg/kg).
Tongue will be dissected and fixed in neutral buffered 10% formalin for histological and histochemical analysis. Paraffin sections (5 μm thick) will be prepared and stained with:
1. Hematoxylin &Eosin stain (H&E).
2. Mallory trichrome stain (MTC).

Results

1. Hematoxylin and Eosin stain results:

- Group I (control group):

Histological examination of H&E stained sections of the control group revealed that the dorsal surface of the tongue had many lingual papillae. The tongue was covered by keratinized stratified squamous epithelium resting on a basement membrane and a lamina propria formed of connective tissue containing blood capillaries. Under the epithelium, a muscular core formed of a mass of skeletal muscles was observed. The muscle fibers of the muscular core were longitudinal, transverse or oblique. The fibers showed acidophilic sarcoplasm and had multiple oval peripheral flattened nuclei. The muscle bundles were separated from each other by ramifying connective tissue endomysium which was continuous with the lamina propria of the mucosa. (F.g.1)

- Group II:

Histological examination of H&E stained sections revealed that the skeletal muscle fibers were widely separated with connective tissue. Some angulated and degenerated skeletal muscle fibers were observed. Some fibers with central or pyknotic nuclei (small, darkly stained) were also seen. Connective tissue showed dilated congested blood vessels and was infiltrated with mononuclear cellular infiltration. (F.g.2)

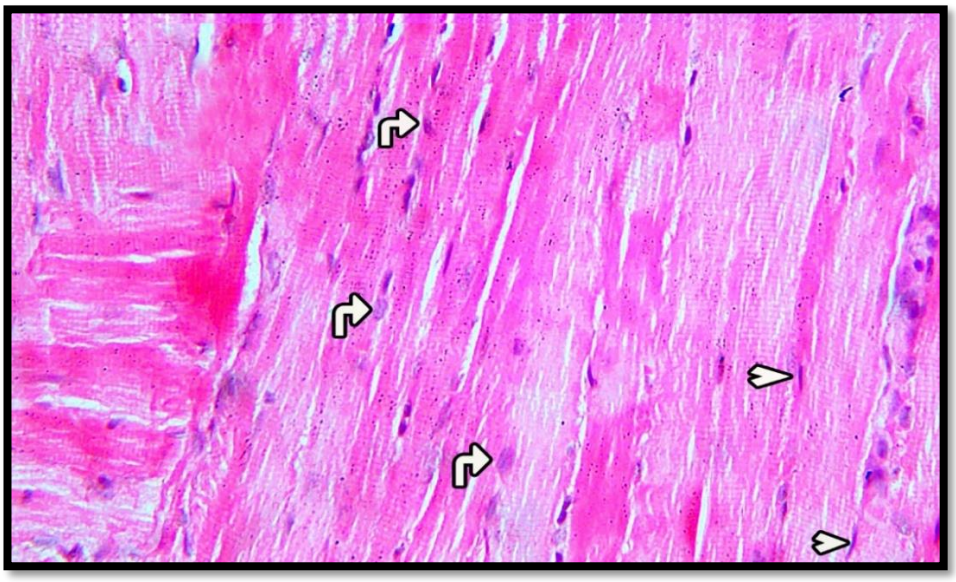

Fig.1. A photomicrograph of the tongue in control group reveling longitudinal bundles of the skeletal muscle fibers with acidophilic sarcoplasm and multiple elongated vesicular nuclei (right angle arrow) which are peripherally located beneath the sarcolemma. Notice the flat nuclei of fibroblasts (arrow head) in the endomysium between the muscle fibers. (H&E stain X400)

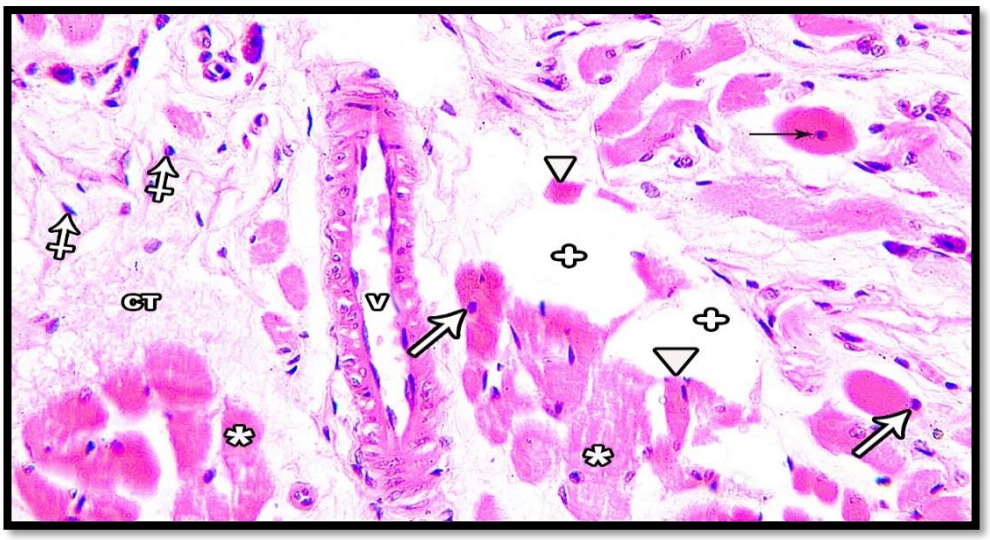

Fig.2. A photomicrograph of the tongue in group II showing some angulated (arrow head) and degenerated (asterisks) muscle fibers are observed. Dilated congested blood vessel are seen (V). Mononuclear cell infiltration is also noticed (crossed arrow) in connective tissue (CT). Some fibers pyknotic nuclei (white arrow) or central nuclei (black arrow) are also observed. (H&E stain X400)

2. Mallory trichrome stain results:

- Group I: Mallory trichrome stained sections of the tongue revealed minimal connective tissue under epithelium and in between skeletal muscle fibers. *(F.g. 3)*

- Group II: histological examination of tongue revealed moderate deposition of connective tissue under epithelium and in between skeletal muscle fibers. *(F.g. 4)*

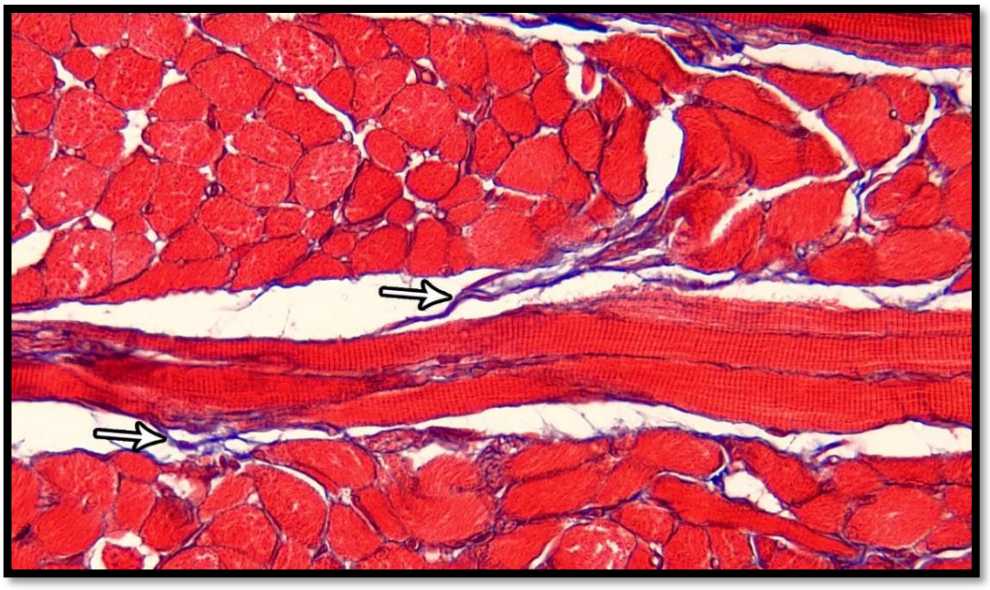

Fig.3. A photomicrograph of group I showing normal amount of connective tissue (white arrow) in lamina propria under the epithelium and in between skeletal muscle fibers. (M.T X400).

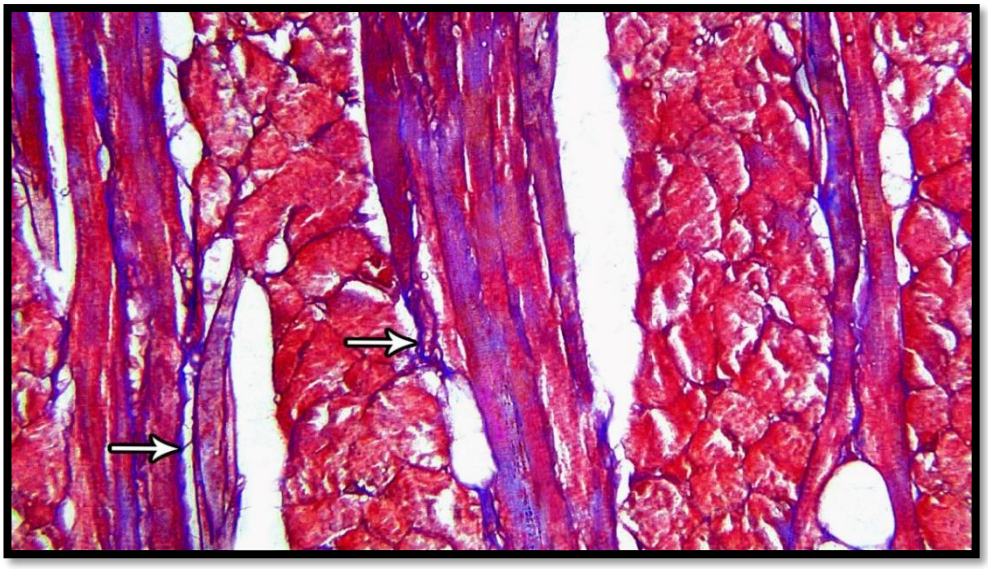

Fig.4. A photomicrograph of the tongue in group II showing moderate deposition of connective tissue (white arrow) under epithelium and in between skeletal muscle fibers. (M.T X400)

Table (1): Comparison of groups after two weeks

		ontrol group	I group	
% area of collagenous fibers	Mean	.7440	.292	0.001*
	SD	.2480	.097	
	ost-hoc		1=0.07	

Data expressed as mean±SD

SD: standard deviation P: Probability *: significance <0.05

Test used: One way ANOVA followed by post-hoc tukey

P1: significance between Control II groups

Discussion

As the tongue is the only muscular organ in the craniofacial region, a number of neuromuscular diseases significantly affect tongue motor functions and several sensational disorders may also occur. [14,15] In addition to the complex network of interwoven fibers and fiber bundles from four intrinsic and four extrinsic tongue muscles, the tongue also has a large network of subdividing nerve branches and blood supply. Studies have shown that the hypoglossal nerve alone has more than 50 primary branches innervating tongue musculature. [16]

Pentylenetetrazole (PTZ) is a central nervous system convulsant. It is a chemical toxin reported to interact with GABA-A receptor complex in the adult brain. It can reach the brain through successful transportation via blood-brain barrier. [17] It is a competitive inhibitor of GABA, and prevents binding of GABA on the $GABA_A$ receptors present on the surface of muscle. In the absence of GABA binding, the excitatory to inhibitory signal ratio increases resulting in a convulsive phenotype. [18]

Minimal data were reported in literature about the effect of PTZ injection on the skeletal muscle fibers. Since, to our knowledge, this is the first study which investigated the effect of PTZ injection on skeletal muscle fibers. However, the degenerative changes observed in the muscle fibers in this work are consistent with that reported by Ahmed S, et al. (2017) in atorvastatin treated rats. [19]

Cullen MJ. Et al. (2002) reported that skeletal muscle fiber splitting is an adaptive response, which occurs when the fiber reaches a critical size at which oxygen supply and metabolite exchange are no longer efficient. Moreover, it was suggested that nuclear migration plays an important role in the pathogenesis of muscle fiber splitting, and accordingly it can be concluded that such changes might occur in muscle fibers that have compensatory hypertrophy or undergone over work. [20]

M Lańcut. Et al. (2004) studied the histological and ultrastructural changes in cross-striation muscle cells of Wistar rats-males, under the influence of atorvastatin-reductase HMG-CoA inhibitor. They confirmed that the progressive increase in the amount of collagen fibers was observed and was concomitant with the degree of muscle injury. It was stated that the increase in intrafasicular CT

usually represents a response to myofibers loss, where in fibroblasts replace the damaged area, with subsequent formation of collagen fibers. [21]

Another explanation of these results was reported by CJ Mann et al. (2011). They related these results to pathophysiologic fibrosis. It is an excessive accumulation of extracellular matrix (ECM) components, particularly collagen, in the end result of a cascade of events proceeding from tissue injury via inflammation. These fibrotic reactions share common cellular and molecular mechanisms, such as cell and tissue degeneration, leukocyte infiltration, persistent inflammation of the tissue, and proliferation of cells with a fibroblast-like phenotype. The interplay and imbalance of different cell types sustains the production of numerous growth factors, proteolytic enzymes, angiogenic factors and fibrogenic cytokines, which together perturb the microenvironment of the damaged tissue, and stimulate the deposition of connective- tissue elements that progressively remodel, destroy and replace the normal tissue architecture. [22]

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