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Quantitative and histological evaluations for the potentiality of pilocarpine on tramadol induced xerostomia and structural alterations in Rats parotid gland

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Abstract---Tramadol is a centrally acting analgesic with weak opioid agonist properties, which also has monoaminergic activity, exerted via inhibition of neuronal uptake of serotonin and norepinephrine. Tramadol is generally well tolerated and the most common adverse events are nausea, dizziness, drowsiness, sweating, vomiting and dry mouth. The effect of intraperitoneal injection of tramadol and pilocarpine were studied in rats on the flow of saliva, histological and histochemical change in the parotid salivary gland. The rats were divided into three groups, first group, rats received saline and used as control. The second group treated with 10 mg tramadol hydrochloride for three days. The third group treated with tramadol hydrochloride for three days followed with 1 mg pilocarpine. The rats were sacrificed at the fourth day after the end of experimental procedure. The administration of tramadol caused decreased in salivary flow rate by approximately 60 %. The parotid histopathological changes include vacuolation and atrophy of acini and excessive widening of the interstitial tissue area which are replaced by extensive fibrosis fibrosis mostly associated with the degenerated ducts and acini and their replaced by inflammatory cells. The

pilocarpine can relatively counteract the tramadol-induced decrease in salivary flow rate and subsequently improves the salivary flow of saliva.

Keywords---Tramadol, Pilocarpine, Salivary gland.

Introduction

The tramadol is a synthetic opioid analgesic that has two chiral centers marketed as a racemic (1:1) mixture of the (+) and (-) enantiomer. It has a multimodal mechanism of action as on one hand, the (+) and (-)-enantiomer which act on the serotonin and noradrenaline reuptake, and on the other hand, the O-desmethyl metabolite of tramadol (called M1 or ODT) acts on the μ -opioid receptor. ⁽¹⁾

Tramadol, a centrally acting analgesic, is used to treat moderate to moderately severe pain. ⁽²⁾ The typical symptoms of opiate agonist overdose, including miosis, vomiting, respiratory depression or arrest, coma, and cardiovascular collapse, are also the expected symptoms of tramadol over dose. ⁽³⁾ Although the naloxone can only partially inhibit the tramadol-mediated analgesia, however, it reverses the respiratory depression. ⁽⁴⁾ Secretion of saliva is almost entirely dependent on the nerve-mediated mechanisms and the parasympathetic impulses activating glandular muscarinic receptors which stimulate the principal stimulus for the fluid secretion in salivary glands. ⁽⁵⁾ The xerogenic drugs may exert conspicuous inhibitory potency by interfering with the neuronal transmission both in the central and the peripheral nervous system. This may occur by affecting the inhibitory pathways in the central nervous system or by blockade of muscarinic or adrenergic receptors in the glands. ⁽⁶⁾ Since the muscarinic receptors may be targets of analgesic drugs and tramadol has been reported to inhibit muscarinic M3 receptor function, ⁽⁷⁾ an antagonism on the glandular muscarinic receptors of tramadol may be attributable for this side effect. The parotid gland in the rat is especially well suited for studies of such an antagonism because muscarinic M3 receptors can mediate the major part, if not the whole of the cholinergic response in this particular gland. ⁽⁸⁾

Pilocarpine, is a parasympathomimetic agent that can stimulate sweat, lacrimal and salivary glands. ⁽⁹⁾ It can be currently used to treat xerostomia caused by Sjogren's syndrome or dryness emerged as a consequence of radiotherapy ^(4,5) or it can also counteract the oral dryness and the xerostomia induced by drug treatment. ⁽⁶⁾

Materials and methods

Thirty adult male Sprague Dawley rats weighing about 200—250 g were kept at animal house of Cairo University and were subjected to the ethical committee of the Faculty of Dental medicine. The rats were randomly divided into one control (10 rats) and two experimental groups (10 rats each). The control group (G I); included 10 rats; received normal saline by intraperitoneal injection without any drug for three days. The rats were sacrificed at the fourth day after the end of experimental procedure, that is, three days. The tramadol group (G II); included

10 rats received 10 mg tramadol hydrochloride (**Tremal, Al Amriya Pharmaceutical Industries, Cairo, Egypt**). for three days by intraperitoneal injection and sacrificed at the fourth day after the end of experimental period. The tramadol and pilocarpine group (G III); included 10 rats were received 10 mg tramadol hydrochloride for three days by intraperitoneal injection followed with 1 mg pilocarpine (**salagen, Sigma Aldrich Company, Cairo, Egypt**) dissolved in 1ml of distilled water by intraperitoneal injection.

Quantitative measurement of Saliva

The amount of saliva was collected before and after injection of the following: Saline, tramadol, tramadol plus pilocarpine. Statistical analysis of the drug effects on the amount of saliva collected, the data gathered from the measured amount of saliva collected from un stimulated condition, following tramadol administration and after combined tramadol and pilocarpine administration was recorded and statistically analyzed. The one way analysis of variance (ANOVA) test was used for comparison between the different groups. Tukey's post hoc test was performed also in order to evaluate the different effects of the injected drugs on the amount of saliva secreted and their significance.

Morphological investigations

The parotid gland was dissected out and microtechnique at the end of the experiment, the animals were sacrificed with overdose of inhaled ether anesthetic solution. The parotid salivary glands were carefully dissected out and immediately fixed in 10% formalin solution for 72 hours. The salivary glands were microtechnically processed in order to obtain paraffinized tissue sections of 5-6 micrometer thickness. The tissue sections were deparaffinized, hydrated then stained with hematoxylin and eosin (H&E) for the morphological examination of the structure of the parotid salivary gland tissue in control and experimental groups of animals. The computer Assisted digital image analysis (Digital morphometric study) were performed to evaluate the pilocarpine efficiency in concentration of the tramadol induced morphological alteration in parotid glands.

Results

(1) Quantitative measurements of saliva collected before and after treatments with tramadol exclusively or combined with pilocarpine.

The amount of saliva secreted before and after drugs administration were calculated and compared to each other. The data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science).

Table (1)
comparison of the mean amount of whole saliva secreted before and after
tramadol or tramadol with pilocarpine treatment.

Amount of saliva	Control Group (n=10)		Tramadol Group(n=10)		Tramadol+ Pilocarpine Group (n=10)		P
	Mean	±SD	Mean	±SD	Mean	±SD	
Before	.00034	.00005	.00032	.000065	.00037	.00006	0.16
After	.00035	.000051	.00013	.000055	.00081	.000064	<0.001* **
P1			<0.001***		<0.001***		
P2					<0.001***		

SD: standard deviation P: Probability

*: mild significance <0.05 **: moderate significance <0.01

***: High significance <0.001

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

P1: significance relative to Control group

P2: significance relative to Tramadol group

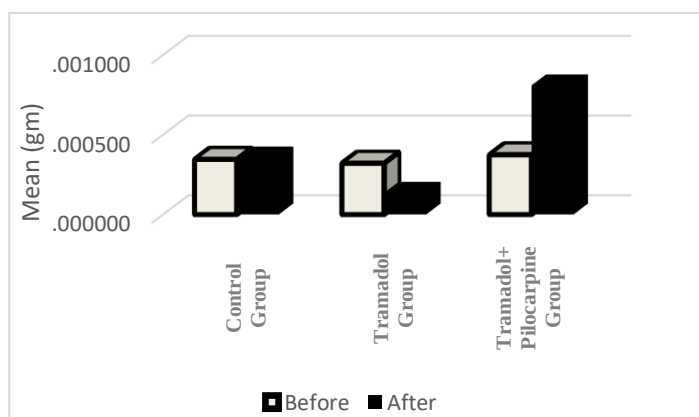


Fig (1): histogram showing the effect of tramadol or tramadol plus pilocarpine on the amount of whole saliva secreted in the three studied groups of animals.

(2) Morphological features of parotid gland before and after treatments with tramadol exclusively or combined with pilocarpine

(a) Histology of parotid glands of control group (G I):

The parotid gland is composed of two main components which are the secretory tissue termed the parenchyma and the supporting connective tissue, termed the stroma embracing the parenchyma and sending connective tissue septae dividing the parenchyma into several lobes and lobules. The parenchymal lobes and lobules of the parotid gland was consisted of a well-defined acinar arrangement of the serous cells forming secretory unites, intercalated ducts, striated ducts and excretory ducts. The intercalated duct cells were cuboidal in shape with large rounded centrally located nuclei, while the striated duct cells were columnar with pale centrally located nuclei and basal striation. (Fig.1)

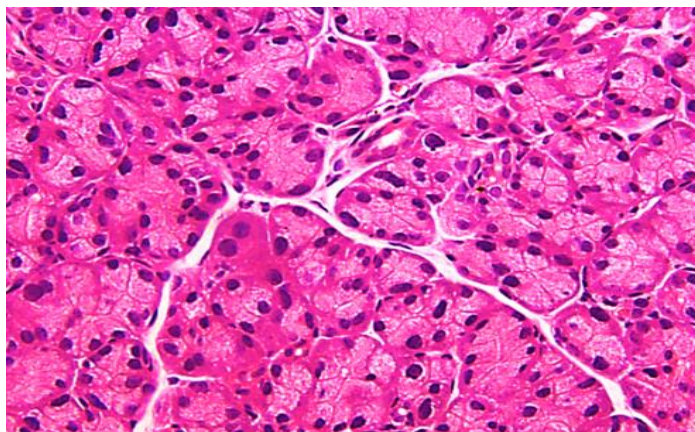


Fig.1: Rat parotid gland of the control group (I) showing typical predominant acinar arrangement of serous cells with basally located deeply basophilic nuclei and eosinophilic cytoplasm, intercatated duct and interstitial connective tissue. (H&E stain, orig. mag. X 200)

(b) Histology of parotid glands of tramadol group (G II): the histological examination and evaluation have evidenced sensitivity of the parotid gland to tramadol. The gland sensitivity has been reflected throughout the different forms of morphological changes. These change include the severe acinar atrophy associated with a reduction of the acinar size and excessive widening of the interstitial tissue area on the expense of acinar elements with sever reduction in the interacinar connective tissue components indicating both the acinar and lobular atrophy. The widened interstitial tissue area is replaced by extensive fibrosis mostly associated with the degenerated ducts and acini and their replaced by inflammatory cells (Fig 3). Other lobules demonstrated both acinar atrophy and degeneration. This degeneration was primarily manifested with the development of extensive acinar cell vacuolation either in the periphery of the gland or the peripheries of the parenchymal lobule of the gland beside interstitial connective tissue capsule or septae. This cytoplasmic vacuoles may coalesced together in the same acinar cell. The vacuoles coalescence ultimately led into the dissolution of the acinar content and hence the acini were replaced with optically clear areas (Fig 2).

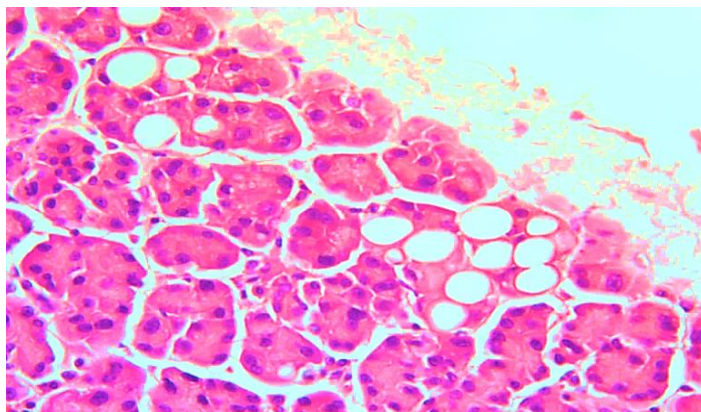


Fig.2: Rat parotid gland of tramadol group (II) showing extensive acinar cell vacuolation in the peripheral acini of the gland and prevailing acinar atrophy. (H&E stain, orig. mag. X 200).

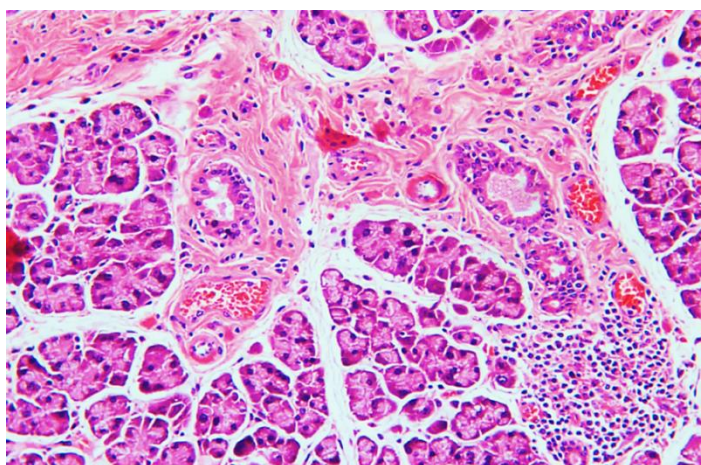


Fig.3: Rat parotid gland of tramadol group (II) showing server acinar atrophy. Widening of the interstitial tissue area on the expense of the acinar elements. The widened interstitial tissue area is replaced by vascular dense fibrosis mostly periductal. The degenerated acini and ducts are replaced by inflammatory cells. (H&E stain, orig. mag.X 100).

(c) Histology of parotid glands of tramadol and pilocarpine group (G III): the administration of pilocarpine after tramadol treatment showed that slight improvement from tramadol harmful effect has occurred. This was manifested in the acinar architecture of the gland which appears near normal. However, this reversal of tramadol effect did not acquire the full normal case of the gland. Though the acinar cell vacuolization was still observed, but with lesser degree than those noted with tramadol group II. This was illustrated in some tissue specimen who showed few acinar cell cytoplasmic vacuolation and slight perinuclear vacuolation in duct or acinar cells. In another tissue specimen a

disappearance of cytoplasmic and perinuclear vacuolation was noted in the vast majority of acinar cells (Fig 4).

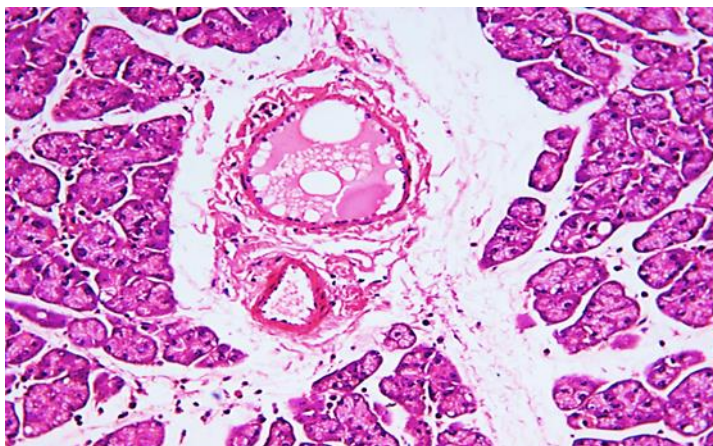


Fig.4: Rat parotid gland of tramadol and pilocarpine group (III) showing normal acinar architecture (blue arrows) and lymph vessel dilatation surrounded by slight fibrosis. (H&E stain, orig. mag. X 100).

(3) Digital morphometric analysis

The morphological features of the parotid gland and interacinar distance were calculated and analyzed by digital morphometric studies.

Table (5)
comparison of the interacinar distance in the parotid salivary gland among three studied groups of animals.

Interacinar distances		Groups			P
		Control Group	Tramadol Group	Tramadol +Pilocarpine Group	
distances	Median	3.84	30.40	19.09	<0.001***
	Minimum	.24	14.02	3.94	
	Maximum	18.88	45.39	41.12	
	P1		<0.001***	0.01*	
	P2			0.03*	

P: Probability *: mild significance <0.05

: moderate significance <0.01 *: High significance <0.001

Test used: Kruskal wallis followed by pairwise comparisons

P1: significance relative to Control group

P2: significance relative to Tramadol group

Discussion

Throughout the available literature that have been accumulated and to our knowledge no researches were noted about the effect of tramadol on salivary gland. In the present study, the tramadol administered for three days has induced severe serous acinar atrophy as manifested with a reduction of the acinar size associated with excessive widening of the interacinar areas. The nuclear changes were also observed which are manifested by the variable size, shape and staining basophilia that is collectively denoting the nuclear pleomorphism. The nuclear pleomorphism and perinuclear halo noted were seen in either ducts or acinar cells. These histological changes were also comparable to those demonstrated in other study who revealed degeneration and pyknosis of the hepatocyte nuclei in the liver cell of tramadol administered rabbits which coincided with the daily administration of tramadol to rats. ⁽¹¹⁾

The liver and kidney are believed to be responsible for the tramadol metabolism and excretion and so the tramadol may induce hepatotoxicity and nephrotoxicity during its metabolism. ^(12, 13) The toxic effects of opioids, at the cellular level, have been explained by the phenomenon termed as the lipid peroxidation, since the biological membranes contain large amount of polyunsaturated fatty acids, which are particularly susceptible to peroxidative attacks by the oxidants resulting in lipid peroxidation so, the lipid peroxidation has been used as an indirect generic marker for the oxidant- induced cell injury. ⁽¹⁴⁾ A significant increase in lipid peroxidation was reported in rats receiving an acute dose of cocaine ⁽¹⁵⁾ and similarly the lipid peroxides were significantly increased among the chronic heroin users. ⁽¹⁶⁾ These results were in agreement with Nehru and Anand ⁽²⁰⁰⁵⁾ who reported that both the reactive oxygen species generation and the lipid peroxidation are responsible for the tramadol-induced nephrotoxicity. ⁽¹⁷⁾

Other etiological factor which may lie behind the tramadol-induced degenerative changes, noted with parotid gland in the present study, beside the possible role of lipid peroxidation may include the widely reported morphine-induced hepatic toxicity, related to its metabolites rather than the morphine alone. ⁽¹⁸⁻²⁰⁾ These metabolic products are believed to induce the free radicals and/or binding with the glutathione (GSH), the natural scavenger of the superoxide radicals. Both the GSH conjugation and its subsequent depletion were believed to induce a free radical accumulation as well as the potentiality of morphine metabolites to induce, directly and indirectly, the cellular toxicity with the enzymatic inactivation, the DNA damage and/or the lipid peroxidation. ⁽²¹⁾

In respect to the periductal fibrosis and inflammation caused by tramadol administration seen in the present study they may be comparable with the morphine sulphate-induced portal-tract fibrosis, bile ductal dilatation and the proliferation of clusters of inflammatory cells surrounding the portal area. ⁽²²⁾ These pathological changes in the liver are reminiscent with similar changes in the present study noted with the parotid gland simulating the periductal collagen fibrosis, venous or lymphatic dilations as well as the proliferation of inflammatory

cells replacing the degenerated acini. Alternatively, the possibilities may exist denoting that the influence of growth factors may be responsible for the parotid periductal fibrosis and the harmful changes in duct cells themselves since these factors are believed to lie behind the fibrogenesis in the liver in which the stellate cells become active to initiate fibrogenesis. ⁽²²⁾

The collagen diseases have been characterized by chronic inflammatory reaction and pathological tissue reaction termed as "fibrinoid collagen necrosis" which form a common feature for most of these diseases. The frequent association of autoantibodies in these diseases has led them to be regarded as autoimmune in origin. Thereby, these autoantibodies may be presumed to play some causative role in the pathogenesis of such lesion in these diseases that is the autoimmune one. ⁽²³⁾ The fibrinoid collagen necrosis lesion led to the consideration of the affected condition to be regarded as collagen disease. The large areas of collagen necrosis were noted to be surrounded by fibroblasts with diffused region of lymphocyte and plasma cells. ⁽²³⁾ The disease processes that affect the connective tissues have been studied and the reactions of the connective tissue to injury were found to follow the generally prescribed patterns. One such pattern of alteration be known as fibrinoid degeneration. This term, was first applied to the appearance of a homogeneous, eosinophilic, relatively acellular, refractile substance with some of the tinctorial properties of fibrin. ⁽²⁴⁾ The fibrinoid was described in inflammations of the serous, mucous, synovial, and endothelial surfaces and it was believed that the alteration of this type was the primary change in the rheumatic fever. However, other literature demonstrated it in a variety of specific and nonspecific inflammations. ⁽²⁴⁾

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