Cytotoxic and Apoptotic Effects of Chronic Amitriptyline Administration on Rat Parotid Salivary Glands

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Abstract: Background: Depression is a chronic disorder that requires long-term treatment. Amitriptyline is one of the more commonly used tricyclic antidepressant drugs. Chronic administration of tricyclic antidepressants has been associated with numerous complains as tremors, nausea, vomiting, tachycardia, blurred vision, urinary retention and dry mouth. **Aim:** The purpose of this study was to determine the histological changes (cytotoxic and apoptotic) resulted from chronic amitriptyline administration for 9 weeks on rat parotid salivary glands. **Methods:** Twenty male albino rats (190 ±10 g) were divided equally into group I (control) and group II (Amitriptyline). The rats of group II received a daily single oral dose of amitriptyline (Tryptizol®) equivalent to the therapeutic dose (10mg/kg b.wt.) using an oro-pharyngeal metallic tube for 9 weeks. At the end of the experimental period, all rats were sacrificed. The parotid salivary glands were dissected out and prepared for histological and Fas immunohistochemical examinations. **Results:** Light microscopic examination of amitriptyline treated group revealed disfigurement, coalescence and shredding of the secretory portions. Some of the serous acini were completely missed leaving large vacuoles. The striated as well as excretory ducts appeared dilated with retained secretion. Widening of the connective tissue septa with numerous vacuolization was also detected. Immunohistochemical examination of experimental group showed increased Fas positive immunoreactivity indicating apoptotic changes. Conclusion: chronic administration of amitriptyline produced cytotoxication and apoptosis of parotid salivary glands.

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Keywords: Amitriptyline; parotid salivary glands; histological changes; apoptosis.

1. Introduction:

Depression is a common chronic disorder that requires long-term treatment [(1)]. It has a significant health and cost implications. Amitriptyline (Tryptizol®) is the most widely used tricyclic antidepressant drugs in treatment of major depression. The use of Amitriptyline is a common practice, especially in developing countries due to its lower price compared to newer antidepressants however, its comparable treatment outcomes [(2)].

Amitriptyline is a derivative of dibenzocycloheptadiene. It has a dual serotonergic and noradrenergic reuptake inhibitor. It is widely used in the management of major depression and different types of pain, including neuropathic pain or migraines [(3)]. There have been reports of amitriptyline potential usefulness as local anesthetics [(4)]. Antidepressants had numerous adverse reactions. Chronic administration of tricyclic antidepressants has been associated with orthostatic hypotension, tremors, nausea, vomiting, tachycardia, dry mouth, blurred vision, urinary retention, and other symptoms and signs of an atropine-like effect[(5)]. Urinary hesitancy and retention were reported as adverse reactions to drugs with anticholinergic properties, including tricyclic antidepressants [(6)]. Acute intoxication of tricyclic antidepressants is most commonly associated with central effects such as agitation, restlessness, hallucinations, seizures, coma, and respiratory depression. Cardiovascular effects of acute intoxication have included hypertension, as well as hypotension (especially postural), tachycardia, bradycardia, ventricular extrasystoles, ventricular tachycardia, congestive heart failure and myocardial infarction [(7)].

Amitriptyline caused significant tissue injury at concentrations less than what would be required to provide clinical effectiveness. This injury affected skin, subcutaneous tissue, muscles and nerves [(8)]. Introduction of amitriptyline also produced acute myocarditis [(9)].

Fas is a member of the tumor necrosis factor. A family of transmembrane receptors involved in cell death signaling. It is a cell surface glycoprotein (about 36 KD molecular weight) that involved in mediation of apoptosis (programmed cell death). Fas has three cystein rich extracellular domains and an intracellular death domain essential for signaling. Ligation of Fas by either agonistic antibody or by its natural ligand transmits a death signal to the target cells potentially triggering apoptosis [(10)]. Amitriptyline was reported to have a dose-dependent toxic effect in neurons (neurotoxicity) that is most likely mediated by apoptosis [(11)].

Although amitriptyline antidepressant drug is effective in treating depressive episodes and preventing relapse, it was reported to cause adverse reactions. Therefore, the aim of the present study was to evaluate the histological and immunohistochemical changes of chronic administration of amitriptyline (widely used antidepressant) on rat parotid salivary glands.

2. Material and Methods:

Twenty healthy adult male albino rats weighing 190 ±10 grams were used in this study. They were kept on normal diet and water. The animals were divided into two main groups (10 rats each) as follows:

Group I (Control group):

The rats were kept on standardized laboratory balanced diet and water for 16 weeks. The rats received a daily single dose of saline using an oro-pharyngeal metallic tube for 9 weeks.

Group II (Amitriptyline group):

The rats were kept on normal diet and water and received a daily single dose of amitriptyline (**Tryptizol- Al Kahira pharmatheutical Co. Egypt)** equivalent to the therapeutic dose (10mg/kg b.wt./day) using an oro-pharyngeal metallic tube for 9 weeks [12].

At the end of the experimental period, the rats were sacrificed by cervical dislocation. The parotid salivary glands were dissected out and cleaned rapidly of any adherent connective tissue. The parotid glands were fixed immediately in 10% calcium formol for 12 hours, washed by tap water, dehydrated in ascending grades of ethyl alcohol, cleared in xylol and embedded in paraffin wax. Then:

I- Sections of 6-7 µm were obtained and mounted on clean glass slides and stained with Haematoxylin and Eosin stain for routine light microscopic examination.

II-Sections of 5µm thick were cut and mounted on poly-L-lysine coated glass slides and prepared for Fas immunohistochemical staining for detection of apoptotic changes in the parotid glands.

Fas Immunohistochemical staining:

Serial 5Mm thick sections were cut and mounted on poly-L-lysine coated glass slides. The slides were dried over night at room temperature. Then sections were deparaffinized and hydrated in descending grades of alcohols. The sections were treated with blocking reagent for 5 minutes and washed in phosphate buffer working solution (PBS) for 10 minutes. After pre-incubation with 1% bovine serum albumin for 15 minutes, two to three drops of Fas protein mouse primary antibody were applied to the sections for 1 hour. Two to three drops of monoclonal mouse linking reagent were added to the slides then incubated for 30 minutes. Slides were incubated over night at 28°C in a humidity chamber. Two to three drops of streptavidin enzyme were placed then several drops of the working color reagent (DAB) were placed. The slides were counterstained with Mayer's hematoxylin, passed through baths of 95% ethyl alcohol, absolute ethyle alcohol and xylene respectively. Two drops of Canada balsam were placed on each slide and covers were mounted.

The immunostained sections were examined using:

a) Ordinary light microscope to assess the prevalence of Fas positive immunoreactivity in the parotid salivary glands.

b) Image analyzer computer system was used to assess the optical density of Fas positive cells and the intensity of the immunostaining. The image analysis was performed using a computer (software Leica Quin500) consisting of color video camera, color monitor, CBU of IBM personal computer connected to the microscope. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The intensity of the reactions within the cells was measured by the optical density in 10 small measuring fields in each specimen using a magnification of 400. After grey calibration, the image is transformed into a grey delineated image to choose the areas exhibiting positive reactivity with accumulation of all grades of reactivity (i.e. minimum, maximum and median grey). Positive areas were masked by a blue binary color. Mean values were obtained for each case **(Fig. 1).**

Statistical analysis:

Paired Student's t-Test was used to compare the mean % values of Fas immunoreactivity between control group and amitriptyline treated group. A p-value $p< 0.01$ was considered significant.

3. Results

I-Light microscopic results:

Group I (Control group):

The Light microscopic examination of the rat parotid glands of control group showed pure serous acini and intercalated ducts in between. The serous acini were uniform in shape, having narrow lumen and lined by pyramidal secretory cells having rounded basophilic nuclei. The intercalated ducts were hardly detected as they were small in size and compressed in between the serous acini. They were lined by small cubiodal cells having central rounded

nuclei. Connective tissue septae that divided the gland into lobes and lobules were also detected **(Fig. 2).**

Group II (Amitriptyline group):

Histological examination of parotid glands of Amitriptyline group revealed disfigurement, coalescence and shredding of the secretory portions. Some of the serous acini were completely missed leaving large vacuoles. Striated ducts showed wide lumen with retained eosinophlic secretory material. There were numerous dilated blood vessels engorged with red blood cells **(Fig.3).** Widening of the connective tissue septa with numerous vacuolization was also detected **(Fig.4).**The excretory ducts appeared dilated with retained eosinophlic secretory material. The fibrous connective tissue surrounding the ducts was thickened and also characterized by presence of congested blood vessels **(Fig. 5).**

II- Immunohistochemical results:

Control group:

Immunohistochemical examination of Fas protein in rat parotid salivary glands of control group revealed negative Fas immunoreactivity in the secretory portions and slight positive Fas immunoreactivity in the duct cells and blood vessels **(Fig. 6).**

Group II (Amitriptyline group):

Immunohistochemical examination of Fas protein in rat parotid salivary glands of amitriptyline treated group showed intense Fas positive immunoreactivity in the secretory portions, striated ducts and excretory ducts **(Fig. 7).** Statistical analysis using Paired Student's t-Test showed a significant increase in the mean optical density of the immunoreactivity of Fas protein in amitriptyline treated group compared with control group **(Table I &Histogram I).**

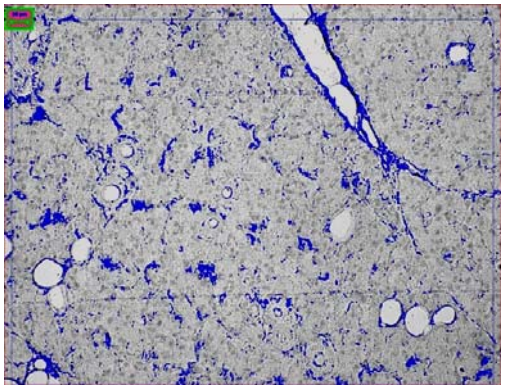


Fig. (1): A copy of display seen on the screen of the image analyzer showing the optical density of Fas immuno-expression after being masked by blue binary color.

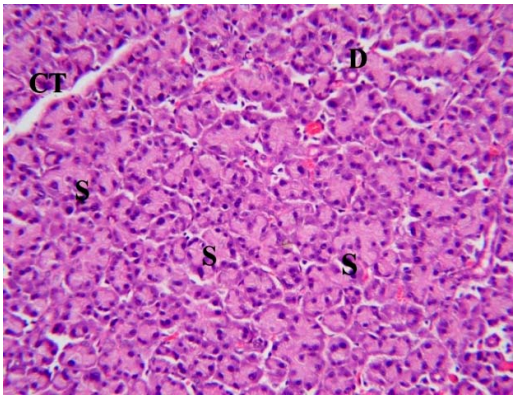


Fig. (2): A photomicrograph of rat parotid glands of control group showing the normal architecture of pure serous acini (S), intercalated ducts in between (D) and connective tissue septa (H & E Orig.mag. X 200).

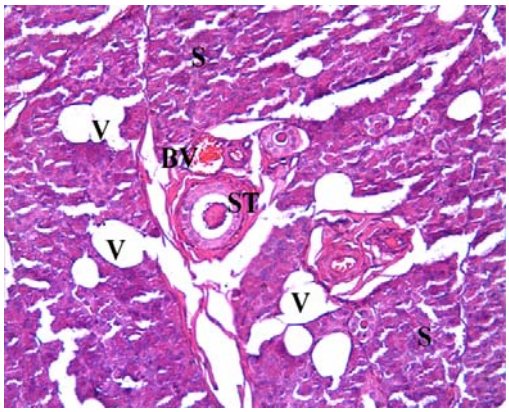


Fig. (3):* A photomicrograph of rat parotid glands of Amitriptyline group showing disfigured, coalesced and shreded serous acini (S), striated ducts (ST) with retained secretory material, numerous vacuoles (V), dilated blood vessels (BV) *(H & E Orig.mag. X 200).

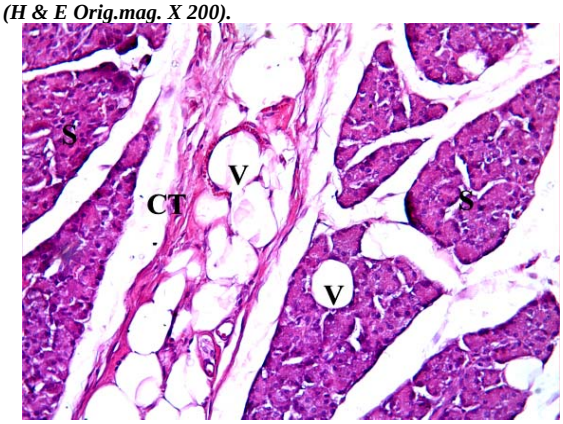


Fig. (4): A photomicrograph of rat parotid glands of Amitriptyline group showing disfigured and coalesced serous acini (S) and wide degenerated connective tissue septae with numerous vacuoles (V) (H & E Orig.mag. X 200).

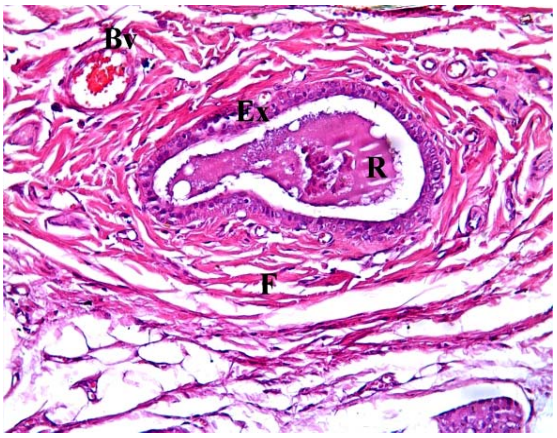


Fig. (5): A photomicrograph of rat parotid glands of Amitriptyline group showing dilated excretory duct (Ex) with retained secretion (R), dilated blood vessels (BV) and extensive fibrosis (F). (H & E Orig.mag. X 200).

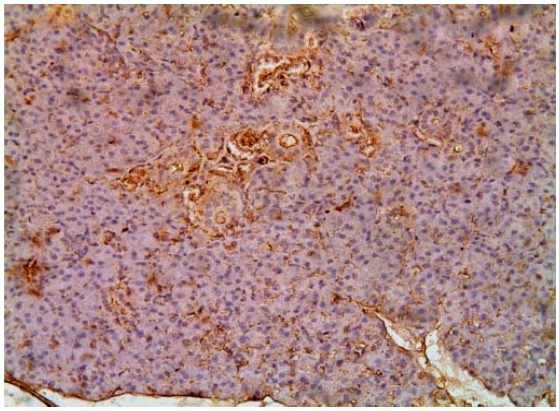


Fig. (6): A photomicrograph of rat parotid glands of control group showing negative Fas immunoreactivity in the acinar cells and slight positive Fas immunoreactivity in the duct cells and blood vessels (Fas Orig.mag. X 200).

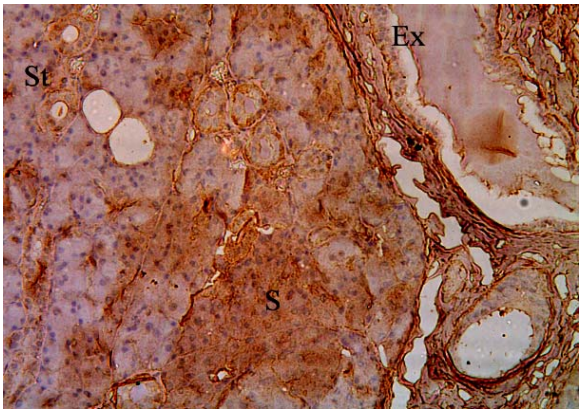
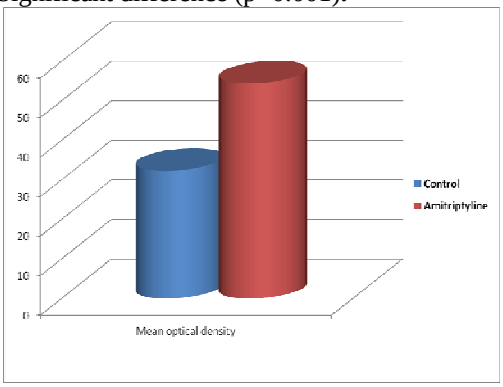


Fig. (7): A photomicrograph of parotid glands of Amitriptyline treated group showing intense Fas positive immunoreactivity in both acinar cells (S), striated ducts (st) and excretory ducts (Ex) (Fas Orig.mag. X 200).

Table I: Showing the difference in mean Fas optical density between control group and Amitriptyline treated group using Paired Student's t-Test

Group	Mean Optical density		
	M ±SD	t-Value	p-Value
Control	32.29± 1.17	11.66	0.0001**
Amitriptyline	54.42± 4.07		

** Significant difference (p<0.001).



Histogram I: Showing the difference in mean Fas optical density between control group and Amitriptyline treated group.

4. Discussion:

The use of antidepressants is associated with major side effects including dry mouth, drowsiness, difficulty in sleeping (insomnia), blurred vision, headache, constipation or diarrhoea, increased appetite or decreased appetite, nausea or vomiting, problems with urination,sexual function, palpitations, feeling light-headed on standing (orthostatic dizziness), feeling like the room is spinning round (vertigo), sweating, increased body temperature, tremor, disorientation, yawning, and weight gain [6, 13].

In the present study, chronic administration of amitriptyline had adversely affected the histological structure of the rats' parotid salivary glands. Light microscopic examination revealed disfigurement, coalescence and shredding of the secretory portions. This finding might be attributed to degenerative changes of the secretory portions. Some of the serous acini were completely missed leaving large vacuoles. This might be due to fatty degeneration and aggregation of the lipid degenerative products into large droplets. However, in the routinely processed hematoxylin and eosin sections the lipid droplets were dissolved during fixation and processing of the tis-

sues leaving large empty vacuoles. Similar findings were detected in the kidney and related to generalized lipidosis induced by ticyclic antidepressants administration [14].

Amitriptyline administration adversely affected the duct system of the parotid salivary glands. Both of the striated and excretory ducts showed dilatation with retaining eosinophilic secretion in their lumen. This finding might be attributed to accumulation of the salivary secretion and failure of exocytosis due to glandular injury and dysfunction. These histological changes in the duct system were in agreement with previous clinical findings. As long term use of amitriptyline produced peripheral side effects such as salivary gland dysfunction manifested as xerostomia [15]. In addition, administration of tricyclic antidepressants (e.g. amitriptyline) decreased whole mouth and parotid salivary output that resulted from blocking of parasympathetic stimulation of the salivary glands [16]. Moreover, amitriptyline reduced parasympathetic evoked salivary secretion by blocking cholinergic receptors as amitriptyline was reported to have atropine like action [17]. Amitriptyline also significantly reduced the flow rate and increased the time for secretion, thus allowing more reabsorption of Na^+ so that, Na^+ concentration decreased and K^+ concentration increased in the final secretion [18].

Light microscopic results revealed widening of the connective tissue septa, extensive fibrosis and vacuolation. Excessive fibrosis might be due to toxic effect of amitriptyline. Numerous congested blood vessels engorged with red blood cells were also detected. The dilatation and congestion of the blood vessels might be attributed to microcirculatory disturbances that developed due to amitriptyline administration that played an important role in glandular degeneration. In addition tricylic antidepressants (e.g. amitriptyline) were reported to produce well characterized areas of coagulative necrosis in skeletal muscles suggesting the possibility that necrosis was due to ischemic side effects of these drugs [19].

Statistical analysis of Fas immunoreactivity showed a significant increase in the mean optical density of amitriptyline treated group compared with control group indicating apoptotic changes in the secretory cells as well as duct cells. This finding coincides with other studies on the neural cells. As amitriptyline exerted a dose-dependent toxic effect on primary sensory neurons that was mediated by apoptosis and is efficiently blocked by an inhibitor of caspase activity [20]. Two mechanisms responsible for initiating apoptosis following tricyclic application have been proposed. First the generation of reactive oxygen species that was described previously in HL-60 leukemia cells as a pivotal step in the elicitation of apoptosis immediately preceding loss of mitochondrial membrane potential [21]. Second, increased cytoplasmic level of Ca2 was reported after application of amitriptyline [22].

In conclusion, chronic administration of amitriptyline adversely affected the histological structure of parotid salivary glands. Amitriptyline produced cytotoxic as well as apoptotic changes leading to glandular dysfunction.

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