

Curcumin Protection against Nicotine Induced Histological Changes of the Chromaffin Cells of Adrenal Medulla in Mice

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Abstract: Introduction: Smoking can affect hormone secretion; some of these effects are associated with important clinical implications. These effects are mainly mediated by the action of nicotine. Curcumin is a well known antioxidant agent. **Aim of the work:** To study the protective role of curcumin against nicotine induced toxic effects on the adrenal medulla. **Methods:** Thirty adult male mice were used and were divided into three groups (10 animals each): The first group (**Group I**) served as control group. The second group (**Group II**) received nicotine (2.5mg/kg b.wt., by subcutaneous injection ,daily for 4 weeks). The third group (**group III**) received curcumin (80 mg/kg b.wt) by intragastric intubation simultaneously along with nicotine for 4 weeks. Specimens of adrenal gland were processed for histological study by light and electron microscopes. **Results:** In nicotine treated mice, the chromaffin cells of the adrenal medulla showed increase in the cytoplasmic vacuolation, mitochondrial degeneration and decreased secretory granules. The nuclei showed abnormalities in the form of shrinkage, pyknosis and marked extended chromatin. These cellular changes have been found to be attenuated by curcumin. **Conclusions:** Curcumin administration may be protective for the chromaffin cells of the adrenal medulla against the toxicity of nicotine.

[Hekmat O. Abdel-Aziz and Sanaa A. Ahmed. **Curcumin Protection against Nicotine Induced Histological Changes of the Chromaffin Cells of Adrenal Medulla in Mice.** Journal of American Science 2011; 7(9): 698-703].(ISSN: 1545-1003). <http://www.americanscience.org>.

Key words: Nicotine, Curcumin. Chromaffin cells.

1. Introduction

Cigarette smoke has enormous negative health consequences worldwide, but the use of tobacco is still rising globally ⁽¹⁾. Although approximately 4000 components occur in the cigarette, nicotine is the alkaloid most active in the tobacco ⁽²⁾. Nicotine is an amine composed of pyridine and pyrrolidine rings ⁽³⁾. The predominant effects of nicotine in the whole intact animal or human consist of an increase in pulse rate, blood pressure, probably through the stimulation of the adrenal medulla and increased secretion of catecholamine ⁽⁴⁾. In addition, nicotine has also been found to disturb the antioxidant defense mechanisms in rats fed a high fat diet ⁽⁵⁻⁷⁾.

It has been shown that nicotine can cross the biological membranes including the blood brain barrier ⁽⁸⁾. By binding to nicotinic acetylcholine receptors (nAChR), nicotine increases the levels of several neurotransmitters. This release of neurotransmitters and hormones is responsible for most of nicotine's effects. It is thought that increased levels of dopamine in the reward circuits of the brain are responsible for the euphoria and relaxation and eventual addiction caused by nicotine consumption ⁽⁹⁾. The stress neurotransmitters adrenaline and noradrenaline are catecholamines that are synthesized and released into the systemic circulation by neurons in response to nicotinic receptor stimulation in the central and peripheral nervous

system and in the adrenal medulla ⁽¹⁰⁾. By binding to (nAChR) in the adrenal medulla nicotine increases flow of adrenaline, by binding to the receptors, it causes cell depolarization and an influx of calcium through voltage-gated calcium channels ⁽¹¹⁾. Calcium triggers the exocytosis of chromaffin granules and thus the release of epinephrine and norepinephrine into the blood stream ⁽¹²⁾. Nicotine also stimulates noradrenergic pathways in diverse regions within the central nervous system ⁽¹³⁾.

Curcumin is the main biological active phytochemical component of turmeric which is a member of the *Curcuma* botanical group (Family Zingiberaceae) ⁽¹⁴⁾. Curcumin firstly was used as a food and later discovered that it also had impressive medicinal qualities ⁽¹⁵⁾. Extensive studies within the last half a century have demonstrated the protective action of curcumin in almost all the disorders of the body. The molecule is known to possess, antiinflammatory ⁽¹⁶⁾, antihypertensive, anticancer, antidiabetic, antipsoriasis, antithrombotic, antihepatotoxic and many other useful properties ⁽¹⁷⁾. Besides its protective action in peripheral organ disorders, the molecule is known to possess neuroprotective properties as well ⁽¹⁸⁾.

Nicotine is a drug and its mechanisms of action on body are not completely understood and curcumin has many protective actions in peripheral organ disorders. So, the aims of the present work were to illustrate the effects of nicotine on adrenaline secretion and whether curcumin prevents these effects or not.

2. Materials and Methods

Chemicals

Nicotine and curcumin (CCM) were used in the present study and were purchased from Sigma chemical company in the form of powder. Nicotine was dissolved in distilled water and was used as 2.5 mg/kg b.wt./day. Curcumin was dissolved in a solution of gum acacia and was used as 80mg/ kg b.wt./day.

Animals and treatment:

The Institutional Animal Care and the Research Ethics Committee of the Faculty of Medicine, Sohag University, Egypt, approved the experimental protocol.

Healthy male mice weighing 30-40 g were housed in clean properly ventilated cages under the same environmental conditions, fed a standard laboratory food and water *ad libitum*. The animals were divided into three groups (10 mice each).

Group I (control group): That served as a control group and received the same volume of saline by subcutaneous injection.

Group II (Nicotine treated group): Where animals were injected subcutaneously with Nicotine in a dose of 2.5mg/kg b.wt.

Group III (Nicotine and Curcumin treated group): The animals of the third group were injected subcutaneously with the same dose of Nicotine as in group II and simultaneously given gavage administration of curcumin at a dose of 80 mg/Kg b.wt. All treatments were carried out daily for 4 weeks.

At the end of the experiment the mice were anaesthetized using ether inhalation and then sacrificed, carefully dissected and the suprarenal glands on both sides were picked up. Some of the specimens were used for preparation of paraffin blocks. Six micrometer thick sections were cut and stained with haematoxylin and eosin. Small pieces of the adrenal gland were fixed in 2.5 % 0.1 M phosphate buffered glutaraldehyde at 4° c for two hours, rinsed in 0.1M phosphate buffer and post fixed in phosphate-buffered 1 % osmium tetroxide for one hour at room temperature then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. Semithin sections (1µm thick) were obtained and stained with 1 % toluidine blue and examined by light microscope. Ultrathin sections (80-90nm) were stained with uranyl acetate and lead citrate to be examined by a JEOL electron microscope at 80 KV in Electron microscopic Units, in Sohag University and Faculty of Medicine, Tanta University.

3. Results

Light Microscopic Results:

Examination of both haematoxylin and eosin and toluidine blue stained semithin sections from the control group revealed the normal structure of the adrenal medulla. The chromaffin cells were arranged in cords separated with blood sinusoids. They were polyhedral in shape, with deeply stained granular cytoplasm and euchromatic nuclei (Figs.1&4).

In group II, mice which received nicotine, most of the chromaffin cells appeared hypertrophied with ill defined cell boundaries. The cytoplasm was pale in staining with very pale nuclei (Figs 2&6).

Examination of sections from group III that received nicotine together with curcumin revealed that most of the chromaffin cells appeared with normal euchromatic nuclei and dark granular cytoplasm (Figs. 3 &11).

Electron Microscopic Results:

Examination of ultrathin sections the adrenal medulla of the control group by transmission electron microscope, showed the normal ultrastructure of chromaffin cells. They appeared having euchromatic nucleus that contained prominent eccentric nucleolus. The cytoplasm was filled with abundant membrane bounded electron dense core granules, which were surrounded with electron lucent area beneath the surrounding membrane. These granules represent the neurotransmitters (adrenaline or noradrenaline) (Fig.5).

In group II, both cytoplasmic and nuclear changes occurred. The cytoplasm of the chromaffin cells showed vacuoles of different sizes with areas of depleted cytoplasmic granules (Figs. 7, 8 and 9) and destroyed mitochondrial cristae (Figs. 9 &10). The nuclei appeared with marked extended chromatin (Fig.8) or pyknotic (Fig.9).

In group III most of the nuclear and cytoplasmic changes observed in group II were improved. Most of the nuclei appeared rounded, euchromatic with prominent nucleolus. The cytoplasm appeared filled with electron dense granules (Figs. 12&13).

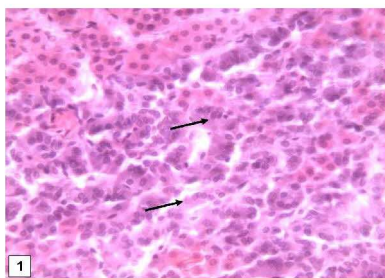


Fig. 1: A photomicrograph of a section in adrenal medulla of control mouse showing irregular shaped chromaffin cells (arrows) with granular cytoplasm. H&EX400

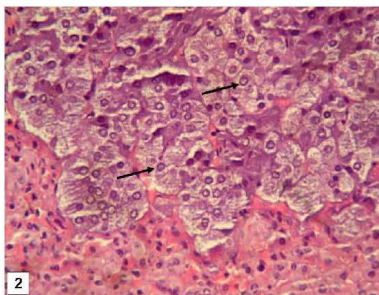


Fig. 2: A photomicrograph of a section in adrenal medulla of nicotine treated mouse showing the vacuolated cytoplasm of most of the chromaffin cells (arrows). H&EX400

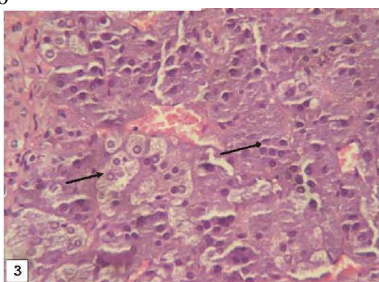


Fig. 3: A photomicrograph of a section in adrenal medulla of nicotine and curcumin treated mouse showing most of the chromaffin cells appeared with granular cytoplasm (diamond arrow) and few cells with vacuolated cytoplasm (arrow). H&EX400

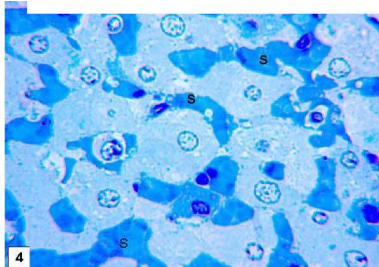


Fig. 4: A photomicrograph of a semithin section in adrenal medulla of control mouse showing cords of chromaffin cells separated by blood sinusoids (S). Toluidine blue, mic.MagXI000

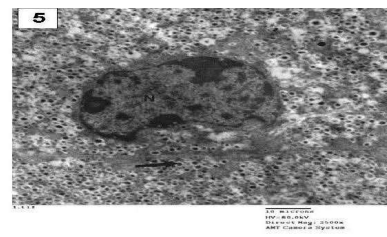


Fig. 5: An electron micrograph of chromaffin cell from the adrenal medulla of mouse from control group showing euchromatic nucleus (N) with eccentric nucleolus. The cytoplasm is filled of abundant membrane bounded electron dense core granules. The granules appear with electron lucent layer beneath the surrounding membrane (arrow). TEMXI0000

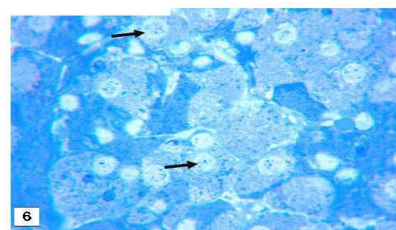


Fig. 6: A photomicrograph of a semithin section in adrenal medulla of mouse from the nicotine treated group showing the hypertrophied cells with ill defined cell boundaries. Most of the cells have pale staining cytoplasm with very pale rounded nuclei (arrows). Toluidine blue, mic.MagXI000

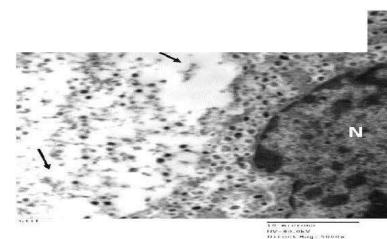


Fig. 7: An electron micrograph of a section of mouse adrenal gland from nicotine treated group showing cells of adrenal medulla showing part of the nucleus (N). The cytoplasm has vacuoles of variable sizes (arrows). TEMX8000

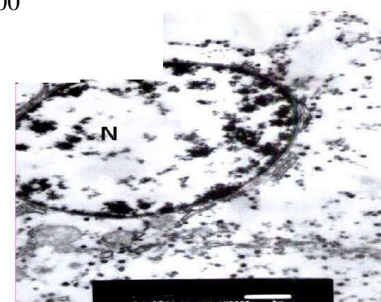


Fig 8: An electron micrograph of a section of mouse adrenal gland from nicotine treated group showing cells of adrenal medulla cells having chromatinolysis of the nucleus (N) and scarce cytoplasmic organelles or granules. TEMX8000



Fig 9: An electron micrograph of a section of mouse adrenal gland from nicotine treated group showing cells of adrenal medulla cells having shrunken pyknotic nucleus (N). The cytoplasm contains vacuoles of variable sizes and mitochondria with destroyed cristae (arrows). TEMXI0000

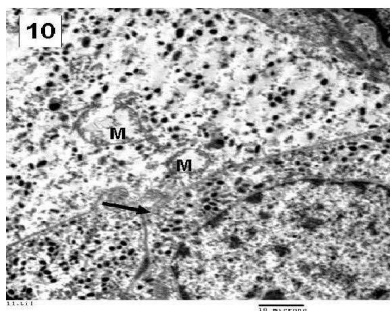


Fig 10: An electron micrograph of a section of mouse adrenal gland from nicotine treated group showing cells of adrenal medulla cells having swollen mitochondria with destroyed cristae (M). Notice the cell junction between the cell membranes of catecholamine producing cells (arrow). TEMXI0000

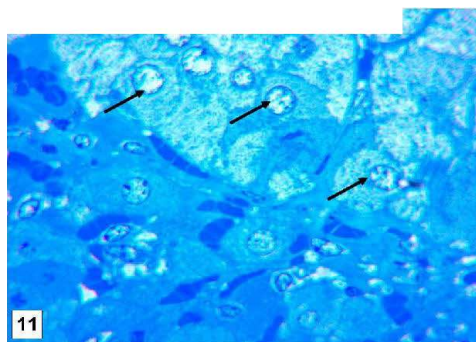


Fig 11: A photomicrograph of a semithin section in adrenal gland of mouse from nicotine and curcumin treated group showing the cells of the adrenal medulla with many of the nuclei appearing large rounded with prominent nucleolus (arrows). Toluidine blue, mic. MagXI000

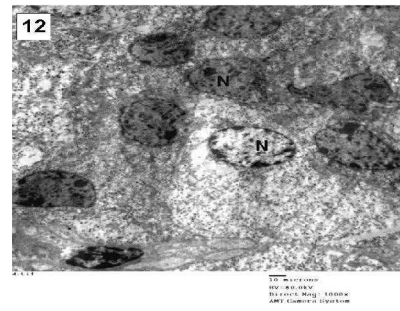


Fig 12: An electron micrograph of a section of mouse adrenal gland of mouse from nicotine and curcumin treated group showing the cells adrenal medulla revealing most of the cells are more or less similar to those of the control having rounded nuclei with extended chromatin (N) prominent nucleolus is also appeared. TEM X4000

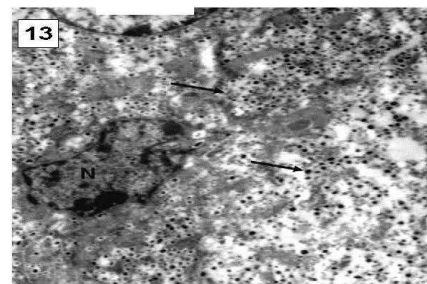


Fig 13: An electron micrograph of a section of mouse adrenal gland in curcumin and nicotine treated group showing adrenal medulla cells. The cytoplasm showed many electron dense granules (arrows). The nucleus (N) appeared euchromatic with prominent nucleolus. TEMXI0000

4. Discussion

In the present work, the histological changes induced by nicotine administration to adult male mice had been studied on chromaffin cells of adrenal medulla. These changes had been investigated after 4 weeks of treatment. We found that nicotine is toxic to the adrenal medulla and curcumin prevented this toxicity when used together with nicotine. The results of the present study are in line with Kalpana *et al.*,⁽⁶⁾ and Perlemuter *et al.*,⁽⁷⁾ who documented that nicotine has been found to disturb the antioxidant defense mechanisms in rats fed a high fat diet. Tatebe and Morita⁽¹⁹⁾ also demonstrated that nicotine in the presence of palmitate enhanced the production of ROS and the expression of TNF- α . In addition nicotine has been reported to produce necrosis, congestion, and fibrosis in the adrenal, brain, and kidney⁽²⁰⁾. Furthermore, Guan *et al.*,⁽²¹⁾ demonstrated that even low doses of nicotine could impact significantly on fetal cardiovascular and central nervous systems, as well as oxygen status, and suggested a toxic risk to fetuses exposed to low levels of nicotine or second-hand smoking during pregnancy.

In our study we noticed that after administration of nicotine, there was decrease in the secretory granules and the cells appeared with their cytoplasm filled with vacuoles of variable sizes. This result was due to toxic effect of nicotine and this observation was in agreement with Bartnicka *et al.*,⁽¹¹⁾ who proved that nicotine increases flow of adrenaline, by binding to the (nAChR) receptors where it causes cell depolarization and an influx of calcium through voltage-gated calcium channels. Also Al-Wadei and Schuller⁽²²⁾ proved that chronic administration of nicotine causing an increase in stress neurotransmitters and a decrease in the inhibitory neurotransmitter as GABA. Also, immunoassays showed a significant increase in serum adrenaline/noradrenaline and increased intracellular cAMP in the cellular fraction of blood of treated hamsters. Nicotine treated group showed cytoplasm with vacuoles of variable sizes; these may be due to dilatation of endoplasmic reticulum where these organelles have an important role in synthesis of catecholamine granules which decreased markedly in this group.

We proved in our study that in the group treated with curcumin together with nicotine, the secretory granules of chromaffin cells were more or less as the control group. The cytoplasm showed many dense granules. The nuclei appeared healthy with prominent nucleolus. Curcumin firstly was used as a food and later discovered that it also had impressive medicinal qualities⁽¹⁵⁾. It has a protective action on many disorders of the body, so it is considered as a promising source of protection against these disorders. Curcumin has antioxidant effects by significantly decreased the lipid peroxidation but significantly increased the reduced glutathione, catalase and glutathione peroxidase level⁽²³⁾. Also previous studies have demonstrated the protective action of curcumin in many disorders of the body⁽¹⁷⁾. Besides its protective action in peripheral organ disorders, the molecule is known to possess neuroprotective properties as well⁽¹⁸⁾.

Conclusion:

From the present study we can conclude that, nicotine which present in cigarette smoke has enormous negative health effects, on the secretion of stress neurotransmitters, adrenaline and noradrenaline, from chromaffin cells of adrenal medulla by depletion of these cells where, these produce most of nicotine's side effects. Curcumin, which is a natural compound, act as antioxidants may be effective in persons who exposed to nicotine due to cigarette smoking or second-hand smoke individuals especially children to protect their chromaffin cells from toxicity of nicotine. Further studies are needed as biochemical and genetic studies.

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20/8/2011