Original Article

Morphological Evaluation on the Protective Effect of Curcumin on Nicotine Induced Histological Changes of the Adrenal Cortex in Mice

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ABSTRACT

Introduction: Nicotine is a major toxic component of cigarette smoke. Cigarette smoking alters the levels of endogenous steroid hormones. 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 cortex. **Materials and 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 2.5mg/kg of body weight (daily for 4 weeks) of nicotine by subcutaneous injection. The third group (Group III) received curcumin (80 mg/kg) by intragastric intubation simultaneously along with nicotine for 4 weeks. Specimens of adrenal cortex were processed for histological study by light and electron microscopes.

Results: In nicotine treated mice, the cells of the three cortical zones (Zona fasciculata in particular) showed apparent increase in the cytoplasmic vacuolation, mitochondrial degeneration and increased lipid droplets. The nuclei showed abnormalities in the form of shrinkage, pyknosis and chromatin extension. These cellular changes have been found to be modulated by curcumin.

Conclusion: Our findings suggested that nicotine has a toxic effect on the adrenal cortex which could be resolved by concomitant administration of curcumin.

Key Words: Nicotine, curcumin, adrenal cortex.

INTRODUCTION

The health consequences of cigarette smoking and of the use of other tobacco products are well known. They became an important cause of increased mortality and morbidity in developed countries and the prevalence is increasing in the developing world as well. Nicotine is the main constituent in tobacco and responsible for its addiction¹. Nicotine is also a major pharmacologically active substance in cigarette smoke². Smoking has an effect on the various metabolic and biological processes in the body including secretion of hormones. Adrenal glands secrete many hormones, including cortisol. Cigarette smoking alters the levels of endogenous steroid hormones. An acute rise in circulating cortisol is observed after smoking³. Cortisol levels drop significantly in people who give up smoking especially during the early withdrawal process. Smoking also has effects on adrenal androgen secretion. Higher levels of androstenedione and dehydroepiandrosterone sulfate (DHEAS) are found in smokers⁴. ACTH-stimulated androstenedione, 17-hydroxyprogesterone and dehydroepiandrosterone (DHEA) levels are reported to be higher in male smokers⁵. In mice on the other hand, cortisol seems to modulate some of the physiological and behavioural effects of nicotine⁶. It also modulates the responsiveness of mice to nicotine7.

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Curcumin (CCM) is a dietary antioxidant derived from tumeric (Curcuma longa) and has been known since ancient times to possess therapeutic properties. It has been reported to be a potent anti-inflammatory, antioxidant and anticarcinogenic compound⁸. Much interest has been focused on the effects of curcumin in helping the body to resist the adverse influences of a wide range of physical, chemical and biological factors and helps the restoration of homeostasis. A promising role of curcumin in the prevention of Alzheimer's disease has been suggested⁹. The ability of curcumin to suppress the anti-tumor antibiotic bleomycin (BLM) induced pulmonary fibrosis in rats has been evaluated¹⁰. Moreover, curcumin induced apoptosis in the breast cancer cell line MCF-7¹¹.

Chemicals inducing cell injury are used in most scientific researches to investigate their effects on the different tissues of experimental animals.

The present study was designed to study the effect of nicotine on the histological structure of adrenal cortex and the possible protective role of curcumin.

49 (1214-2010)

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/day. Curcumin was dissolved in a solution of gum acacia and was used as 80mg/kg/day. Toxicity studies performed at 2000 mg/kg, which is 25-fold greater than our low-dose curcumin treatment (80 mg/kg), revealed no mortalities in any group of mice tested; the compound also had a low ulcerogenic index¹².

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 propylene oxide, the specimens were embedded in epoxy resin mixture. Semithin sections (1um 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 Unit, Sohag University¹³ and in Faculty of Medicine, Tanta University.

RESULTS

Light Microscopic Results:

Examination of both haematoxylin and eosin and toluidine blue stained sections from control group revealed the normal histological architecture of the adrenal cortex. The cells of zona glomerulosa were separated by blood sinusoids and arranged in the form of rounded or arched clusters beneath the adrenal gland capsule (Fig.1). The cells of zona fasciculata were arranged in long straight cords separated by blood sinusoids. These cells were large and polyhedral with pale vacuolated cytoplasm and vesicular rounded nuclei (Figs. 1 &5). Zona reticularis cells were disposed in the form of cords anastomosing with one another. Some cells appeared with pale nuclei and others with dark nuclei and blood sinusoids were observed in-between the cords (Fig. 1).

In group II which received nicotine, zona glomerulosa showed irregular orientation of the cells (Fig. 2). Most of zona fasciculata cells showed swelling and marked increase in cytoplasmic vacuolation (Figs. 2, 3) and lipid droplets (Figs. 6, 7). The nuclei appeared either pyknotic shrunken (Fig. 6) or very faintly stained (Fig. 7).

Examination of different sections from group III that received nicotine with curcumin revealed that most of the changes which were observed in the group II decreased. Zona fasciculata cells were revealing more or less a picture similar to those of the control (Figs. 4, 8).

Electron Microscopic Results:

Transmission electron microscopic examination of ultrathin sections from control group revealed the normal ultrastructure of the cells of the three zones of the adrenal cortex. Zona fasciculate cells appeared with spherical nuclei having both euchromatin and heterchromatin and well apparent nucleolus. Their cytoplasm contained numerous mitochondria and some lipid droplets (Fig. 9).

In group II which received nicotine, most of the changes were well apparent in the Zona fasciculata cells. These cells showed marked increase in the cytoplasmic vacuolation and lipid droplets (Fig. 10). They showed nuclear changes that included either shrinkage with condensation of their chromatin and widening of perinuclear space (Figs. 10-12, 14) or rounded nucleus with overextended chromatin (Fig. 13). The mitochondria were swollen with destroyed cristae (Fig. 10-12). Dilated smooth endoplasmic reticulum also found (Fig. 11)

In group III most of the changes observed in group II were improved. Most of the cells of the three zones of

adrenal cortex appeared more or less similar to those of the control with much decrease in the lipid droplets and normal nuclear appearance (Figs. 15, 16).



Fig. 1: Photomicrographs of a section of the adrenal gland of control group showing the capsule (C) and underlying arches of zona glomerulosa (G), parallel cell cords of zona fasciculate (arrow) separated by blood sinusoids(S) and zona reticularis cells (R) with anastomosing cords. (M) is part from the medulla. H&E X400



Fig. 2: Photomicrographs of a section of the adrenal cortex of nicotine treated group showing irregular orientation of cells of zona glomerulosa (G) swelling and vacuolation of some of the cells of zona fasciculata (arrows). H &E X400



Fig. 3: Photomicrographs of a section of the adrenal gland of nicotine treated group showing swelling and vacuolation of the cells of zona fasciculate (arrows) and dilated blood sinusoids (S). H &E X400



Fig. 4: Photomicrographs of a section of the adrenal gland of nicotine and curcumin treated group exhibited that most of the cells of the zona fasciculata are like those of the control group (arrows). Some cells are binucleated (arrow heads). H &E X400



Fig. 5: A photomicrograph of a semithin section in adrenal cortex of control mouse showing parallel cell cords of zona fasciculata separated by blood sinusoids (S). The cells are large and polyhedral with vacuolated cytoplasm and rounded nuclei with prominent nucleoli. Toludine blue, X1000



Fig. 6: A photomicrograph of a semithin section in adrenal cortex of mouse from the nicotine treated group showing the cells of zona fasciculata with increased lipid droplets in the cytoplasm (L). Most of the nuclei appeared indented and shrunken (oval arrows). Few of them appeared large rounded with prominent nucleolus (arrows). Toludine blue, X1000



Fig. 7: A photomicrograph of a semithin section in adrenal cortex of mouse from the nicotine treated group showing the cells of zona fasciculata with increase in the cytoplasmic vacuolation (arrows) and very pale staining nuclei. Toludine blue, X1000



Fig. 8: A photomicrograph of a semithin section in adrenal cortex of mouse from nicotine and curcumin treated group showing zona fasciculata having few lipid droplets (L). Many of the nuclei appeared large rounded with prominent nucleolus (arrow heads) and few of them appeared dark (arrows). Toludine blue, X1000



Fig. 9: An electron micrograph of a section of mouse adrenal cortex from control group showing cells of zona fasciculata having rounded nucleus (N) with prominent nucleolus and both extended and condensed chromatin, numerous mitochondria (m) and lipid droplets (L). Mic. Mag X6000



Fig. 10: An electron micrograph of a section of mouse adrenal cortex from nicotine treated group showing cells of zona fasciculata cells revealing shrunken nucleus (N) marked increase in lipid droplets (L) and numerous mitochondria with destroyed cristae (arrows). Mic. Mag X8000



10 microns HV=80.0kV Direct Mag: 2500x AMT Camera System

Fig. 11: An electron micrograph of a section of mouse adrenal cortex from nicotine treated group showing cells of zona fasciculata cells revealing shrunken pyknotic nucleus (N), obvious increase in lipid droplets (L), numerous mitochondria with destroyed cristae (arrow) and dilated smooth endoplasmic reticulum (oval arrow). Mic. Mag X10000



Fig. 12: An electron micrograph of a section of mouse adrenal cortex from nicotine treated group showing cells of zona fasciculata cells having nucleus (N) with dilated perinuclear space, increased lipid droplets (L), numerous mitochondria (arrow heads) some of them appeared swollen (m). Mic. Mag X12500



Fig. 15: An electron micrograph of a section of mouse adrenal cortex in curcumin and nicotine treated group showing zona fasiculata cells revealing that most of the cells are similar to those of the control having rounded nuclei with extended chromatin and prominent nucleolus (N) and cytoplasm showed few lipid droplets (L). Blood sinusoid (S) containing RBCs was also shown Mic. Mag X3000



HV=80.0kV Direct Mag: 2000x AMT Camera System

Fig. 16: An electron micrograph of a section of mouse adrenal cortex in curcumin and nicotine treated group showing zona fasciculata cells revealing the cells are similar to those of the control having rounded nuclei with extended chromatin (N) prominent nucleolus is also appeared. The cytoplasm showed few lipid droplets (L) and many mitochondria (m). Mic. Mag X10000



Fig. 13: An electron micrograph of a section of mouse adrenal cortex from nicotine treated group showing cells of zona fasciculata having rounded nuclei with markedly extended chromatin (arrows). Mic. Mag X3000



Fig. 14: An electron micrograph of a section of mouse adrenal cortex from nicotine treated group showing cells of zona fasciculata cells revealing many indented nuclei (arrow) and marked increase in lipid droplets (L). Mic. Mag X3000

DISCUSSION

Cigarette smoking has multiple effects on hormone secretion. It affects pituitary, thyroid, adrenal, testicular and ovarian function, calcium metabolism and the action of insulin³. Moreover it causes generalized disturbance in adrenal cortical hormone levels¹⁴. Nicotine is one of hundreds of substances contained in cigarette smoke and had been evaluated for their toxicity. The present study showed that treatment of adult male mice with nicotine induced degenerative changes in the adrenal cortex in the form of vacuolation of the cytoplasm, increase in lipid droplets swollen destroyed mitochondria, pyknosis, shrinkage and degradation of the nuclei.

The zona fasciculata and zona reticularis regions of the adrenal cortex are the site for synthesis and secretion of glucocorticoids (corticosterone) involved in the regulation of carbohydrate, protein and lipid metabolism¹⁵. Changes caused by nicotine observed in the adrenal cortex could bring about impairment in secretion and synthesis of corticosterone, leading to accumulation of lipid droplets and appearance of cytoplasmic vacuolation which were observed in this work. This was consistent with the results of previous investigators who demonstrated increase in lipid droplets in zona fasciculata and zona reticularis cells after inhibition of steroidogenesis by dexamethasone administration¹⁶. However, Nicotine, like adrenocorticotrophin (ACTH), can cause a dosedependent increase in steroidogenesis¹⁷. This can explain the extended chromatin as a consequence for the over stimulation of the adrenal gland.

Cell toxicity exhibited by nicotine in the present study may be due to combined action of this compound inducing many cellular processes mediated through reactive oxygen species (ROS). It is known that nicotine induces oxidative stress both in vitro and in vivo. Increased free radical production and cooperative lipid peroxidation levels in pancreatic tissue of rats incubated with nicotine¹⁸ and increased lipid peroxidation in Chinese hamster ovary cells incubated with nicotine and smokeless tobacco extract19 have been reported. In addition, elevated oxidative DNA damage in various tissues of mice exposed to side-stream cigarette smoke²⁰ and increased lipid peroxidation levels in tissues of intraperitoneal nicotine administered rats have been found. Furthermore, increased lipid peroxidation in blood of smokers has also been reported²¹. It seems that people who smoke and also who are exposed to cigarette smoke indirectly by breathing the air in the same environment are exposed to nicotine-induced oxidative stress²². Oxidative stress would result in increased free radical injury in the tissue leading to extensive tissue damage with subsequent derangement of cell physiology. ROS also causes the peroxidation of membrane phospholipids, which can alter membrane fluidity and lead to loss of cellular integrity. Thereby, the impaired activities of mitochondrial enzymes lead to decreased energy levels²³.

These changes lead eventually to cell degeneration with appearance of cytoplasmic vacuolation, degeneration of the mitochondria and nuclear changes as indentation and pyknosis that were observed in this work.

Nicotine is one of the compounds that induce intracellular oxidative stress and recognized as the important agents involved in the damage of biological molecules. Experiments using animal and cell culture model systems suggested that moderately higher concentrations of some forms of ROS like NO and H_2O_2 can act as signal transducing agents. Nuclear transcription factor kappa B (NF- κ B) an inducible transcription factor detected in neurons found to be involved in many biological processes such as inflammation, innate immunity, development, apoptosis and antiapoptosis²⁴.

In the present study, curcumin was found to be effective in protecting the adrenal cortex from the damaging effect of nicotine as most of the morphological changes induced by nicotine were greatly improved. This was consistent with the results of some researchers who reported that curcumin therapy modulated ion channels and cortisol secretion from bovine adrenal zona fasciculata cells²⁵. It is well known that curcumin is an antioxidant alternative for vitamin E^{26} . Curcumin is several times more potent than vitamin E as a free radical scavenger²⁷, protects the brain from lipid peroxidation²⁸ and scavenges NO-based radicals²⁹. Furthermore, curcumin can inhibit nuclear factor Kappa B (NF- κ B) mediated transcription of inflammatory cytokines³⁰.

CONCLUSION

In conclusion, the present study indicated that curcumin suppressed nicotine induced oxidative stress in adrenal cortex. The multiple beneficial effects make curcumin a promising dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress.

REFERENCES

- 1. Benowitz NL, Schultz KE, Haller CA, Wu AHB, Dains KM and Jacob P. (2009): Prevalence of smoking assessed biochemically in an urban public hospital: A rationale for routine cotinine screening. Am.J.Epidemiol. ;170(7):885-891.
- 2. Ford CL and Zlabek JA. (2005): Nicotine replacement therapy and cardiovascular disease. Mayo Clin.Proc. May;80(5):652-656.
- 3. Kapoor D and Jones TH. (2005): Smoking and hormones in health and endocrine disorders. Eur.J.Endocrinol. Apr;152(4):491-499.
- Baron JA, Comi RJ, Cryns V, Brinck Johnsen T and Mercer NG. (1995): The effect of cigarette smoking on adrenal cortical hormones. J.Pharmacol.Exp.Ther. Jan;272(1):151-155.
- Hautanen A, Adlercreutz H. (1993): Hyperinsulinaemia, dyslipidaemia and exaggerated adrenal androgen response to adrenocorticotropin in male smokers. Diabetologia Dec;36(12):1275-1281.

- Caggiula AR, Donny EC, Epstein LH, Sved AF, Knopf S, Rose C, McAllister CG, Antelman SM and Perkins KA. (1998): The role of corticosteroids in nicotine's physiological and behavioral effects. Psychoneuroendocrinology Feb;23(2):143-159.
- Fuxe K, Janson AM, Jansson A, Andersson K, Eneroth P and Agnati LF. (1990): Chronic nicotine treatment increases dopamine levels and reduces dopamine utilization in substantia nigra and in surviving forebrain dopamine nerve terminal systems after a partial di-mesencephalic hemitransection. Naunyn Schmiedebergs Arch.Pharmacol. Mar;341(3):171-181.
- Khar A, Ali AM, Pardhasaradhi BV, Varalakshmi CH, Anjum R and Kumari AL. (2001): Induction of stress response renders human tumor cell lines resistant to curcumin-mediated apoptosis: Role of reactive oxygen intermediates. Cell Stress Chaperones Oct;6(4):368-376.
- Lim GP, Chu T, Yang F, Beech W, Frautschy SA and Cole GM. (2001): The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J.Neurosci. Nov 1;21(21):8370-8377.
- Punithavathi D, Venkatesan N and Babu M. (2000): Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats. Br.J.Pharmacol. Sep;131(2):169-172.
- Choudhuri T, Pal S, Agwarwal ML, Das T and Sa G. (2002): Curcumin induces apoptosis in human breast cancer cells through p53-dependent Bax induction. FEBS Lett. Feb 13;512(1-3):334-340.
- Srimal RC and Dhawan BN. (1973): Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. J.Pharm.Pharmacol. Jun;25(6):447-452.
- Bozzola JJ and Russell LD. (1999): Electron microscopy: Principles and techniques for biologists. 2nd ed.Jones and Bartlett Publishers: Boston.
- Kirschbaum C, Wust S and Strasburger CJ. (1992): 'Normal' cigarette smoking increases free cortisol in habitual smokers. Life Sci. ;50(6):435-442.
- **15.** Vinson GP. (2009): The adrenal cortex and life. Mol.Cell. Endocrinol. ;300(1-2):2-6.
- Thomas M, Keramidas M, Monchaux E and Feige JJ. (2004): Dual hormonal regulation of endocrine tissue mass and vasculature by adrenocorticotropin in the adrenal cortex. Endocrinology ;145(9):4320-4329.
- Rubin RP and Warner W. (1975): Nicotine-induced stimulation of steroidogenesis in adrenocortical cells of the cat. Br.J.Pharmacol. Mar;53(3):357-362.
- Wetscher GJ, Bagchi M, Bagchi D, Perdikis G, Hinder PR, Glaser K and Hinder RA. (1995): Free radical production in nicotinetreated pancreatic tissue. Free Radic.Biol.Med. May;18(5):877-882.

- Yildiz D, Liu YS, Ercal N and Armstrong DW. (1999): Comparison of pure nicotine- and smokeless tobacco extractinduced toxicities and oxidative stress. Arch.Environ.Contam. Toxicol. Nov;37(4):434-439.
- Howard DJ, Briggs LA and Pritsos CA. (1998): Oxidative DNA damage in mouse heart, liver and lung tissue due to acute side-stream tobacco smoke exposure. Arch.Biochem.Biophys. Apr 15;352(2):293-297.
- Helen A, Krishnakumar K, Vijayammal PL and Augusti KT. (2000): Antioxidant effect of onion oil (Allium cepa. Linn) on the damages induced by nicotine in rats as compared to alphatocopherol. Toxicol.Lett. Jul 27;116(1-2):61-68.
- 22. Suleyman H, Gumustekin K, Taysi S, Keles S, Oztasan N, Aktas O, Altinkaynak K, Timur H, Akcay F, Akar S, Dane S and Gul M. (2002): Beneficial effects of Hippophae rhamnoides L. on nicotine induced oxidative stress in rat blood compared with vitamin E. Biol.Pharm.Bull. Sep;25(9):1133-1136.
- 23. Barr J, Sharma CS, Sarkar S, Wise K, Dong L, Periyakaruppan A and Ramesh GT. (2007): Nicotine induces oxidative stress and activates nuclear transcription factor kappa B in rat mesencephalic cells. Mol.Cell.Biochem. Mar;297(1-2):93-99.
- 24. Chen LF and Greene WC. (2004): Shaping the nuclear action of NF-kappaB. Nat.Rev.Mol.Cell Biol. May;5(5):392-401.
- Enyeart JA, Liu H and Enyeart JJ. (2008): Curcumin inhibits bTREK-1 K+ channels and stimulates cortisol secretion from adrenocortical cells. Biochem.Biophys.Res.Commun. ;370(4):623-628.
- 26. Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, Kopelovich L, Hawk ET, Lieberman R, Lawrence JA, Ali I, Viner JL and Sigman CC. (2000): Progress in cancer chemoprevention: Development of diet-derived chemopreventive agents. J.Nutr. Feb;130(2S Suppl):467S-471S.
- 27. Zhao BL, Li XJ, He RG, Cheng SJ and Xin WJ. (1989): Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals. Cell Biophys. Apr;14(2):175-185.
- 28. Martin Aragon S, Benedi JM and Villar AM. (1997): Modifications on antioxidant capacity and lipid peroxidation in mice under fraxetin treatment. J.Pharm.Pharmacol. Jan;49(1):49-52.
- **29.** Sreejayan and Rao MN. (1997): Nitric oxide scavenging by curcuminoids. J.Pharm.Pharmacol. Jan;49(1):105-107.
- Gonzales AM and Orlando RA. (2008): Curcumin and resveratrol inhibit nuclear factor-kappaB-mediated cytokine expression in adipocytes. Nutr.Metab. ;5(1):Art. No. 17.

الملخص العربى

تقييم مورفولوجى للتأثير الوقائى للكركم على التغيرات النسيجية التى يسببها النيكوتين فى قشرة الغدة الكظرية فى الفئران حكمت عصمان عبد العزيز قسم الهستولوجيا - كلية الطب - جامعة سوهاج

إن النيكوتين هوالعنصر الرئيسي من العناصر الضارة في دخان السجائر. كما أن التدخين يغير مستويات هرمونات الستيرويد الذاتية و يعتبر الكركم عامل معروف من مضادات الأكسدة. و إستهدف هذا البحث دراسة الدور الوقائي للكركم من الأثار السمية التي يسببها النيكوتين على القشرة الكظرية. و لقد تم إستخدام ثلاثون من الفئران الذكور البالغين حيث قسمت الى ثلاث مجموعات رئيسية (١٠ فئران لكل مجموعة): المجموعة الضابطة. ومجموعة تم علاجها بالنيكوتين عن طريق الحقن تحت الجلد بجر عة ٢,٥ مجم من وزن الجسم (يوميا لمدة ٤ أسابيع) و مجموعة تم علاجها بالكركم (١٠ مجم / كجم) مع النيكوتين بنفس الجرعة السابقة لمدة ٤ أسابيع. تم تجهيز عينات من قشرة الغذة الكظرية لفحصها بالميكر سكوبين الضوئي و الإلكتروني وقد أوضحت النتائج: أن خلايا الثلاث طبقات في قشر الكظر في المجموعة المعالجة بالنيكوتين قد تكدست بالفجوات السيتوبلازمية و الأنوية غير منتظمة و متكثفة الصبغيات او الأنوية ذات الكروماتينات المتحللة. و عند إضافة الكركم فإن معظم هذه التغيرات و الأنوية غير منتظمة و منكثفة الصبغيات او الأنوية ذات الكروماتينات المتحللة. و عند إضافة الكركم فإن معظم هذه التغيرات