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Original Article

Hazards of nicotine on the uterus of albino rat and the possible ameliorative role of human chorionic gonadotropin (hCG)

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ABSTRACT

Introductions: Smoking is an important cause of increased mortality and morbidity in the developing countries. Nicotine in cigarette smoke is one of the toxic substances which impair the fertility.

So, the aim of this work was to study the histological changes induced by nicotine hydrogen tartrate on the endometrium of albino rat and the possible ameliorative effect of human chorionic gonadotropin (hCG).

Material and Methods: Forty females of Wister albino rats in the normal estrous cycle were selected and used for the experiment. The experimental animals were randomly divided into four groups, each one including 10 female rats. The experiment period was extended to four weeks.

Group I was (control group). Group II (Nicotine treated group) was subcutaneously (SC) injected by nicotine hydrogen tartrate as 8 mg/rat three times/week for four weeks. Group III (Nicotine and hCG) was subcutaneously injected with the previous dose of nicotine together with hCG (pregnyl). HCG was subcutaneously injected as 2 IU/rat/day for four weeks. Group IV (hCG group) was subcutaneously injected only with hCG as 2.0 IU/rat/day for four weeks. The specimens were prepared for light and scanning electron microscopic examinations, and morphometric measurements.

Results: Nicotine induced decrease in the thickness of the endometrium. The height of the surface epithelium was decreased, the endometrial stromal cells were disrupted and had congested blood vessels. The endometrial glands became small and atrophied. The endometrium showed apparent increase in collagen fibers, a weak PAS reaction and highly positive immunohistochemical staining KI 67. Scanning electron microscopic examination revealed flattening of the surface epithelial cells with absent apical microvilli and apparent decrease in the number of pits of the glands. Concomitant administration of nicotine and hCG showed marked improvement in the structures of the endometrium as compared with that of the nicotine-treated group.

Conclusion: Nicotine altered the structure of the endometrium and concomitant administration of hCG with nicotine improved it.

Key Words: Endometrium, hCG, nicotine

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INTRODUCTION

Over the past few decades the use of tobacco among women of reproductive ages has increased (Singha and Kazi, 2015). Cigarette smoke contains a mixture of 4000 toxic chemicals, including nicotine, addictive components, carbon monoxide and several recognized carcinogens and mutagens (Sanders *et al*., 2002). Nicotine is the major constituents of tobacco (Wu *et al*., 2002). Smoking has deleterious effects on cardiovascular, pulmonary physiology and reproductive system. Nicotine is a highly toxic substance and it is quickly absorbed through the respiratory tract, mouth mucosa and skin. Nicotine is extensively metabolized to a number of metabolites by the liver. Quantitatively, the most important metabolite of nicotine in mammalian species and humans is the lactam derivative cotinine. In humans, about 70 to 80% of nicotine is converted to cotinine (Hukkanen *et al*., 2005).

Follicular levels of cotinine, a biomarker for nicotine exposure and cotinine incorporates into human ovarian granulosa-lutein cells, interfere with development of ovarian follicles (Zenzes and Reed, 1997). Smoking causes spontaneous abortion and delayed conception (Hughes and Brennan, 1996; Hull *et al*., 2000).

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The smoking during pregnancy is usually associated with increase in infant mortality, early neonatal mortality and risks of stillbirth. Also, low birth weight and abnormalities in development of infant brain is common (Salihu *et al*., 2003; Lavezzi et al., 2013 and Baba *et al*., 2014). Each phase of reproductive function, folliculogenesis, steroidogenesis, embryo transplant, endometrial receptivity, endometrial angiogenesis, uterine blood flow and uterine myometrium is susceptible to the harmful effects of smoking (Dechanet *et al*., 2011). In addition, smoker women are more liable to have an earlier menopause than non-smokers (Henningfield, 1995). Experimental studies in rodents support the human correlational data that nicotine alters normal processes involved in reproduction. For example, when delivered orally nicotine alters ion transport in the uterus in rats and mice, causing changes in the ionic composition in uterine fluid and epithelium which may affect hatching and outgrowth of blastocysts (Jin and Roomans, 1997). Injection of nicotine impairs endometrial decidualization, prolonged gestation, inhibited cervical ripening and induces degeneration of the endometrium in the uterus and ovarian follicles in female rats. Also, injection of nicotine produces abnormalities in the testicular ultrastructure in male rats (Aydos *et al*., 2001; Iranloye and Bolarinwa, 2009 and Yang *et al*., 2014).

Tobacco smoking alters the duration of the follicular phase of the human menstrual cycle (Liu *et al*., 2004). Subcutaneous injection of nicotine decreases fertility and increases follicular atresia in female rats (Holloway *et al*., 2006). The monitoring of estrous cycle is a basic method of evaluating the reproductive capacity of a female animal. Abnormality in reproductive function is often associated with estrous disruption. Estrogen is usually involved in regulation of estrous cycle in their reproductive age. Both in humans and rodents, estrogens stimulate endometrial growth (Benowitz *et al*., 2006). Also, nicotine may interfere with estrogen mediated neuroprotection in the brain (Raval, 2011).

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced in pregnancy by the developing embryo after conception and later by the syncytiotrophoblast. Its role is to prevent the disintegration of the corpus luteum of the ovary and thereby maintain progesterone production and pregnancy (Kayisli *et al*., 2003). Because of its similarity to luteinizing hormone, hCG can also be used clinically to induce ovulation in the ovaries through its action on the hypophyseal–gonadal axis. Similar to other gonadotropins, hCG can be extracted from urine (pregnyl) or by genetic modification (ovidrel) (Michels *et al*., 2007). Previous studies have proved the protective effect of hCG against lithium chloride and cyclophosphamide induced changes in the ovary (Ghosh *et al*., 2001). Thus the aim of this work was to study the histological changes induced by nicotine hydrogen tartrate on the endometrium of albino rat and the possible ameliorative effect of hCG.

MATERIAL AND METHODS

Forty females Wister albino rats were obtained from the Egyptian organization for biological products and vaccines (Cairo, Egypt). The average weight of them was about 200250-grams and the age of experimental rats was about 22.5- months old. All animals were housed for one week in suitable cages for acclimatization on the laboratory conditions before the experiment. Fresh water adlibitum and standard rodent food pellets were always available. Animals in the normal oestrous cycle were selected and used for the experiment. The experimental animals were divided into four groups, each one including 10 female rats. The experiment period was extended to four weeks.

The design of the experiment was as the follow:

Group I (Control): The animals of this group were divided into two subgroups. Group (1A) consisted of 5 rats which were used as negative control where the animals didn't receive any injected substances. Group (1 B) consisted of 5 rats which were used as positive control where the animals were injected with 0.4ml of normal saline subcutaneously (S/C) three times /week.

Group II (nicotine treated group): Nicotine hydrogen tartrate (Sigma USA) were prepared by dissolving 100mg nicotine in 5ml saline. So, each rat was injected with 0.4ml saline containing 8mg nicotine three times/week according to (Leach et al., 2013). The working solutions were stored in foil-wrapped glass bottle at 4°C for no longer than ten days.

Group III: (nicotine and hCG group): the animals of this group was subcutaneously injected with the same previous dose of nicotine together with hCG (pregnyl). HCG was subcutaneously injected as 2.5 IU/rat/day for four weeks. The later was purchased from Nile Pharmaceuticals. It was in the form of ampoules; each ampoule contained 5000 IU of hCG. One ampoule was dissolved in 1000 ml distilled water (DW) to obtain a concentration of 2.5 IU/0.5 ml DW. So, each rat of this group was also injected subcutaneous with 0.5ml distilled water containing 2.5 IU of hCG every day for four weeks (Chattopadhyay and Ghosh, 2010).

Group IV (hCG group): the animals of this group were subcutaneously injected with hCG (pregnyl as previously mentioned in group III.

Tissue preparation for light microscope

At the end of the experiment, the animals were sacrified by cervical dislocation. They were dissected and the uterine horns from female rats were excised to cut into two halves and one sample from each horn was taken. The specimens were fixed in 10% formol saline, dehydrated, cleared, and embedded in paraffin. Serial sections for light microscopy were cut (5-µm-thick) to becomes suitable for histological techniques. The sections were stained with haematoxylin and eosin (H&E), Masson's trichrome and periodic acid–Schiff (PAS) (Bancroft and Gamble, 2002).

Immunohistochemistry (IHC) for KI 67

Immunohistochemistry was carried out on paraffin sections (4 µm). Immunohistochemistry was done using a staining kit (Vectastain, Santa Cruz Biotechnology, USA). The sections were stained by avidin biotin peroxidase method for detection of KI 67 expression. Briefly, sections were deparaffinized, hydrated and then incubated overnight with the mouse monoclonal primary antibody to KI 67 (Ab-7, Mouse Mab. MS.) Using a universal detection kits (Dakocytomation), biotinylated secondary antibodies form a complex with peroxidase conjugated streptavidin molecules. Sections were rinsed in phosphate buffered saline (PBS) and few drops of biotinylated secondary antibodies were applied for 15 minutes. Then, sections were rinsed and treated with the prepared diaminobenzidine tetra-hydrochloride (DAB) substrate chromogen solution for 15 minutes until the desired brown colour obtained. Finally, sections were counterstained with Mayer's haematoxylin (Ramos- Vara *et al*., 2008).

Tissue preparation for scanning electron microscopy (SEM)

For scanning electron microscopic (SEM) examination, small pieces of uterine horn tissue (1 mm3) were freshly cut and rinsed with phosphate buffered saline (PBS) and fixed with 2.5% gluteraldehyde in PBS (pH 7.4) for 2 h. Specimens were postfixed in 1% osmium, dehydrated in acetone, and then subjected to drying. Dried specimens were coated with gold, and then examined and photographed (Adams *et al*., 2002).

Morphometric and statistical study

Using the image analyzer Leica Q 500 MC (Leica) program installed on a PC (Dell), the mean thickness of the lining epithelium of the uterus, the endometrial thickness and the perimeter of the endometrial glands/high power field were measured. Measurements were taken from five different fields from each section. Five sections from five different animals of each group were measured. The mean for each animal was calculated (n=5). The parameters were compared using analysis of variance tests between the different groups. Calculations were performed using SPSS (version 16 (IBM-USA). P was considered significant if less than 0.05.

RESULTS

Light microscopic examination

Group I (control)

Examination of H&E stained sections of both control groups (1A) and (1B) rats showed that no difference in histological study. The uterine wall was formed of endometrium, myometrium and very thin connective tissue perimetrium. The endometrium consisted of a surface columnar epithelium lining the uterine lumen and overlying a thick lamina propria containing compact stromal cells, blood vessels and few endometrial glands. The myometrium was organized into inner circular and outer longitudinal layers of smooth muscles with an intervening vascular layer (figure1 A). The simple columnar epithelium lining the endometrium was formed of secretory epithelial

cells with pale vacuolated cytoplasm. There were two types of secretory cells: dark cells with dark irregular nuclei and pale cells with pale vesicular nuclei. Most of the cells had brush borders. The endometrial stroma had a well organized thick population of stromal cells. Some of stromal cells were large cells with vesicular nuclei and pale basophilic cytoplasm. Other cells were spindle shaped cells with darkly stained nuclei (fibroblast-like cells) and few of them with deep acidophilic cytoplasm. The endometrial stroma, also contained blood vessels and thick population of well-defined endometrial glands embedded in a highly cellular stroma. Some glandular epithelial cells showed vesicular nuclei, whereas others showed darkly stained nuclei. Some of the cells had pale vacuolated cytoplasm (figure 1 B).

Masson's trichrome-stained sections showed the extracellular collagen content of the endometrial stroma with a few fibers beneath the surface epithelium (figure 1 C).

PAS-stained sections revealed PAS-positive reaction in the brush border and the basement membrane of the surface epithelial cells, and in the endometrial stroma (figure 1 D).

Group II (nicotine-treated group)

H&E-stained sections revealed a marked reduction in the thickness of endometrium, myometrium and permetrium with nicotine treatment (figure 2 A). The luminal epithelium is reduced in height with disorganization of the epithelial lining and irregular shaped nuclei with deeply acidophilic cytoplasm as compared with tall columnar cells with oval nuclei in the control group. The underlying connective tissue stroma was disrupted and had wide intercellular spaces and dilated congested blood vessels. The stromal cells showed disorganization in the cellular arrangement with less population than normal. They were formed mainly of spindle-shaped cells with darkly stained nuclei. Some of the stromal cells showed cellular necrosis in the form of vacuolated cytoplasm with karyolysis and others had pyknotic nuclei. Also there were some red cellular infiltrations. The epithelium lining the uterine glands was reduced in thickness with occasional apoptotic, degenerated cells. (figure 2 B).

Masson's trichrome stained sections showed marked increase in the green collagen fibers in the stroma and in between the smooth muscle fibers. The fibers appeared compact with minimal interstitial stromal spaces in between and extended beneath the surface epithelium (figure 2 C).

PAS-stained sections revealed a weak PAS-positive reaction in the brush border and the basement membrane of the surface epithelial cells, and in the endometrial stroma (figure 2 D).

Group III (the nicotine and human chorionic gonadotropin treated group)

Examination of H&E-stained sections of group III revealed that the endometrium, myometrium and primetrium were more or less similar to those of the control one (plate3 A). The height of the surface epithelium was increase in comparison to the nicotine treated group. Nearly all surface epithelial cells were tall columnar with oval vesicular nuclei with some parts of disorganization. The endometrial stromal cells became dense and infiltrated by polymorph nuclear leucocytes as compared with group I. The stromal cells attained their rounded shape with vesicular nuclei and a few of them were spindle shaped with spindle shaped nuclei. Some of the stromal cells contained many vacuoles and dark irregular apoptotic nuclei. The endometrial glands embedded in highly cellular stroma. The epithelium lining the uterine glands had a normal thickness nearly similar to the control group. They had large rounded vesicular nuclei and some of them had dark irregular apoptotic nuclei (figure 3 B).

Masson's trichrome-stained sections of the uterus showed a marked decrease in the collagen fibres in the uterus, which became less than group II. It was decreased in the endometrial stroma and in between the muscle fibres (figure 3 C).

PAS-stained sections of the uterus showed PAS-positive reaction in the brush border and the basement membrane of the surface epithelial cells, and in the endometrial stroma (figure 3 D).

Group IV (human chorionic gonadotropin treated group)

Examination of H&E-stained sections revealed that the thickness of the endometrium, myometrium and perimetrium were nearly similar to those of the control one (figure 4 A). The height of the surface epithelium was nearly similar to the control one with some areas of disorganization. Most of the surface epithelial cells were tall columnar with oval vesicular nuclei and acidophilic vacuolated cytoplasm. Some cells showed vacuolations and apoptotic changes. The endometrial stroma was dense nearly similar to the control one. The blood vessels of the stroma appeared normal. There were well-defined glands embedded in highly popular cellular stroma. Most of the epithelium lining the glands had normal thickness and showed vesicular nuclei with pale vacuolated cytoplasm (figure 4 B).

Masson's trichrome-stained sections of the uterus showed marked decrease in the collagen fibres in the endometrial stroma and in between muscle layers (figure 4 C).

PAS-stained sections of the uterus showed PAS-positive reaction in the brush border and the basement membrane of the surface epithelial cells and in the endometrial stroma (figure 4 D).

Immunohistochemical staining KI 67

Immunohistochemical staining KI 67 of control uterus showing negative expression of nuclei of the lining epithelium of the endometrium, underlying stromal cells of the connective tissue and in the nuclei of the glandular epithelium (figure 5 A). There was a marked positive expression in the nuclei of connective tissue stromal cells, the lining epithelial cells and the glandular epithelium in nicotine treated group (group II) (plate5 B). There was a decreased positive expression in endometrial tissue of both groups; group III which treated by nicotine and human chorionic gonadotropin (figure 5 C) and in human chorionic gonadotropin treated group (group IV) (figure 5 D).

SEM examination

SEM examination of the control rats group I showed the endometrial epithelial cells and multiple pits of glands in between. It also showed pinopodes, which appeared as smooth membrane projections from the epithelial cells inside or around the glandular orifices. Some mucous patches were also seen on the surface of the epithelial cells. The endometrial epithelial cells had bulged apices appeared dome shaped and were covered by short dense microvilli, and some of them showed fully developed pinopodes, which projected inside the glandular orifice. Some epithelial cells showed depressed umbilicated surface, whereas other secretory cells were more or less flattened with dense apical microvilli (figure 6 A).

SEM examination of group II showed that the surface epithelial cells became flattened with few or absent apical microvilli. The pits of glands in the endometrial surface were not detected. Mucous patches and the cells with pinopodes were hardly detected (figure 6 B).

SEM examination of group III showed that the endometrial surface became mucous patches, and mucous threads that appeared on the surface of epithelial cells. Some surface epithelial cells are covered with dense microvilli, while others were flattened with few microvilli. there were also absent microvilli in some cells. Mucous patches and cells with few pinopodes were detected around the pits of the glands (figure 6 C).

SEM examination of group IV showed that the surface epithelial cells had the dome shaped appearance and were covered by dense microvilli with many pits of the glands. There were many mucous patches and mucous threads on the surface of epithelial cells. There was also few well developed pinopodes around the pits of the glands (figure 6 D).

Morphometric and statistical results

All parameters (mean thickness of the surface epithelium of the endometrium, mean endometrial thickness and perimeter of the endometrial glands) showed a significant reduction in the nicotine treated group (group II) as compared with the control group. In contrast, group III (the hCG and nicotine group) revealed a significant increase in all parameters when compared with group II (Tables 1).

Table 1: Changes in epithelial thickness, endometrial thickness and perimeter of endometrial glands among different groups after four weeks.

	Epithelial Thickness Mean + SD	Endometrial thickness Mean + SD	Perimeter of endometrial glands Mean + SD
G I (Control)	27 + 5.2	500 + 114	216.1025
G II (Nicotine only)	20 + 3.2	375 + 150*	197.192*
G III (Nicotine + hCG)	20 + 3.1*	364 + 115*	209.966
G IV (hCG)	25 + 7.1*	1143 + 165*	213.563*

* = significant = $p < 0.05$

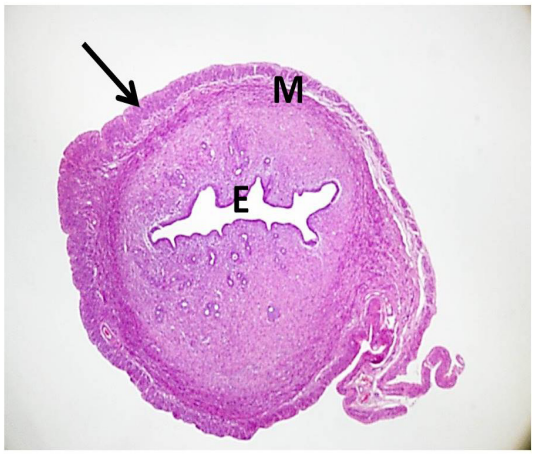

Fig. 1A: A Photomicrograph of a section in the control uterus of albino rat. The uterine wall is thin formed of endometrium (E), myometrium (M), and very thin CT perimetrium (arrow). H&E × 4.

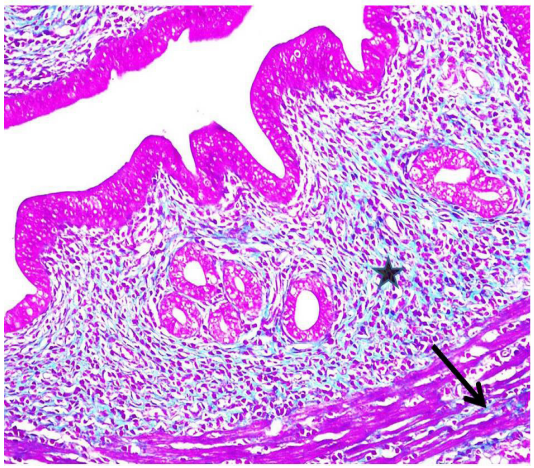

Fig. 1C: A Photomicrograph of a section in the control uterus of albino rat showing green stained collagen fibers in the stroma (star) and small amounts inbetween smooth muscle fibers(arrow). Masson's trichrome X 100.

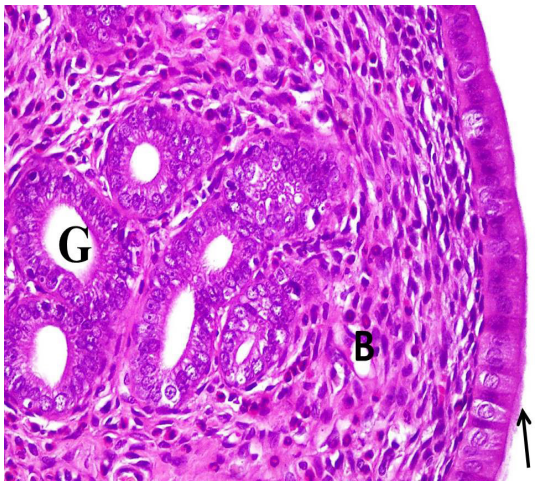

Fig. 1B: A Photomicrograph of a section in the control uterus of albino rat showing the surface epithelium with its brush border (arrow), underlying connective tissue stroma containing blood vessels (B) and endometrial glands (G). H&E × 400.

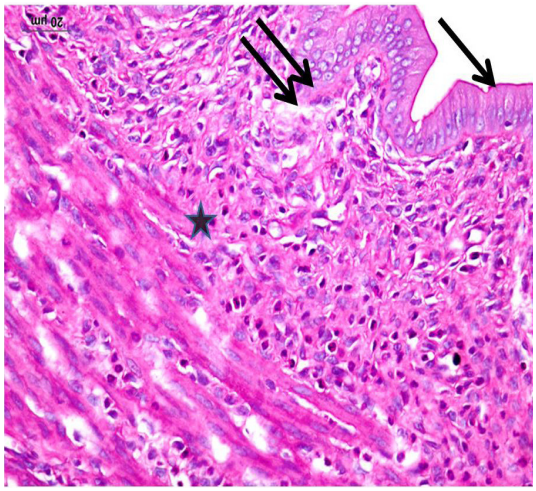

Fig. 1D: A Photomicrograph of a section in the control uterus of albino rat showing (PAS)-positive reaction in brush border (arrow) and in basement membrane of the surface of the epithelial cells that appears thin and regular (2 arrows). Note also, the PAS-positive reaction in the endometrial stroma (star). PAS Stain, X400.

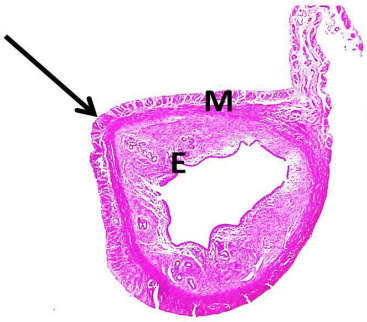

Fig. 2A: A Photomicrograph of a section in the uterus of albino rat group II showing marked reduction in the thickness of endometrium (E), myometrium (M) and thin CT perimetrium (arrow). H&E × 40.

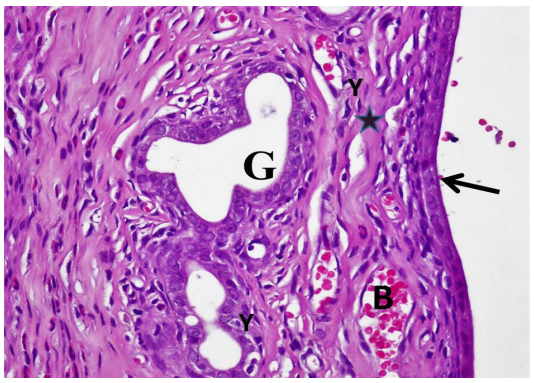

Fig. 2B: A Photomicrograph of a section in the uterus of albino rat group II showing that the luminal epithelium is reduced in height with disorganization of the epithelial lining and irregular shaped nuclei(arrow). The underlying connective tissue stroma has dilated wide spaces (star), congested blood vessels (B). Some of the cells shows cellular necrosis in the form of vacuolated cytoplasm with karyolysis (Y) while, others have pyknotic nuclei. The epithelium lining and of uterine glands (G) is reduced in thickness with occasional apoptotic and karyolitic cells (Y). H&E × 400.

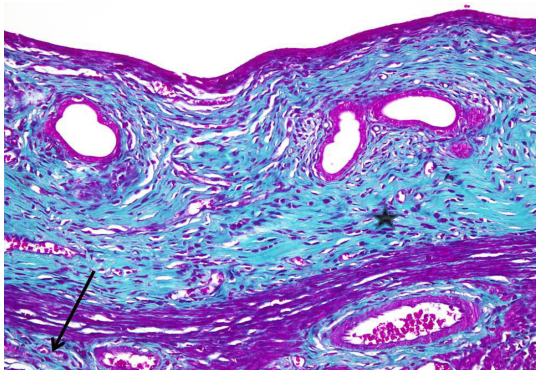

Fig. 2C: A Photomicrograph of a section in the uterus of albino rat group II showing marked increase in the green stained collagen fibers in the stroma (star) and inbetween the smooth muscle fibers (arrow). The collagen fibers appeared compact with minimal interstitial spaces and extended beneath the surface epithelium Masson's trichrome X 100.

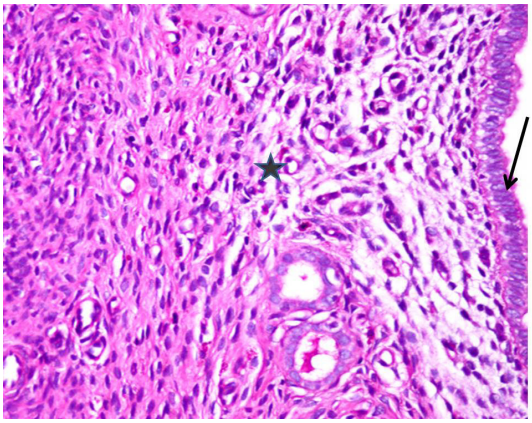

Fig. 2D: A Photomicrograph of a section in the uterus of albino rat group II showing (PASweak positive reaction in brush border of the surface epithelium (arrow). Note also, (PAS) negative reaction in the endometrial stroma (star). PAS Stain, X400.

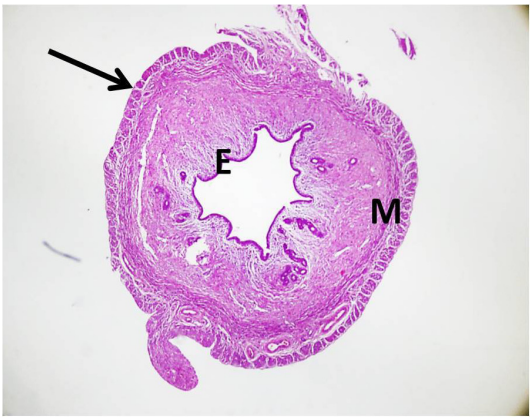

Fig. 3A: A Photomicrograph of a section in the uterus of albino rat group III showing that the uterine wall is normal and is formed of endometrium (E), myometrium (M), and thin CT perimetrium (arrow). H&E × 40.

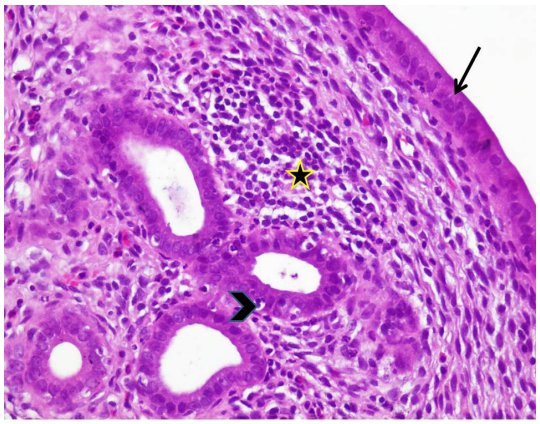

Fig. 3B: A Photomicrograph of a section in the uterus of albino rat group III showing an increase in the height of both surface epithelium and endometrial glands (arrow). The stromal cells contain an infiltration of polymorph nuclear leucocytes (star) and showing many vacuoles with dark irregular apoptotic nuclei (arrow). The glandular epithelial cells have large rounded vesicular nuclei and few apoptotic nuclei (arrow head). H&E × 400.

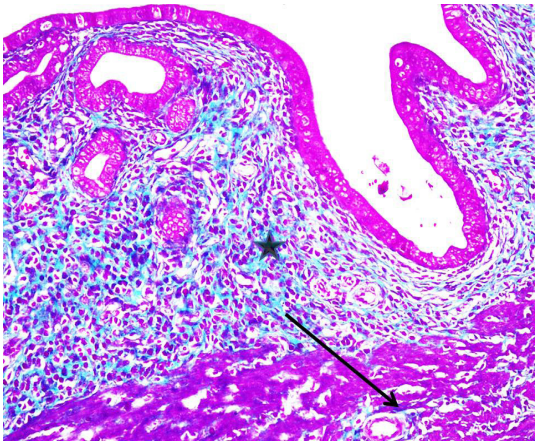

Fig. 3C: A Photomicrograph of a section in the uterus of albino rat group III showing a decrease in the collagen fibres in the endometrial stroma (star) and inbetween the muscle fibres (arrow). Masson's trichrome X 100.

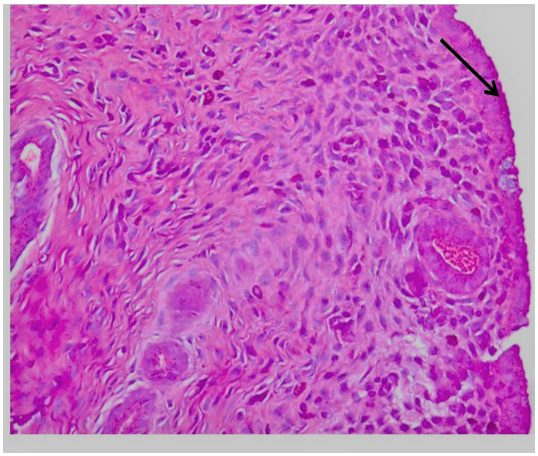

Fig. 3D: A Photomicrograph of a section in the uterus of albino rat group III showing PAS-positive reaction in brush border of the surface epithelium (arrow) and in the endometrial stroma.PAS Stain X400.

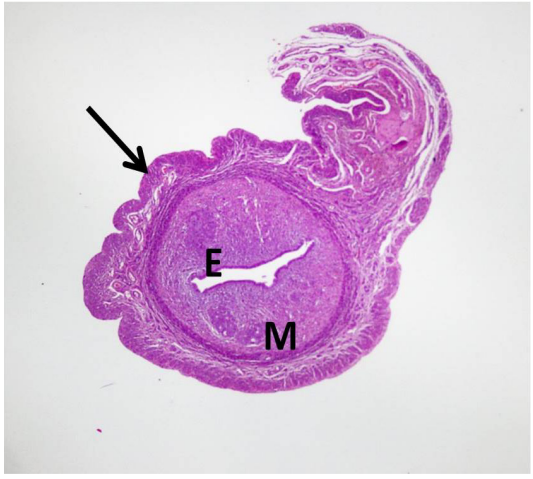

Fig. 4A: A Photomicrograph of a section in the uterus of albino rat group IV showing that the uterine wall is formed of endometrium (E), myometrium (M), and thin CT perimetrium (arrow) which is similar to the control group. H&E × 40.

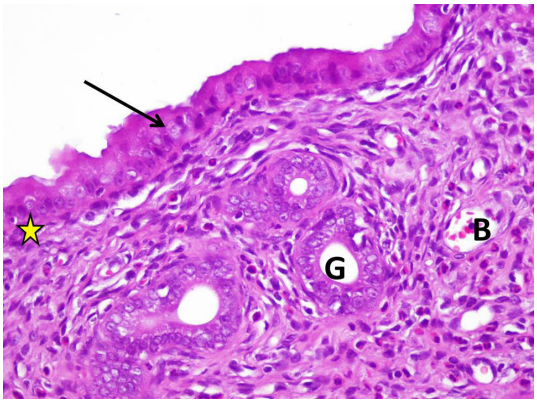

Fig. 4B: A Photomicrograph of a section in the uterus of albino rat group IV showing the surface epithelium has brush border and lined by tall columnar epithelium with oval vesicular nuclei and acidophilic cytoplasm (arrow). Some cells shows vacuolations and apoptotic changes (star). The endometrial stroma is dense. The blood vessels of the stroma are dilated (B). Most of the epithelial cells lining the glands (G) have vesicular nuclei with pale vacuolated cytoplasm. H&E × 400.

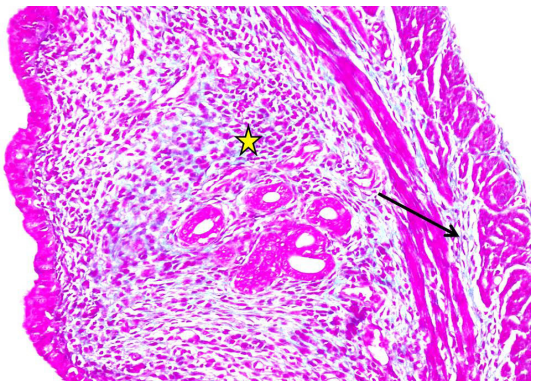

Fig. 4C: A Photomicrograph of a section in the uterus of albino rat group IV showing a decrease in collagen fibres of the endometrial stroma (star) and inbetween muscle fibres (arrow). Masson's trichrome X 100.

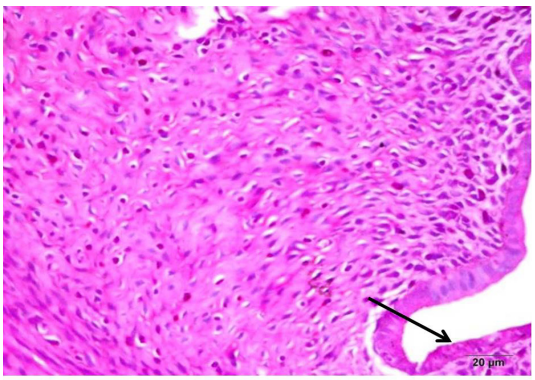

Fig. 4D: A Photomicrograph of a section in the uterus of albino rat group IV uterus of albino rat showing (PAS)-positive reaction in brush border of the surface epithelium (arrow). Note also, PAS-positive reaction in the endometrial stroma (star). PAS Stain X400.

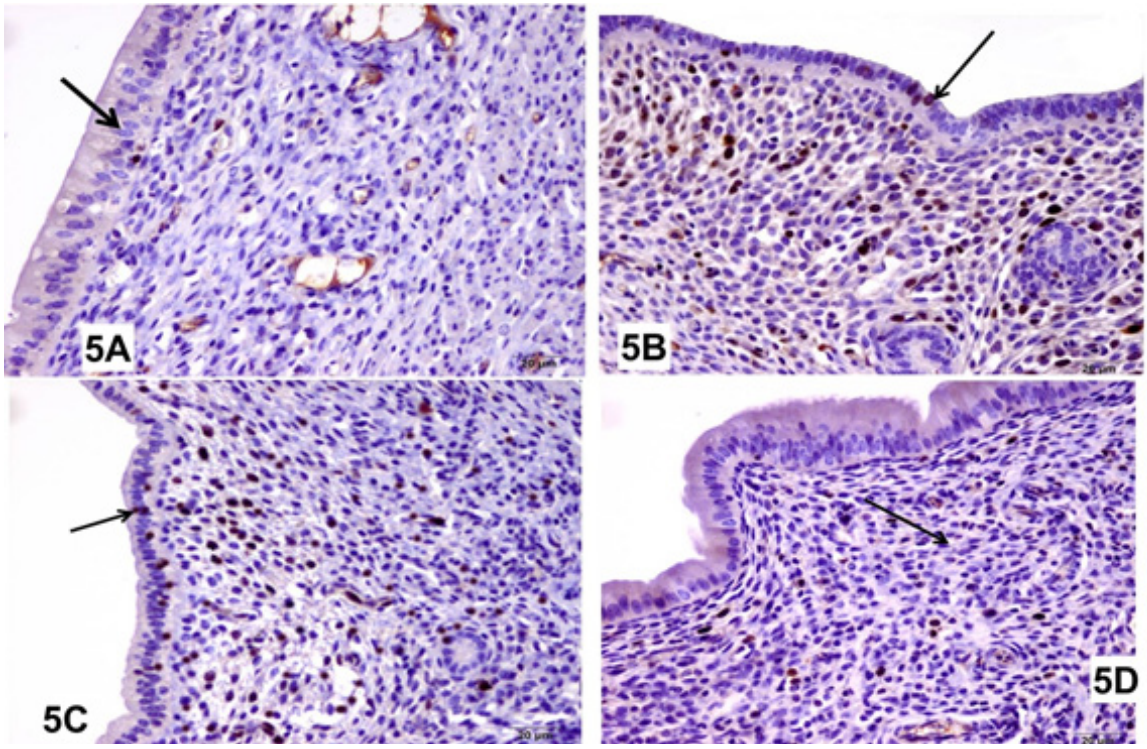

Fig. 5: Photomicrographs of sections of Immunostained KI 67 in the uterus of the control albino rat (group I) (5A) showing negative expression of the nuclei of the lining epithelium of the endometrium (arrow) and underlying connective tissue stroma. Nicotine treated rat (group II) (5B) showing marked positive expression in the brown stained nuclei of the lining epithelium of the endometrium (arrow) and underlying connective tissue stroma. Nicotine and hCG (group III) (5C) showing decreased expression of the nuclei of the lining epithelium of the endometrium (arrow) and underlying connective tissue stroma. HCG (group IV) (5D) showing also decrease in positive expression of the nuclei of the lining epithelium of the endometrium (arrow) and underlying connective tissue stroma. KI 67X400.

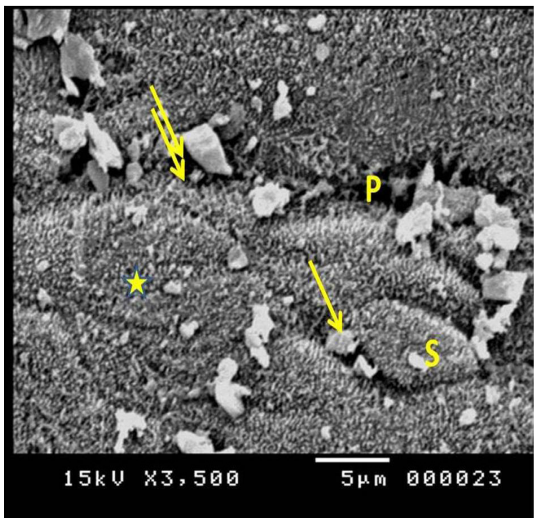

Fig. 6A: An Electron micrograph of the control uterus of albino rat showing multiple pits of glands (P) in between the endometrial epithelial cells (S) which is covered by short dense microvilli (star). Apical cell projections (pinopodes) (arrow) are noted around the pits (2 arrows) and inside the glandular orifice. SEM × 3500.

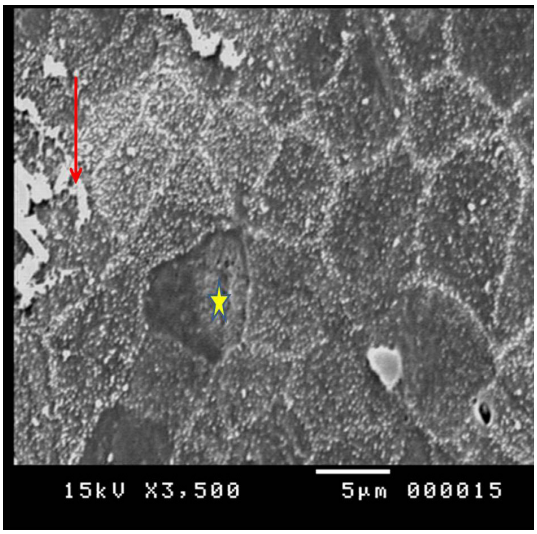

Fig. 6B: An Electron micrograph of the uterus of albino rat group II showing that the surface epithelial cells are flat with few or absent apical microvilli (star). The pits of glands in the endometrial surface are not detected. Mucous patches (arrow) and cells with pinopodes are few. SEM × 3500.

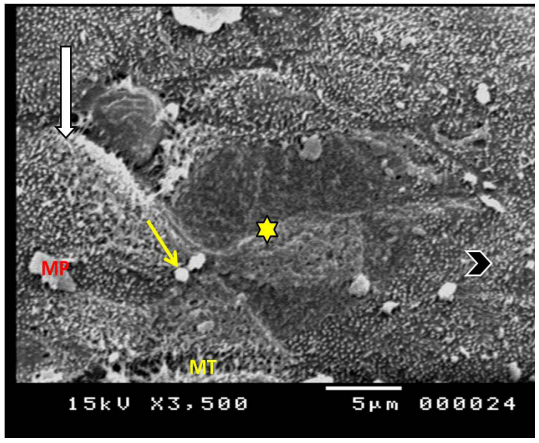

Fig. 6C: An Electron micrograph of the uterus of albino rat group III showing that the endometrial surface have mucous patches(MP), and mucous threads (MT) that appeared on the surface of epithelial cells. Some surface epithelial cells are covered with dense microvilli (arrow), while others are flat with few microvilli (arrow head). Notice also in some cells shows absent microvilli (star), while, others show Mucous patches. The cells with pinopodes are hardly detected around the pits of the glands (yellow arrow). SEM × 3500.

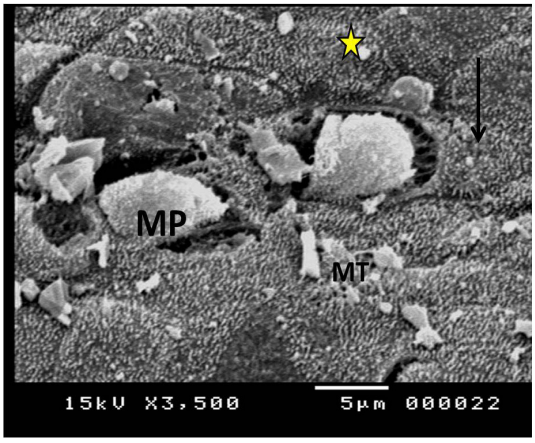

Fig. 6D: An Electron micrograph of the uterus of albino rat group IV showing that the surface epithelial cells have dome shaped surfaces and are covered by dense microvilli (arrow) with many pits of the glands. There are many secretory mucous patches (MP) and mucous threads (MT) on the surface of epithelial cells. There is also few well developed pinopodes (star) around the pits of the glands. SEM × 3500.

DISCUSSION

The Nicotine was an important alkaloid identified in the endometrial fluid in smokers female. Animal models exposed to toxic metabolites of tobacco had shown abnormal endometrial maturation (Cooke and Bitterman, 2004).

In this study the endometrial structure was affected with nicotine treatment. It induced reduction in the thickness of endometrium and in the height of the surface epithelial cells. The epithelium lining the uterine gland was also reduced in thickness with degenerated epithelial cells. The previous findings were in concordance with El-Meligy *et al*. (2007); Iranloye and Bolarinwa (2009) and Camargo *et al*. (2014). They had similar findings and observed that the uterus of the animals administrated nicotine exhibited marked reduction in the thickness of both endometrium and myometrium with an observable reduction in the endometrial glands. Anastasia *et al*. (2009) mentioned that the reduced thickness of endometrium and reduction in the size of endometrial glands indicated the inhibition of ovarian steroid biosynthesis necessary for growth of the uterus and reproductive cyclicity. It was well known that through neural stimulus to gonadotrophic releasing hormone (GnRH), hypothalamus regulates the rhythmic release of pituitary gonadotrophins, i.e., FSH, LH and prolactin. Investigations on constituents of the tobacco indicated that nicotine being a central nervous system influencing drug inhibited the release of gonadotrophins from pituitary. Uterine growth was depended on the ovarian estrogen secretion. Estrogen primarily acted upon the surface epithelium and the glands within endometrium. Progesterone acts on estrogen primed uterus and prepares the uterine epithelium from proliferative to secretory state.

In this study the presence of apoptotic cells in the endometrial lining, stoma and in the uterine glands is explained by Petrik *et al*. (2009) and Wang *et al*. (2000) who reported that nicotine induced an increase in the number of apoptotic cells in the epithelium of the uterus and oviduct. The increased level of apoptosis in the luminal epithelium of the uterus and oviduct is presumably due to a reduced level of estradiol and its receptor.

In this study there were dilated congested blood vessels in the stoma of endometrium of nicotine treated group, this was explained by Xiao *et al*. (2007), and Singha and Kazi (2015) who suggested that the toxins present in the tobacco interfere with the endometrial receptivity, endometrial angiogenesis and uterine blood flow. Also, Bordel *et al*. (2006) stated that nicotine produced a direct action in the release of mediators and growing factors responsible by angiogenic dynamic and vascular development.

In the present work there was an increase in the amount of collagen fibers in the stroma and in between smooth muscle fibers, it was in agreement with a study in vitro by Shan *et al*. (2009) who reported an increase in the collagen synthesis from fibroblast exposed to nicotine during 30 days.

In this work there was wide inter cellular spaces indicating edema in the underlying connective tissue stroma. The stromal cells showed disorganized cellular arrangement and were formed mainly of spindle-shaped cells with darkly stained nuclei and some of them had vacuolated cytoplasm and pyknotic nuclei. The epithelial lining the uterine glands were reduced in thickness with occasional apoptotic, degenerated cells. These findings were in agreement with the study done by Zhang *et al*. (2007) who stated that the compounds found in tobacco were capable of altering the epithelial cell layer of endometrium and myometrium. Tobacco was producing stromal inflammation and cellular edema. The nicotine induced impairment in hormonal support (estrogen and progesterone). The progesterone was responsible mainly for the development of the uterine glands and glycogen deposition, while the estrogen was responsible for the proliferation of the uterine muscles. In addition, the authors mentioned that the direct toxicity of nicotine or its related metabolites might induce degenerative and atrophic changes.

In this study Immunohistochemical staining KI 67 reaction in group II treated by nicotine showed strong positive expression of many cells lining the endometrium, connective tissue stromal cells and glandular epithelium. In contrast a weak positive expression was observed in group III treated by nicotine and hCG and group IV (group. The previous findings were supported by Bullwinkel *et al*. (2006) who described the KI 67 protein as a nuclear and nucleolar protein and an excellent marker to determine the growth fraction of a given cell population. Another study by Pier *et al*. (2013) who supported the results in this study. They found an increase in cell proliferation in the tubal epithelium of smokers. Smoking might affect cell fallopian tube epithelial cell turnover. Therefore it was associated with structural changes of the fallopian tube.

In the present study, coadministration of hCG with nicotine in group III was nearly preserved the normal endometrial histoarchitecture. The significant protective role of hCG on the female genital system was explained by previous researchers who proved that hCG had a direct effect on the ovarian tissue as it stimulated folliculogenesis and an indirect effect on the uterus through increased estradiol production (Akifcam and Kuran 2004). Estradiol acts as a regulator of uterine histometry (Cagnacci *et al*., 2000). Moreover, Kavitharaj and Vijayammal (1999) revealed that the toxicity of nicotine could be caused by direct action on the reproductive structures or by indirect action through the endocrine system. This could lower gonadotropin synthesis and secretion. Low gonadotropin levels could in turn decrease activities of ovarian steroidogenesis. In the present study in group III the height of the surface epithelium showed a significant increase after hCG coadministration. The stroma became dense and the endometrial glands were embedded into the highly cellular stroma. A previous study described the morphological and molecular changes of the endometrium after in-vivo infusion of hCG. It reported that hCG induced differentiation of stromal fibroblasts and induced synthesis of progestogen-associated endometrial protein, the most abundant secretory product of early pregnancy (Sherwin *et al*., 2010). However, apoptotic figures in both surface epithelium and stromal cells were still observed in group IV. This was in agreement with many reports, which stated that hCG played a role in cellular differentiation and proliferation (Adams *et al*., 2002).

In this study the scanning electron microscope examination of the nicotine-treated group revealed that the pinopodes and microvilli were hardly seen, while the pits of the glands were lost which interfere with fertility as suggested by

Meekera and Benedict (2013). The authors stated that nicotine adverse reproductive outcomes including spontaneous abortions, still births, and early pregnancy loss which had already been reported in different areas exposed to nicotine.

In this study there were improvement of group III and IV after administration of hCG where few pinopodes were detected around the pits of the glands. The previous findings were supported by Adams *et al*. (2002) who recorded that pinopode expression appeared to advance or retard depending on hormonal treatment. They added that detection of pinopodes might be of high chemical utility in preparation of the endometrium before embryo transfer. The expected positive effect of hCG on endometrium was suggested by Nishigai *et al*. (2002) who stated that the improvement was due to hCG direct action on corpus lutea leading to increasing progesterone secretion, which change intrauterine environment. HCG was stimulated the proliferation of the uterine cells and secretions to enhance the uterine receptivity (Kane *et al*., 2009). Many studies also supported that hCG treatment increased implantation and pregnancy rates (Drakakis *et al*., 2009 and Jarvela *et al*., 2010).

CONCLUSION

It was concluded that hCG could protect the uterus against nicotine induced endometrial changes. Nicotine was a uterine toxicant and major threat to female reproductive health. Therefore, hCG could be beneficial in cases of subfertility and infertility especially among smoker females.

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مخاطر النيكوتين على رحم الجرذ ألابيض والتاثير الوقائى المحتمل لهرمون الكريونيك جونادوتروفين البشرى (HCG)

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ملخص البحث

المقدمة: التدخين هو أحد أهم أسباب زيادة الوفيات والأمراض فى البلدان النامية. يوجد النيكوتين في دخان السجائر وهي واحدة من المواد السامة التي تضر الخصوبة. لذلك، كان الهدف من هذا العمل هو دراسة التغيرات النسيجية الناجمة عن النيكوتين ترترات الهيدروجين على بطانة الرحم فى الجرذ الابيض والتأثير الوقائى المحتمل لهرمون الكريونيك جونادوتروفين البشرى (hCG)

الطرق والوسائل: تم استخدام 40 أنثى من الجرذان البيضاء البالغة ولقد قسمت هذه الحيوانات الى اربع مجموعات متساوية:**المجموعة الأولى:** (المجموعة الضابطة) و **المجموعة الثانية:** (المجموعة المعالجة بالنيكوتين) وتم حقن حيوانات هذه المجموعة تحت الجلد ب 8مجم من النيكوتين ترترات الهيدروجين لكل جرذ ثلاث مرات فى الأسبوع لمدة 4 اسابيع

و**المجموعة الثالثة:** (النيكوتين و هرمون الكريونيك جونادوتروفين البشرى و تم حقن حيوانات هذه المجموعة تحت الجلد بهرمون الكوريونيك جونادوتروفين البشرى بجرعة 2 وحدة لكل جرذ يوميا تحت الجلد ولمدة 4 اسابيع وذلك اضافة إلى الجرعة السابقة من النيكوتين و**المجموعة الرابعة:** (مجموعة هرمون الكريونيك جونادوتروفين البشرى حيث تم حقن هذة المجموعة بجرعة 2 وحدة تحت الجلد لكل فأر يوميا ولمدة 4 اسابيع و تم إعداد العينات للفحص بالميكروسكوب الضوئى والميكروسكوب الإلكترونى الماسح وعلاوة على ذلك اجريت مجموعة من القياسات المورفومترية.

النتائج: تسبب النيكوتين فى انخفاض سمك بطانة الرحم و انخفاضا كبيرا في ارتفاع الخلايا الطلائية السطحية لبطانة الرحم و خلل فى شكل النسيج تحت الطلائى لبطانة الرحم مع احتقان فى الاوعية الدموية و ظهور انكماش وضمور فى الغدد الموجودة فى بطانة الرحم بالمقارنة بالمجموعة الضابطة. وكما وجد زيادة ظاهرية فى الياف الكولاجين.وتفاعل ضعيف لل PAS فى بطانة الرحم وزيادة فى دلالات KI67 . و قد أظهر الفحص بالمجهر الإلكترونى الماسح وجود تسطيح للخلايا المبطنة ونقص فى الزوائد المبطنة للرحم مع نقص فى عدد فتحات الغدد. و عند اعطاء هرمون الكوريونيك جونادوتروفين البشرى مع النيكوتين لوحظ وجود تحسن فى أصبحت دورة الإسترس منتظمة وتحسن التركيب لبطانة الرحم مقارنة با لمجموعة المعالجة بالنيكوتين.

الخلاصة: الاشعه المقطعيه و من النتائج السابقة، ثبت أن النيكوتين احدث تغير ضار فى تركيب بطانة الرحم ومع إعطاء هرمون الكوريونيك جونادوتروفين البشرى مع النيكوتين ادى الى تحسن واضح بالرحم.