Histopathological observations about the effect of pure nicotine in different doses on ovaries of albino rats

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Abstract:
The aim of present study was detected the effect of pure nicotine on the histological structure of rat ovaries cortex. In this study was used (24) good healthy adult female rats were divided into three groups each group includes (8) animals: The 1st experimental group was injected (4) mg of pure nicotine subcutaneously (s/c) twice daily. The 2nd experimental group was injected (8) mg of pure nicotine (s/c) twice daily, while the 3rd control group was injected physiological normal saline (0.9 NaCl 2). The experimental period was (8) weeks.

The histopathological observations in the 1st. experimental group was appeared as follow, degenerative changes and disarrangement in the follicular cells & distribution of granulose cells in the follicular fluid. In the 2nd experimental group the histopathological changes was include, failure in development of the primary follicles into growing follicles & spread of granulose cells in the antrum within the follicular fluid, as well as vasculization of the follicular fluid & fibrosis was happened in some growing follicles, also the number of atretic follicles was found in the cortex of the ovary.
جربيات نامية، وانتشار الخلايا الحبيبية للغار في السائل الجريبي، علاوة على تفجي السائل الجريبي و حدوث التليف في بعض الجربيات النامية مع وجود عدد من الجربيات الرائقة في قشرة المبيض.

Introduction:
Many reports in the previous studies pointed out that the smoker women with diseases caused by cigarette smoking including lung cancer & coronary heart disease and the smoking habit adversely affects the pregnant women & lead to still birth & fetal hypoxia [1]. The new born of smoker women had mild growth retardation comparing with non – smokers, the growth impairment might be due to suboptimal nutritional intake of the mother during pregnancy & vasoconstriction of the uterine blood vessels that then decrease the utero placental perfusion [2].

There are several effects of nicotine on ovaries of laboratory animals & women was observed by several investigators [3, 4, 5, 6]. The earlier studies were determined several polycyclic aromatic hydrocarbons containing benzopyrene (Bp) which is an organic toxin found in cigarette & industrial smoke, can initiate ovarian tumors in the mice & cause damage of oocytes at low dose of Bp (20 mg / Kg body weight) [7], while other study was observed the decrease in folliculogenesis of the mice ovaries when when exposed to (100 µg Bp / Kg B.w) [8].

The mutagenic factors in cigarette smoke may be induce of deoxy ribonucleic acid synthesis (DNA) in the oocytes & then affect the growth & development of ovarian follicles & lead to infertility [9]. The chromosomes aberration & sister chromatid changes was indicated in the oral mucosa due to chronic tobacco smoking [10]. The smoker women had anti estrogentic effects associated with cystic ovaries due to cigarette smoking [11, 12].

The purpose of this study is to understand the effect of pure nicotine injection on the rat ovaries & investigate the histo-pathological changes of the ovarian follicles in albino rats.

Materials & methods:
Twenty four females of albino rats were used in this study, the average weight of them was 210 grams & the age of experimental rats was reached to three months old. The experimental animals were randomly divided into three groups, each one including eight female rats. All groups of experiment were given ration & drinking water ad libtum. The experiment were conducted at 20 – 25 C with light period 12 hours followed by dark period 12 hours daily. The experiment period was extended to eight weeks.

The design of the experiment was as the follow:
- Control group: injected with normal saline (0.9 NaCl 2)
subcutaneously (S/C) twice daily in morning & afternoon.

- The experiment group 1: injected with 4 mg of pure nicotine S/C twice daily.
- The experiment group 2: inject with 8 mg of pure nicotine S/C twice daily.

At the end of experiment female rats were dissected & the ovaries were removed from their bodies, then fixed in 10% formalin for 48 hours. The routine histological processing was carried out on the ovaries specimens, the histological blocks were cutting by micrometer in 5-6 thickness. then the histological sections were stained with hematoxylin & eosin stain[13].

Result & discussion:

The present study was showed the normal histological structure of the rat ovary in the control group which is covered with simple squamous epithelium & consist of cortex & medulla. The cortex is composed of different stages of ovarian follicles (primary, secondary & Graffian follicles) & surrounded by stromal cells which is represent the type of fibroblasts (figure 1).

The histopathological observations in the rats ovaries of the 1st. experimental group (treated with 4 mg pure nicotine) was revealed failure of the folliculogenesis & desquamation or sloughing of the epithelium that covering the rat ovary, disarrangement of the cellular elements of the theca interna, thickening & irregular pattern of the connective tissue in the theca externa & from other hand spreading of granulosa cells in the follicular fluid of the growing ovarian follicles (figure 2).

These histopathological finding was identical to previous observations were reported by [3, 14], they mentioned the the distribution of the follicular cells in the follicular fluid & reduce the number of developing ovarian follicles due to folliculogenesis failure when the rat & mice was exposed to nicotine & cigarette smoke. Our suggestions may be due to the effect of pure nicotine on the blood vessels of the rat ovaries & the histopathological alterations could be interpreted due to vasoconstriction of the ovaries & ovarian follicles & the toxic effect of pure nicotine was cause the decrease in number of corpora lutei & atretic follicles.

The histopathological alterations in the rat ovaries of 2nd. experimental group (treated with 8 mg of pure nicotine) were constricted in the superficial layer of the ovarian cortex which included the failure of development of the primordial & primary ovarian follicles, distribution of follicular cells in the antrum of growing follicles, vacuolization that appeared with follicular fluid & atretic follicles were found in the ovarian cortex, from other hand degenerative changes & fibrosis.
which occurred in some primary & growing follicles (figure 3).

The same observations was reported by [15], he was detected reducing in the ovulatory efficiency due to administration of the nicotine to female rats & it effect on the germinal epithelium of the ovaries causing damage to the follicles & lead to infertility. While workers [16, 17] were reported the lengthen of follicular phase & need to gonadotrophin requirement for ovarian hyper stimulation, from other hand there is decreasing in the serum peak & follicular fluid estradiol (E2).

The present study results were due to effect of pure nicotine in different doses on hormonal disturbance specially sex hormones by effect of pure nicotine on pituitary gland cause inhibition of the gonadotropins releasing. These suggestion were corresponding with previous study was conducted on healthy menopause women due to cigarette smoking which exposed to hormonal imbalance & cause infertility [18].

Figure 1: Normal histological structure of cortex in rat ovary, showed different developmental stages of ovarian follicles & corpus lutei.
Hematoxylin & eosin, 450 x.
Figure 2: Ovarian cortex of rats in 1st. experimental group (treated with 4 mg of pure nicotine): show the histopathological changes including sloughing of epithelial lining ovary, failure in development of ovarian follicles, elongation in some growing follicles & distribution of the granulosa cells in the follicular fluid. 
Hematoxylin & eosin, 450 x.

Figure 3: The histopathological section of the rat ovarian cortex in the second experimental group (treated with 8 mg of pure nicotine), show the following changes: the most primary follicles not develop into growing follicles, some growing follicles revealed vacuolization in follicular fluid & spreading of follicular cells in the antrum, irregularity of cellular elements in the theca interna & theca externa beside to fibrosis of several growing follicles.
Hematoxylin & eosin, 450 x.
References:


