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CROCIN EFFECTS ON THE NICOTINE-INDUCED OVARY INJURIES IN FEMALE RAT

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ABSTRACT

Nicotine is an alkaloid found in tobacco plants. Nicotine decreased estrogen, reduced uterine weight and reduced thickness of the uterus. The Saffron has a special position at people's eating pattern. Suggesting that saffron may be useful for the treatment of psychological dependence to opioids in humans. Therefore, due to the effects of nicotine on ovary tissue and regard to Crocin properties the aim of this study is to determine the effects of crocin on nicotine induced damage on the female rat ovaries. In this study, 56 wistar immature female rats weighing 30-27 g were used. The mice were randomly assigned to 8 groups. For each ovary, the total follicle was measured after the injection in each groups. Histological, hormonal measurement were done and nitric oxide measurement was performed.The effective dose of nicotine (1 mg/kg) caused a significant decrease in the ovary weight. The stained tissue sections showed that nicotine causes ovarian follicle atresia and administration of Crocin decreased the percentage of atretic follicles. In the Crocin (25,50, 100mg/kg) groups have a significant increase in estrogen, LH and FSH levels in the rat blood serum. By increasing the dose in crocin groups significantly decrease the levels of nitric oxide in blood serum.It seems that nicotine reduces the gonadotropin releasing hormones in hypophysis by exerting its effects on hypothalamus and decreases the growth and development of secondary follicles by reducing the LH and FSH hormones, as a result. Moreover antioxidant properties of Crocin can partly prevent these dysfunctions

Keywords: **nicotine, crocin, ovary, rat**

INTRODUCTION

Nicotine is an alkaloid found in tobacco plants. It is the primary psychoactive chemical in tobacco and therefore cigarettes are very addictive[1]. There are more than 4000 chemicals in tobacco smoke, of which at least 250 are known to be harmful and more than 50 are known to cause cancer [2].The tobacco epidemic is one of the biggest public health threats the world has ever faced, killing around six million people a year [3]. The rise in drug abuse, including smoking during the past few decades is not only have detrimental effects on the vital organs such as the heart and brain, but also it has negative effects on the reproductive system and glands [4]. Nicotine decreased estrogen, reduced uterine weight and reduced thickness of the uterus. The in vitro studies on human ovarian cells have shown that nicotine is factor to induce the disorder in luteal phase by inhibit the release of progesterone. While nicotine has an inhibitory effect on the production of androgens by internal cells in the ovaries, In addition, a significant reduction in the weight of the ovaries and testes will develop [5]. One of the major problems of modern medicine, despite its apparent advantages compared with traditional

medicine, is unfortunately the increasing use of synthetic drugs. Although synthetic drugs in the treatment of certain diseases are useful, but long-term use cause side effects that may be more dangerous than the disease itself. Therefore, more attention to the use of medicinal plants is expected [6]. Given the unwanted side effects of some chemical drugs, must be pay more attention to the possible effects of medicinal plants on the function of different parts of the body [7]. The Saffron has a special position at people's eating pattern. Saffron is a spice derived from the flower of Crocus sativus. Saffron contains more than 150 perfume compounds [8]. However saffron's golden yellow-orange color is primarily due to crocin. Crocin ($C_{44}H_{64}O_{24}$) is effective compound on different cell types and has anti-cancer effect on a range of tumors. Saffron, crocin and crocetin have the liquidator effect of free radicals and antioxidants [9]. Suggesting that saffron may be useful for the treatment of psychological dependence to opioids in humans [10]. Alcoholic extract of saffron and crocin in the prevention of ischemia-induced oxidative damage in rats is useful to re-establish blood [11]. Therefore, due to the effects of nicotine on ovary tissue and regard to Crocin properties the aim of this study is to determine the effects of crocin on nicotine induced damage on the female rat ovaries.

MATERIALS & METHODS

Animals

In this study, 56 wistar immature female rats weighing 30-27 g were purchased from Tehran Razi Institute. This experimental study was conducted under approval of Ethics Committee of Kermanshah University of Medical Sciences. 7 mice were placed in each cage and were kept under light/dark cycle and free access to water and food [12].

Chemicals

Crocin (digentiobiosyl 8, 8'-diapocarotene-8, 8'-oate; $C_{44}H_{64}O_{24}$) powder was purchased (Merk-Germany). The powder was diluted by normal saline (0.9%) to prepare different doses. Also, the Nicotine solution (1-Metyl-2-3-pyridel-Pirolidin; $C_{10}H_{14}N_2$) was purchased from (Merk-Germany) and was diluted by normal saline (0.9%) for administration [13].

Study design

The effects of nicotine on ovarian tissue damaging dose of 1 mg/kg [14] which injected interaperitoneally for 4 weeks. The mice were randomly assigned to 8 groups. The group was administered saline (1 ml/kg). The group was administered nicotine (1 mg/kg). The groups were administered Crocin (25, 50, 100 mg/kg). The groups were administered Crocin (25, 50, 100 mg/kg) plus nicotine (1 mg/kg) interaperitoneally (IP). Injection time for all groups was around 8am, once a day for 28 days [15].

Histological analysis

After the injection in each groups, the rats were anesthetized with chloroform, killed and ovarian tissue is removed. Ovaries weight were measured by scales. Briefly ovarian tissue to be prepared according to routine laboratory H & E staining. These steps including 10% formalin fixation, dehydration, paraffin permeability, molding, thin sections (5 μm) were cut using a microtome, and stained with hematoxylin and eosin (H&E). More than 20 sections were prepared from each block. The sections numbered 5, 10, 15, and 20 were selected and photographed separately from three random scopes. The preparation was examined with 3.34-million pixel resolution camera and Olysia Bio software [16].

Morphometric measurements

For each ovary, the total follicle was measured. The outline of each follicle was measured after taking an image with a 40X objective. The longest and shortest axis were measured in the drawing of each follicles in order to estimate the mean diameter (mean axis). At least 10 follicles from each zone (total 50) were measured in each ovary [17].

Hormones Measurement

After the rats were anesthetized by chloroform, blood samples collected from heart and blood samples were incubated at 37 ° C for 15 minutes to clot. Then the blood was centrifuged for 15 minutes at 3000 rpm to separate serum. Separated serum was stored at -20 ° C until hormones measurement. Serum levels of LH, FSH and estrogen levels using Enzyme- linked Immunosorbant Assay was assessed using STAT FAX machine[18].

Nitric oxide measurement

Nitric oxide was measured based on Griess colorimetric assay. Accordingly, NEED, sulfonamide solutions and Nitrite standards were prepared. After de-freezing the serum samples 100 µl of the sample serum was deproteinized by zinc sulfate and transferred to the wells so measure Nitrite concentration in serum. 100 µl chloride vanadium, 50 µl sulfonamide, and 50 µl NEED solutions were added afterwards. The samples were

incubated darkness at 30 °C . The optical density (OD) of samples at the wavelength of 540 nm was measured by ELISA reader (Hyperion, Germany) [19].

STATISTICAL ANALYSIS

All the quantitative data were presented as mean ± standard deviation. Due to determine the statistical significance between different groups using SPSS software package 16.0. One-way analysis of variance (ANOVA) followed by LSD post-hoc test were done. P<0.05 was considered significant.

RESULTS

Changes in rat ovarian weight

The effective dose of nicotine (1 mg/kg) caused a significant decrease in the ovary weight compared to saline group (p<0.05). By increasing the dose, Crocin increased the ovary weight in comparison with nicotine groups (p<0.05). Further, in crocin plus nicotine (100 mg/kg) has a significant increase the ovary weight compared with nicotine group (Fig.1).

Morphometric measurements

The results of the primordial and primary follicles diameter in the experimental group showed a significant decrease in primordial follicles diameter at the nicotine group compared with the saline group. Also follicles diameter increase in the Crocin (25, 50 and 100 mg/kg) groups and the nicotine plus Crocin (100 mg/kg) group compared with the nicotine group but this difference was not significant(Fig.2). The results of the secondary and antral follicles diameter show a significant decrease in secondary follicles diameter at the nicotine group compared with the saline group. By increasing the dose, Crocin significantly increased the secondary and antral follicles diameter in comparison with nicotine groups (p<0.05). Further, in Crocin plus nicotine (100 mg/kg) group have increasing secondary and antral follicles diameter compare with nicotine group but this difference was not significant(Fig.3).

Histological analysis

The stained tissue sections showed that nicotine causes ovarian follicle atresia and Crosin could not prevente toxic properties of nicotine (Fig.4).

Nitric oxide measurement

The results of measurements of the levels of nitric oxide in blood serum showed a significant increase in nicotine group compared with saline group. By increasing the dose in crocin (25, 50 and 100 mg/kg) groups significantly decrease the levels of nitric oxide in blood serum comparison with nicotine groups (p<0.05). Further, in crocin plus nicotine (50,100 mg/kg) groups have decrease of the levels of nitric oxide in blood serum compare with nicotine group (Fig.5).

Hormones Measurement

The results of the study showed that estrogen, LH and FSH levels decreased significantly in nicotine group compared with the saline group. In the Crocin (25 100, 50 mg/kg) groups have a significant increase in estrogen, LH and FSH levels in the rat blood serum. Further, in Crocin plus nicotine (100 mg/kg) group have increasing of the levels of estrogen and FSH in blood serum compare with nicotine group but this difference was not significant (Fig.6).

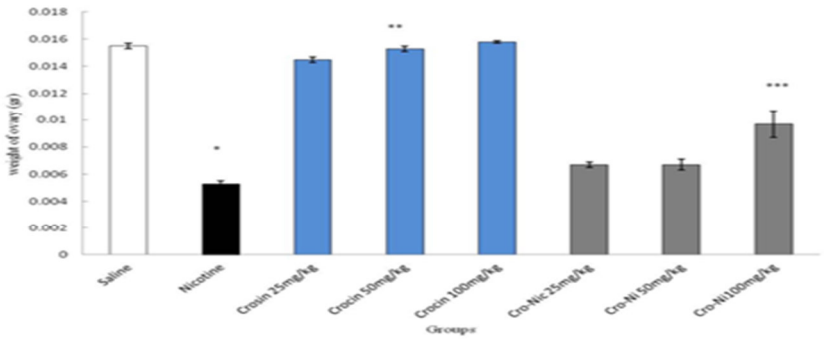

Figure 1

Analysis of the effect of nicotine, Crocin and Crocin plus nicotine on ovary weight. * Significant decrease of ovary weight in nicotine group compared to saline group (p<0.05). ** Significant increase in Crocin (25, 50 and 100 mg/kg) groups compared to nicotine group (P<0.05). *** Significant increase in Crocin plus nicotine (100 mg/kg) compared to nicotine group.

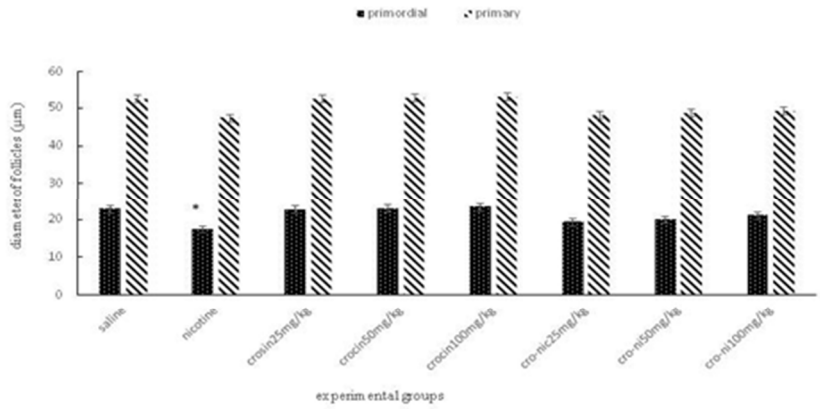

Figure2

Analysis of the effect of nicotine, Crocin and Crocin-nicotine on primary and primordial follicles diameter. * Significant decrease of primordial follicles in nicotine group compared to saline group (p<0.05).

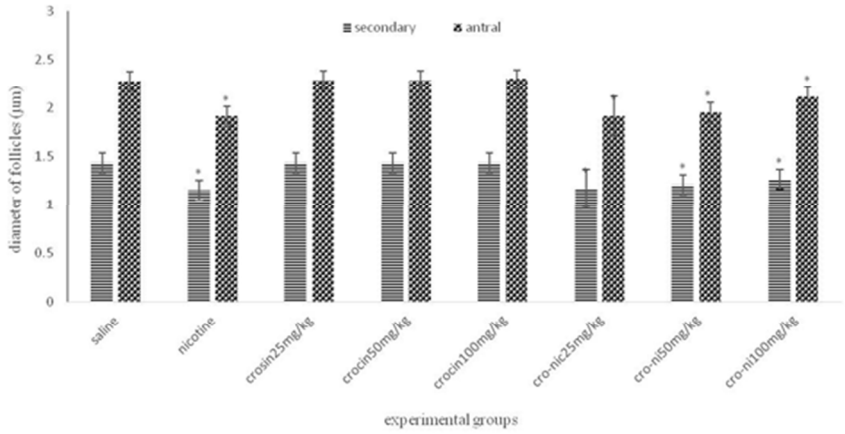

Figure3

Analysis of the effect of nicotine, Crocin and Crocin plus nicotine on secondary and antral follicles diameter. *Significant decrease of secondary and antral follicles in nicotine group compared to saline group (p<0.05). ** By increasing the dose, Crocin significantly increased the secondary and antral follicles diameter in comparison with nicotine groups (p<0.05).

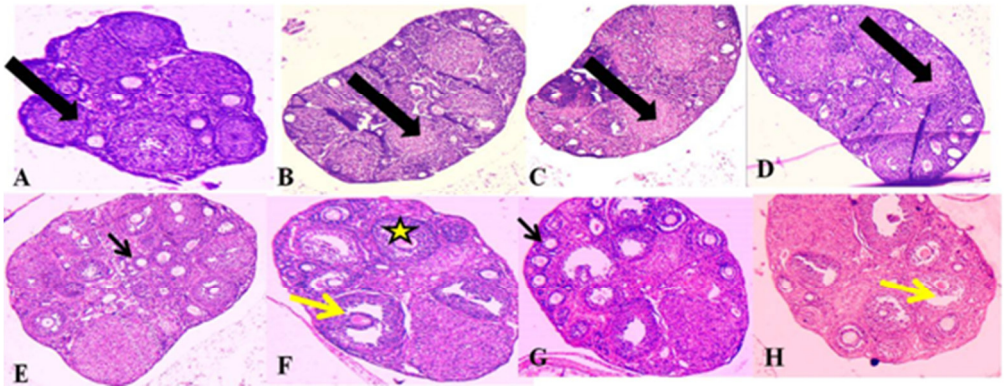

Figure4

The histological images of rat ovary. A: nicotine group. B: nicotine plus Crocin 25 mg/kg C: nicotine plus Crocin 50 mg/kg. D: nicotine plus Crocin 100 mg/kg. E:control group. F: Crocin 25 mg/kg. G: Crocin 50 mg/kg. H: Crocin 100 mg/kg. The thick black arrow indicates atretic follicles. The thin black arrow indicates the primary follicle, the yellow arrow is graph follicle and star represents secondary follicle. (Magnification 100 ×) (Stained H & E).

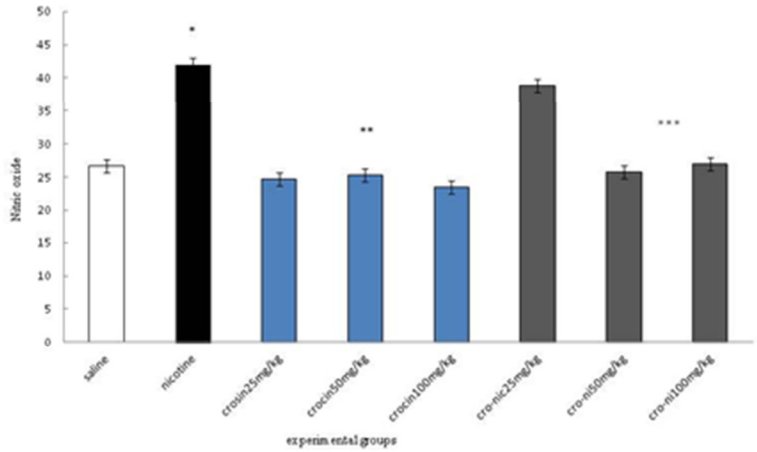

Figure5

The effect of different concentrations of Crocin and nicotine on nitric oxide levels of nitric oxide in blood serum in study groups. * Significant increasing of serum nitric oxide in nicotine group compared to saline group (p<0.05). ** Significant decreasing of serum nitric oxide in Crocin (25, 50 and 100 mg/kg) groups compared to nicotine group (p<0.05). *** Significant decreasing of serum nitric oxide in Crocin plus nicotine (50,100 mg/kg) groups compared to nicotine group (p<0.05).

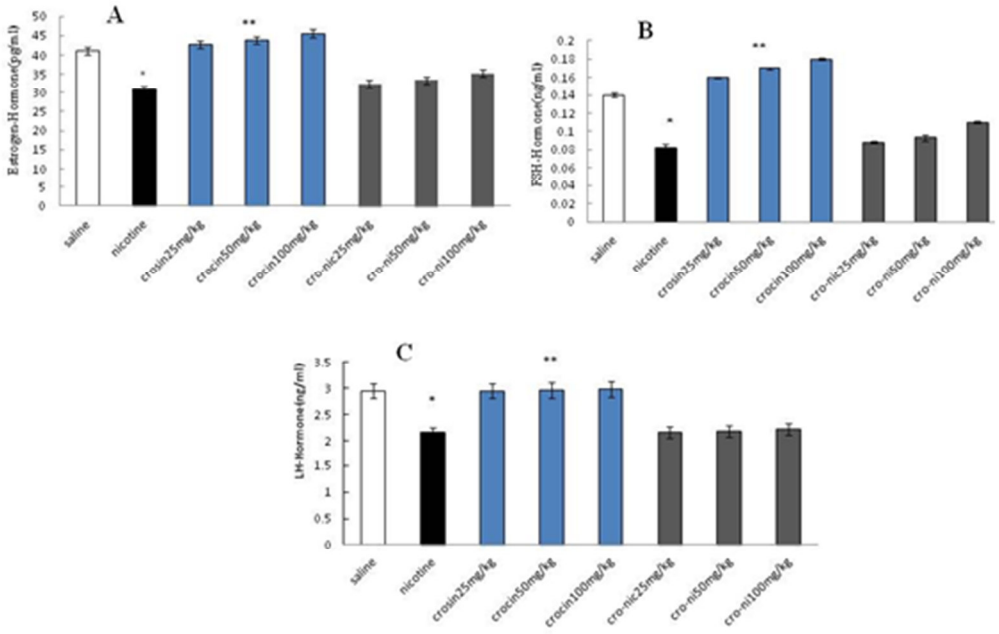

Figure6

The effect of different concentrations of Crocin and nicotine on estrogen, FSH & LH hormone levels in blood serum in study groups. * Significant decrease of estrogen and FSH & LH hormone levels in nicotine group compared to saline group (p<0.05). ** Significant increase of estrogen and FSH & LH hormone levels in Crocin (25 100, 50 mg/kg) groups compared to nicotine group (p<0.05).

DISCUSSION

There has been increasing concerns about the incidence of reproductive abnormalities among the human population in recent years. Exposure to external factors before and after pregnancy and during the early stages after birth can endanger the reproductive ability and health of the neonates [20-21].

Medicinal plants are highly significant and valuable in providing the healthcare of the communities through treatment and prevention of diseases [22]. In the present study, the effects of Crocin on the histological disorders of ovary due to nicotine administration in female rats were investigated. Analyzing the size of follicles can yield important information about the performance of ovary, especially the relationship between folliculogenesis

and its regulatory factors [23]. The findings obtained in this study indicated the reduced diameter of primordial, primary, secondary and antral follicles in the group receiving nicotine. High-dose administration of nicotine (100 mg/kg) caused a reduction in the toxic effects of nicotine to decrease the diameter of ovarian follicles. It seems that nicotine reduces the gonadotropin releasing hormones in hypophysis by exerting its effects on hypothalamus and decreases the growth and development of secondary follicles by reducing the LH and FSH hormones, converting them to mature follicles, as a result [24]. Moreover, Crocin can enhance folliculogenesis via affecting gonadotropins [25]. The results of present study were in line with the findings of some studies that showed nicotine and morphine can cause a reduction in the growth and development of ovarian follicles and death of granulosa cells [26-27]. The findings of the current research revealed that administration of Crocin decreased the percentage of atretic follicles. Reactive oxygen species (ROS) seem to have reduced some reproductive physiologic functions such as oocyte maturation, ovarian steroidogenesis, ovulation, implantation, blastocyst formation and corpus luteum function, and antioxidant properties of Crocin can partly prevent these dysfunctions [28-29]. In addition, the results demonstrated that nicotine administration decreased the weight of ovary and high-dose Crocin (100 mg/kg) could neutralize the effects of nicotine on weight reduction. It seems that nicotine can reduce the diameter of ovarian follicles by reducing the hormones, thereby decreasing the ovarian weight [30]. On the other hand, Crocin can increase ovarian weight by increasing the diffusion of tissue oxygen and enhancing the number of follicles [31]. Nicotine exerts its effect on estrogen production through reducing the ovarian weight, and irregular ovarian cycle causes the progression of all these dysfunctions to disturb and decrease the egg production and to reduce reproduction consequently [30]. Our results showed that nicotine significantly increased serum nitric oxide level in the group receiving nicotine, which was reduced by Crocin administration. Nicotine can induce oxidative stress invivo and invitro and consequently increase serum nitric oxide [32]. On the other hand, Crocin, with its antioxidant properties [33], neutralizes the effects of nicotine to raise nitric oxide level. The results of the present study were in agreement with the findings of the study carried out by Salahshoor et al in which curcumin, as an antioxidant, reduced serum nitric oxide [34]. Furthermore, the results of this study showed that nicotine caused a reduction in estrogen, FSH and LH hormones in blood. Nicotine seems to have reduced 17-beta estradiol and progesterone level, which have anti-estrogenic effects [35]. Also, nicotine and other opioids can decrease the gonadotropin releasing hormones (FSH and LH) by affecting hypothalamus [36]. The study conducted by Kasson et al indicated that nicotine can decrease FSH/LH synthesis, which confirms the results of current study [37]. Apparently, Crocin releases gonadotropin hormones through stimulation of noradrenergic and estrogenic neurons and locus ceruleus. These neurons play a role in increasing FSH receptors in the primary follicles as well as estradiol synthesis [38]. The findings of the current research, however, were in contrast with the results of Gocze et al that showed nicotine caused a gradual increase of estradiol in granulosa cells [39]. On the other hand, the findings of the present study were compatible with the results of the study performed by Barbieri et al in which they reported the reduced activity of aromatase in the cells treated with nicotine [40].

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