



Role of *Falcaria vulgaris* on Nicotine Toxicity in Rat Hippocampus

Shiva Roshankhah^a, Babak Arji Rodsari^a, Cyrus Jalili^b, Mohammad Reza Salahshoor^{a*}

^aDepartment of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran, ^bMedical Biology Research Center, Department of Anatomical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Abstract

Falcaria vulgaris (*F. vulgaris*) is a vegetable that contains antioxidant ingredients. Nicotine is a major risk factor in the development of functional disorder of several organ systems. In this study, 64 male rats were randomly assigned to 8 groups: Sham (saline) group, nicotine group (0.5 mg/kg), *F. vulgaris* groups (50, 100, and 150 mg/kg), and nicotine + *F. vulgaris* groups (10, 30, and 60 mg/kg). Treatments were administered intraperitoneally daily for 28 days. FRAP method was applied to determine the total antioxidant capacity. The number of dendritic spines was investigated by Golgi staining technique. Cresyl violet staining method was used to determine the number of neurons in hippocampal region CA1. Also, Griess technique was used to determine serum nitrite oxide level. Nicotine administration increased significantly nitrite oxide level and total antioxidant capacity and a decreased number of neuronal dendritic spines and neurons compared to the sham group ($P < 0.05$). In the *F. vulgaris* and nicotine + *F. vulgaris* groups, in all dosages, the number of neurons and neuronal dendritic spines increased significantly while nitrite oxide level and total antioxidant capacity decreased compared to the nicotine group ($p < 0.05$). It seems that *F. vulgaris* administration improves hippocampal region CA1 injury in rates because of nicotine.

Keywords: *F. vulgaris*, Hippocampus, Nicotine.

Corresponding Authors: Mohammad Reza Salahshoor,
Department of Anatomical Sciences, Medical School,
Kermanshah University of Medical Sciences,
Kermanshah, Iran.
Tel: (+91) 9052983544
Email: bhu_rabi@rediffmail.com
Cite this article as: Roshankhah Sh, Arji Rodsari B,
Jalili C, Salahshoor M, Role of *Falcaria vulgaris* on
Nicotine Toxicity in Rat Hippocampus, 2020, 16 (3):
33-44.

1. Introduction

The *F. vulgaris* is a vegetable belonging to the Apiaceae (*Umbelliferae*) family that grows in the west and southwest of Iran as an annual wild plant [1]. *F. vulgaris* has anti-oxidant properties [2]. This plant has a stem and its height is 30 cm on average. In the west of Iran, this plant has been traditionally used to treat skin ulcers, gastric disorders, including gastric

ulcer, liver disease, kidney stones, and bladder [3]. Phytochemical studies on this medicinal plant have shown the presence of *Tannins* and *Saponin* [4, 9]. This plant contains vitamin C, phytosterol, protein, and starchy materials and is a rich source of tannins and ascorbic acid. Similar to antibiotics, it can be used for the treatment of skin ulcers [5].

Tobacco consumption has declined dramatically in industrialized countries over the past three decades due to raising awareness of the health risks and effective control policies, while it has increased in developing countries over the same period [6]. The overall nicotine content of tobacco and percentage nicotine be an average of 10.2 mg [7]. Nicotine spreads rapidly in brain tissue through pulmonary circulation within 10-20 seconds and attaches to the nicotinic acetylcholine receptors (nAChRs) [8]. Nicotine rapidly passes through the blood-brain barrier and stimulates the mesolimbic dopamine system. This substance can regulate brain neurotransmitters, including catecholamine and serotonin A [9].

However, nicotine can induce oxidative stress in some organs including the brain. Pathologic changes associated with neuronal apoptosis have been reported due to the use of nicotine [10]. Also, nicotine can induce increased oxidative stress and neuronal apoptosis, destroy DNA, produce reactive oxygen species, and increase the production of lipid peroxidation [11, 12]. This compound seems to activate the areas of the brain that play an important role in drug addiction and learning process.

Among the brain areas greatly affected by nicotine is mesocorticolimbic region that contains nucleus accumbens, ventral tegmental area, the hippocampus, and the amygdala. In this region, the amygdala and hippocampus have structures that play an important role in the formation of long-term memory, and their function is associated with stimulation reward system [13].

Hippocampus is a part of the limbic system and seems to be essential in the formation of different types of learning and memory [14]. The CA1 is a part of the hippocampus, and its role is creating long-term memory [15]. Considering the effects of nicotine toxicity on the brain and the therapeutic properties of *F. vulgaris*, the present study was designed and conducted to investigate the effects of *F. vulgaris* on nicotine-induced toxicity in the hippocampus CA1 region of male rats.

2. Materials and Methods

2.1. Animals

In this experimental study, 48 male *Wistar* rats (weighing 220-250 g) were purchased from the Pasteur Institute and transferred to the animal house in medical school. During the study, the animals were kept under standard conditions (i.e., 12 h light/12 h dark and $22 \pm 2^\circ\text{C}$), humidity of 50%–60%, in special cages and on a straw bed. Water and food were freely available to the animals. Standard food was including plate and treated municipal water was used to feed the animals. All investigations conformed to the ethical and human principles of research and were

approved by the Ethics Committee (ethics certificate No. 97501) [16].

2.2. Extract Preparation

F. vulgaris plant was obtained from a local store (time to pick and buy this plant in the spring) and its impurities were removed. After endorsement by a botanist, the plant was cleaned. The leaves and stems were desiccated in shadow for 5 days and ground using a grinder. Next, 100 g of the powder was added to 70% ethanol. The acquired solution was reserved in a warm water bath (36°C) under dark condition. Thereafter, the solution was progressively poured on Buchner funnel filter paper and cleaned by a vacuum pump. It was then transferred to a rotary device to obtain the extra solvent. The isolation process continued until the concentrated extract was obtained. The extract was dissolved in distilled water and administered intraperitoneally per a kilogram of animal's weight. It was sterilized after double filtration through a 0.2- μ m filter [2].

2.3. Study Groups and Treatment of Animals

Vial of nicotine (Sigma, USA) with a dose of 0.5 mg/kg of body weight was dissolved in 0.9% normal saline solution. A total of 64 male rats were randomly divided into 8 groups and 8 rats were placed in each group. The first group was sham group, which received normal saline through intraperitoneal injection equivalent to the amount of experimental groups. In the second nicotine group, each animal received 0.5 mg/kg single dose course of nicotine, intraperitoneally and the solvent of

nicotine was normal saline [10]. The third to fifth groups included the *F. vulgaris* groups, in which each animal respectively received 50, 100, and 150 mg/kg of *F. vulgaris* intraperitoneally for 28 days at 10 a.m. [1, 2, 4]. The sixth to eighth groups include nicotine + *F. vulgaris* groups, in which each animal received a single dose of 0.5 mg/kg nicotine in order to induce damage and then respectively received 50, 100, and 150 mg/kg of *F. vulgaris* intraperitoneally for 28 day at 10 a.m.

2.4. Transcardiac Perfusion

The transcardiac method was used for fixation. In this process, 24 h after the last injection of the drug, animals were anesthetized with *Ketamine* (100 mg/kg) and *Xylazine* (10 mg/kg). The chest was opened in the midline and the apex of the left ventricle was pierced after the completion of thoracotomy. Next, a glass cannula of 1 mm diameter was inserted and fixed in the ascending aorta. The pericardium and the right ventricle were cut. The left ventricle pathway was cut and the ascending aorta was connected to a plastic tube by the glass cannula and descending aorta was clamped right above the diaphragm. The cannula linked to the normal saline solution was implanted into the aorta through making an incision in the left ventricle. The descending aorta was fastened and after washing the brain, the solution was removed through the incision made in the right atrium. Formalin 5% and buffer phosphate 7% were inoculated into the brain by the cannula and the brain were fixed in 15 min. After perfusion, the brains were separated from the

skull and stored in the same perfusion solution for 3 days [14].

2.5. Golgi Methods

The Golgi method was used to observe neuron dendrites in the hippocampus CA1 region. This method was applied using potassium dichromate followed by silver nitrate. After brain fixation, tissue blocks were put inside 3% potassium dichromate solution for 48 h in a dark environment. The blocks were washed in 0.75% silver nitrate solution and were put inside the solution for 72 h. The tissues were washed in 1% silver nitrate solution. Then, tissue processing, counting dehydration, clearing, and embedding were performed. Microscopic sections (5 μ m) were prepared and examined morphologically [15].

2.6. Cresyl Violet Method

The Cresyl violet staining method was used to determine the number of live cells in the hippocampus CA1 region. For this purpose, eight rat heads from each group (8 groups) and 5 slides from each rat were taken to be stained. Subsequently, after producing 5 μ m cuts by microtome and performing tissue processing, the left hemispheres were stained using Cresyl violet staining technique. In brief, the slips were stable again (10 min) in 4% paraformaldehyde solution. Slide was immersed in 70% (5 min) and 100% (15 min) ethanol and in xylene for 20 min. They were then immersed back through the ethanol

descent concentrations. They were stained for 5 min in filtered Cresyl violet solution, and then washed in distilled water. The slides were then dehydrated again in ethanol. They were located in xylene for another 10 min and then cover slipped. After preparing the photo, the number of cells was counted in one square millimeter (mm^2). In the slides stained by means of Cresyl violet technique, the round cells without peak nose were considered as live cells [14].

2.7. Dendritic Thorns

The dendritic thorn count was made via microscopic examination with an optical microscope and Motic software and Image tool IT (version 3) software. In the slides stained through Golgi staining technique, neurons entirely stained with cell bodies in the central part of the tissue slices distant from the surrounding stained neurons were included. The dendritic tree of pyramidal neurons was demonstrated through camera lucida at 750 \times magnification and the dendritic exclusion order from the cell body was used for counting the dendritic pieces [14].

2.8. Griess Technique

Nitrite oxide, measurement by Griess assay using microplate technique. Through this process, zinc sulfate powder (6 mg) was mixed with serum samples (400 μ l), and vortexed for 1 min. The samples were centrifuged at 4 $^{\circ}$ C for 10 min at 12,000 rpm and supernatant was used to measure the nitrite oxide. Briefly, 50

μl of sample was added to 100 μl of Griess reagent (Sigma; USA) and the reaction mixture was incubated for about 30 min at room temperature. The optical density (OD) of the sample was measured by an ELISA reader (Hyperion; USA) at a wavelength of 540 nm according to manufacturer protocol [16].

2.9. FRAP Method

FRAP method was used in order to the measure total antioxidant capacity of the serum (Abcam, ab234626). Before transcardiac method, venipuncture from the animals' hearts (right ventricle) was done using a 5cc syringe. The blood sample was incubated for 15 minutes at 37° C to clot. Then the clotted blood was then centrifuged for 15 minutes at 3000 rpm until the serum was separated. The separated serum was stored at the temperature of -70° C until antioxidant capacity level was measured. In this technique, the ability of the plasma to reinstate ferric ions was measured. This process required a great quantity of Fe^{III} . A blue stain was formed when the compound of Fe^{III} -TPTZ in acidic pH returned to Fe^{II} and absorption at the maximum wavelength of 593 nm. The only factor defining the speed of the Fe^{II} -TPTZ and the blue color was the vitalizing power of the sample. Total antioxidant capacity values were strategized by means of the standard curve with diverse concentrations of iron sulfate [17].

2.10. Statistical Analysis

After extracting the information, Kolmogorov-Smirnov test was first conducted to confirm the data compliance of the normal distribution. The One-way analysis of variance (one-way ANOVA) was used for statistical analysis and Tukey post hoc test was used to determine the difference between the groups. SPSS 16 was used for data analysis. The obtained results were expressed as mean \pm standard error and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

Temporal lobe and hippocampal organization are involved in transferring short-term memory to long-term memory [18]. Nicotine produces many implications and side effects by influencing the central and peripheral nervous system [7, 10]. The present study was aimed to investigate the effects of *F. vulgaris* on nicotine-induced disorders in the hippocampus CA1 region. The results of a number of neurons in the hippocampal region CA1 showed a significant decrease in nicotine group compared to the sham group ($p < 0.05$). The mean number of neurons was not significant in all *F. vulgaris* groups compared to the sham group ($P > 0.05$). Also, the mean of pyramidal neurons increases significantly in *F. vulgaris* and nicotine + *F. vulgaris* groups in all doses compared to the nicotine group ($p < 0.05$) (Figure 1 and 2).

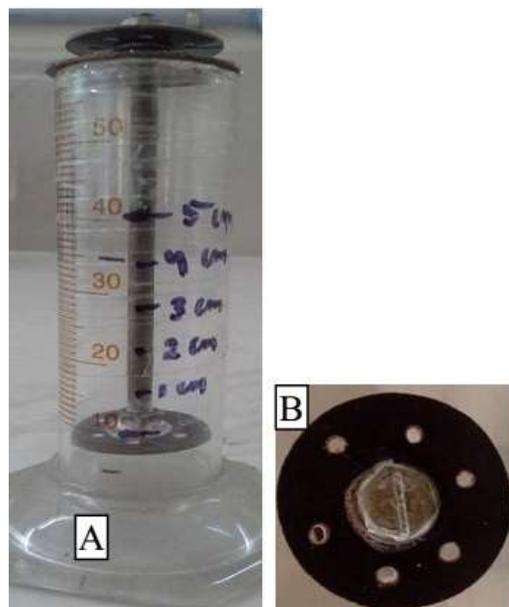


Figure 1. Gel strength measurement device. (A) Measuring cylinder with device inside; (B) Device.

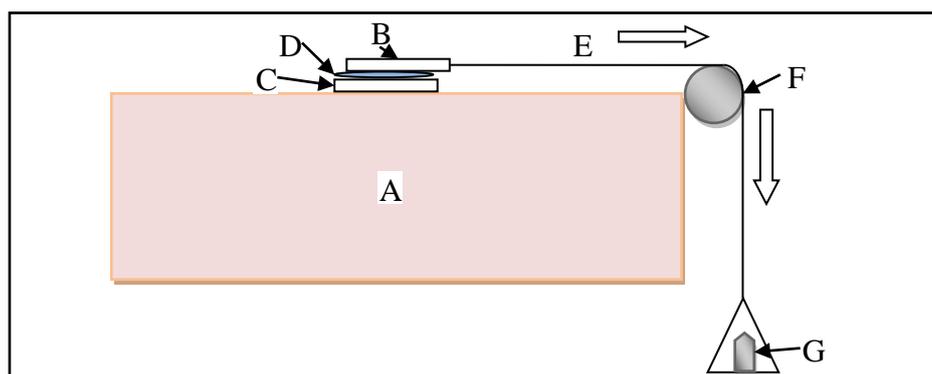


Figure 2. Modified spreadability testing apparatus. (A) Table; (B) Upper glass slide; (C) Lower glass slide; (D) Thin layer of IBP gel; (E) Nylon thread; (F) Glass rod; (G) Weights.

The mean number of neuronal dendritic spines in experimental groups showed a significant decrease between the sham group and nicotine group ($p < 0.05$). Also, the mean number of neuronal dendritic spines was not significant in all *F. vulgaris* groups compared to the sham group ($P > 0.05$). Further, at the *F. vulgaris* and nicotine + *F. vulgaris* groups, the mean number of neuronal dendritic spines increases significantly in all treated groups

compared with the nicotine group ($p < 0.05$) (Figure 3 and 4).

The results may indicate the control of apoptosis and neurodegeneration by administering different doses of *F. vulgaris*. The results of Tewari et al. were consistent with those of the present study that showed nicotine could damage the cells in the hippocampus by increased protein accumulation in the membrane and reduced

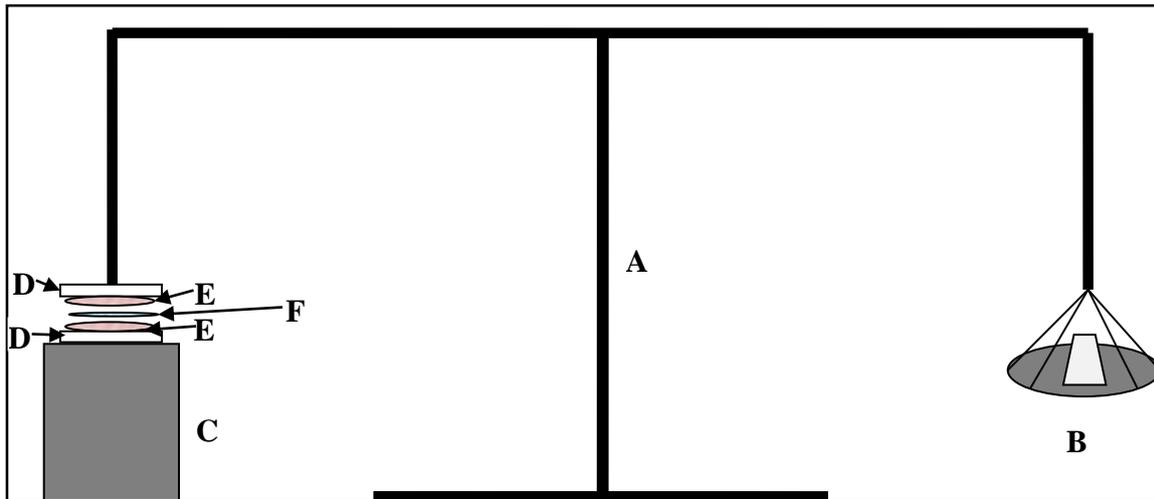


Figure 3. Device used for the measurement of bioadhesive strength. (A) Modified balance; (B) Weights; (C) Platform; (D) Glass plate; (E) Goat skin; (F) IBP gel [32].

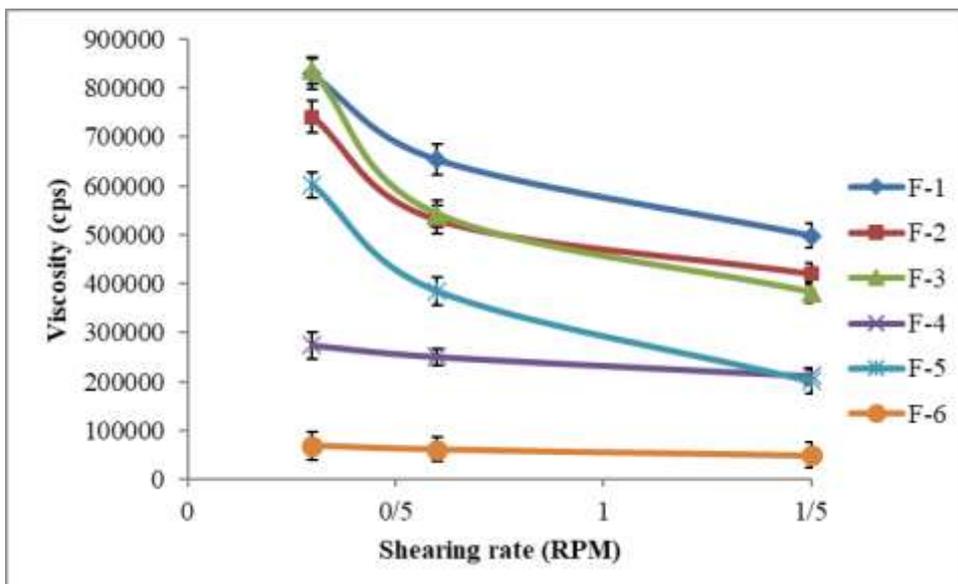


Figure 4. Graph of Viscosity vs Shearing rate in RPM

cell size [19]. It seems that nicotine induces oxidative stress and, consequently, the production of free radicals such as superoxide and hydroxyl radicals, which can cause cell damage [10]. Generated free radicals following oxidative stress may have the potential to damage cellular compositions, including proteins, lipids, and DNA [16]. The

lipid in the membrane of the nerve cells has a high content of oxidized unsaturated fatty acids. Therefore, it seems that nicotine can produce reactive oxygen species via P-450 enzyme and cause the destruction of the nucleus in neurons [20]. Dendritic thorns play a major role in synaptic transmission. In this regard, many brain diseases are associated

with changes in the morphology and density of dendritic thorns [15]. Nicotine can reduce the length and the number of dendritic thorns in nucleus *Accumbens* by affecting the neurotrophic factors in the striatum [21]. A study by Brown et al. showed that nicotine injections could reduce the length and the number of dendritic thorns; that is consistent with the results of our study [22]. It seems that nicotine can destroy dendritic thorns by $\beta 2$ -*nAChRs* deactivation at postsynaptic cells in hippocampus region [23]. Moreover, nicotine can reduce the number of thorns by deactivating $\alpha 4\beta 2$ -*nAChRs* in the pre-synaptic membrane and by disrupting the release of glutamate neurotransmitters [24]. *F. vulgaris* is a purifier of reactive oxygen species and has the potential to destroy oxidative stress and prevent lipid peroxidation [2]. The results of the study by Rafiey et al. confirmed the results of the present study that *F. vulgaris* could prevent cell death and development oxidative

stress due to STZ administration [25]. *F. vulgaris* seems to inhibit lipid peroxidation of *quinolinic acid* and control the production of cyanide-induced superoxide [4]. *F. vulgaris* extract can increase the effects of antioxidant enzymes such as catalase and superoxide dismutase and reduce ROS production [16].

The results of blood serum NO measurement showed a significant increase in nicotine group compared to the sham group ($p < 0.05$). The mean nitrite oxide in the blood serum was not significant in all *F. vulgaris* groups compared to the sham group ($P > 0.05$). Also, the mean of nitrite oxide in blood serum declined significantly in *F. vulgaris* and nicotine + *F. vulgaris* groups in all doses compared to the nicotine group ($p < 0.05$) (Figure 5).

Nitric oxide is a free radical and can regulate angiogenesis, apoptosis, cell cycle, invasion, and metastasis in cells [26]. Nicotine can stimulate nicotinic receptors in the brain

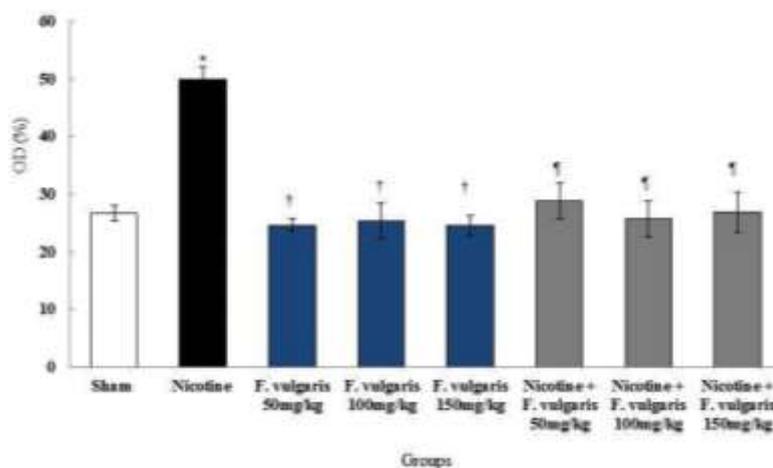


Figure 5. Effects of *F. vulgaris*, nicotine, and nicotine + *F. vulgaris* on the mean of No level. *Significant increase of No in the nicotine group compared to the sham group ($P < 0.05$). †Significant decrease in all *F. vulgaris* groups compared to the nicotine group ($P < 0.05$). Significant decrease in all nicotine + *F. vulgaris* groups compared to the nicotine group ($P < 0.05$).

and increase glutamate release and NMDA activation. The activation of NMDA may increase the formation of nitric oxide in the hippocampus [27]. The results of a study by Keser et al. showed that exposure to nicotine increases the activity of nitric oxide in the frontal cortex in the mouse brain; consistent with the results of the present study [28]. Kahnmoiei et al. showed that total antioxidant level was significantly reduced in smokers in proportion to non-smokers; which is consistent with the results of the current study [29].

The results displayed that the total antioxidant capacity serum level reduced significantly in the nicotine group compared to the sham group ($p < 0.05$). The total antioxidant capacity level enhanced significantly in all *F. vulgaris* and nicotine + *F. vulgaris* groups compared to the nicotine group ($p < 0.05$) (Figure 6).

The reduction in total antioxidant capacity level in this study shows the effects of oxidative stress from nicotine on the hippocampal neuron. Nicotine induces oxidative stress in neural tissue that is demonstrated as a growth in the levels of ROS and lipid peroxidation and a reduction in the action of antioxidant enzymes like total antioxidant capacity [30]. In the present study, improved levels of total antioxidant capacity in rats treated with *F. vulgaris* highlight the antioxidant effects of *F. vulgaris* [3]. A total antioxidant level increase due to the administration of nicotine indicates the positive impact of *F. vulgaris* on magnified antioxidant effects and also the inhibition of nicotine-induced inflammation and destruction

process on neurons in the brain. Further, it is assumed that saponins in the extract of *F. vulgaris* inhibit the synthesis of nitric oxide induction enzyme (iNOS) [4]. The results of the study by Jalili et al. are consistent with the results of the present study, which indicated that *F. vulgaris* administration could inhibit nitric oxide production [4].

The results of the present study showed that *F. vulgaris* administration in all doses studied might have a positive effect on nicotine-induced toxicity, as an oxidative stress, in the neurons of the hippocampus CA1 region and these effects were not associated with an increase in the dose of *Falcaria* extract.

4. Conclusion

It appears that *F. vulgaris* provides protection against oxidative stress resulting from nicotine in the CA1 region. Such an ability of the *F. vulgaris* might be due to its strong potential antioxidant attributes. *F. vulgaris* administration moderates the antioxidant agents in the extract. As a result, it leads to CA1 tissue recovery and prevention of nicotine adverse effects on total antioxidant capacity, nitric oxide, number of neurons, and dendritic spines as evidenced in the male rats.

Acknowledgements

We gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (No: 97501) for the financial support.

References

- [1] Salahshoor MR, Mohammadi MM, Roshankhah S, Najari N, Jalili C. Effect of *Falcaria vulgaris* on

oxidative damage of liver in diabetic rats. *J. Diabetes Metab. Disord.* (2019): 1-9.

[2] Rafiey Z, Jalili F, Sohrabi M, Salahshoor MR, Jalili C. Effects of hydro-alcoholic extract of *Falcaria vulgaris* on pancreas tissue in streptozotocin-induced diabetic rats. *Iranian J. Endocrin Metab.* (2017) 19 (2): 91-8.

[3] Hamideh J, Khosro P, Javad ND. 2012. Callus induction and plant regeneration from leaf explants of *Falcaria vulgaris* an important medicinal plant. *J. Med. Plant Res.* (2012) 6 (18): 3407-14.

[4] Jalili C, Kamani M, Roshankhah S, Sadeghi H, Salahshoor MR. Effect of *Falcaria vulgaris* extracts on sperm parameters in diabetic rats. *Androl.* (2018) 50 (10): e13130.

[5] Fazly Bazzaz BS, Haririzadeh G, Imami SA, Rashed MH. Survey of Iranian plants for alkaloids, flavonoids, saponins, and tannins [Khorasan Province]. *Int. J. Pharm.* (1997) 35 (1): 17-30.

[6] Esfandiari E, Roshankhah S, Mardani M, Hashemibeni B, Naghsh E, Kazemi M, Salahshoor MR. The effect of high frequency electric field on enhancement of chondrogenesis in human adipose-derived stem cells. *Iran J. Basic Med. Sci.* (2014) 17 (8): 571.

[7] Kozlowski LT, Mehta NY, Sweeney CT, Schwartz SS, Vogler GP, Jarvis MJ, West RJ. Filter ventilation and nicotine content of tobacco in cigarettes from Canada, the United Kingdom, and the United States. *Tob. Control.* (1998) 7 (4): 369-75.

[8] Filbey FM, Gohel S, Prashad S, Biswal BB. Differential associations of combined vs. isolated cannabis and nicotine on brain resting state networks. *Brain Struct. Func.* (2018) 7: 1-0.

[9] Timmerman W, Westerink BH. Brain microdialysis of GABA and glutamate: what does it signify?. *Synapse.* (1997) 27(3): 242-61.

[10] Jalili C, Khani F, Salahshoor MR, Roshankhah SH. Protective effect of curcumin against nicotine-induced damage on reproductive parameters in male mice. *Int. J. Morphol.* (2014) 32 (3): 844-9.

[11] Decina P, Caracci G, Sandik R, Berman W, Mukherjee S, Scapicchio P. Cigarette smoking and neuroleptic-induced parkinsonism. *Biol. Psychiatry.* (1990) 28 (6): 502-8.

[12] Salahshoor MR, Mohamadian S, Kakabaraei S, Roshankhah Sh, Jalili C. Curcumin Improves liver Damage in Male Mice Exposed to Nicotine. *J. Tradit. Complement Med.* (2016) 176-183.

[13] Changeux JP. Nicotine addiction and nicotinic receptors: lessons from genetically modified mice. *Nature Rev. Neurosci.* (2010) 11 (6): 389.

[14] jalili. C, Salahshoor MR, Pourmotabbed A, Moradi S, Motaghi M, Shabanizadeh Darehdori A, Motaghi M. The Effects of Aqueous Extract of *Boswellia Serrata* on Hippocampal Region CA1 and learning deficit in kindled Rats. *Res. Pharm. Sci.* (2014) 9 (5): 351-358.

[15] Salahshoor MR, Khazaei M, Jalili C, Keivan M. Crocin improves damage induced by nicotine on a number of reproductive parameters in male mice. *Int. J. Fertil. Steril.* (2016) 10 (1): 71.

[16] Jalili C, Ahmadi S, Roshankhah S, Salahshoor M. Effect of Genistein on reproductive parameter and serum nitric oxide levels in morphine-treated mice. *Int. J. Reprod. Biomed.* (2016) 14(2): 95.

[17] Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* (1996) 239(1): 70-6.

[18] Huhn S, Beyer F, Zhang R, Lampe L, Grothe J, Kratzsch J, Willenberg A, et al. Effects of resveratrol on memory performance, hippocampus connectivity and microstructure in older adults—A randomized controlled trial. *NeuroImage.* (2018) 174: 177-90.

[19] Salahshoor MR, Roshankhah S, Hosseni P, Jalili C. Genistein Improves Liver Damage in Male Mice Exposed to Morphine. *Chin. Med. J.* (2018) 131(13): 1598-604.

[20] Oddo S, Caccamo A, Green KN, Liang K, Tran L, Chen Y, Leslie FM, et al. Chronic nicotine administration exacerbates tau pathology in a transgenic model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA.* (2005) 102 (8): 3046-51.

- [21] Androuin A, Potier B, Nägerl UV, Cattaert D, Danglot L, Thierry M, Youssef I, et al. Evidence for altered dendritic spine compartmentalization in Alzheimer's disease and functional effects in a mouse model. *Acta. Neuropathol.* (2018) 25: 1-6.
- [22] Brown R. Nicotine sensitization increases dendritic length and spine density in the nucleus accumbens and cingulate cortex. *Brain. Res.* (2001) 899: 94-100.
- [23] Blum R, Kafitz KW, Konnerth A. Neurotrophin-evoked depolarization requires the sodium channel Na V 1.9. *Nature.* (2002) 419(6908): 687.
- [24] Oda A, Nakagomi S. Nicotine induces dendritic spine remodeling in cultured hippocampal neurons. *J. Neurochem.* (2014) 128: 246-55.
- [25] Shin HJ, Son E, Lee DH, Kim HJ, Kang SS, Cho GJ, Choi WS, et al. Curcumin attenuate the Kainic acid Induced hippocampal cell death in mice. *Neurosci. Lett.* (2007) 416 (1): 49-54.
- [26] Ying L, Hofseth LJ. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. *Cancer Res.* (2007) 67(4): 1407-10.
- [27] Piri M, Nasehi M, Shahab Z, Zarrindast MR. The effects of nicotine on nitric oxide induced angiogenic-like behaviors in the dorsal hippocampus. *Neurosci. Lett.* (2012) 528(2): 93-8.
- [28] Keser A, Nesil T, Kanit L, Pogun S. Brain nitric oxide metabolites in rats preselected for nicotine preference and intake. *Neurosci. Lett.* (2013) 545: 102-6.
- [29] Kahnmoeei JR, Maleki F, Nasirzadeh M, Kishizadeh F. The Effects of Cigarette Smoking on Plasma MDA And TAC in University Students. *Bull. Env. Pharmacol. Life Sci.* (2014) 3:95-8.
- [30] Nickander KK, Mcphee BR, Low PA, Tritschler H. Alpha-lipoic acid: antioxidant potency against lipid peroxidation of neural tissues in vitro and implications for diabetic neuropathy. *Free Radic. Biol. Med.* (1996) 21 (5): 631-9.

ONLINE SUBMISSION

www.ijps.ir