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Extended Abstract
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Pharm Sci 2020-Curcumin Recovers the Toxic Effects of Nicotine on Hippocampus Cornu Ammonis 1 in Rats-Kermanshah University of Medical Sciences, Iran

Mohammad Reza Salahshoor, Amir Abdolmaleki, Shiva Roshankhah, Amir Jalali[1] , Cyrus Jalili[2]

Department of Anatomical Sciences, Medical School, Kermanshah University of Medical Sciences,

[1] Department of Nursing, Substance Abuse Prevention Research Center, Kermanshah University of Medical Sciences,
[2] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Introduction

Tobacco consumption is increasing in developing countries over the same period. Each cigarette contains an average of 10–14 mg nicotine (NIC). Through pulmonary circulation, the NIC spreads rapidly across the brain tissue within 10–20 s. NIC available in the cigarette is an alkaloid agent which rapidly passes through the blood–brain barrier and stimulates the mesolimbic dopamine system. This substance reduces the activity of superoxide dismutase, glutathione peroxidase, and glutathione reductase in the hippocampus. Pathologic changes associated with neuronal apoptosis have been reported due to the use of NIC. Furthermore, NIC can induce the increased oxidative stress levels and neuronal apoptosis rate, destroy deoxyribonucleic acid (DNA), and produce reactive oxygen species (ROS). Among the brain areas where the NIC has the greatest effect, the mesocorticolimbic is a region which contains nucleus accumbens, ventral tegmental area, hippocampus, and amygdala. In this region, the structures of the amygdala and hippocampus play a crucial role in the formation of long-term memory, whose function is associated with stimulation of the reward system. Hippocampus is a part of the limbic system and seems to be essential in the formation of different types of learning and memory. Cornu ammonis 1 (CA1) area, which belongs to the hippocampus, plays an essential role in converting short-term memory to long term. Meanwhile, in recent years, herbal medicines have gained popularity. For centuries, India and China have been used turmeric as an anti-inflammatory substance in the treatment of colic pain, toothache, chest pain, jaundice, anorexia, and menstrual disorders. The rhizome extract contains mainly curcumin (CUM). CUM has antioxidant and anti-inflammatory properties. The results of a study by Jayaprakasha et al. revealed that in Alzheimer's experimental model, the CUM could improve the memory through intravenous injection of streptozotocin. CUM can encourage the neurogenesis process in elderly female rats. The results of a study by Mashayekhi et al. on various antioxidants showed that the CUM has far more potent in breaking down of free radicals. In addition, it can protect the brain against lipid peroxidation and breaking down nitrite oxide (NO)-induced free radicals. CUM seems to prevent the destructive effects of NIC in the brain through the inhibition of oxidative stress mechanism. According to the effects of NIC brain toxicity and the therapeutic properties of CUM, and considering that no study has evaluated the antioxidant effects of CUM on NIC-induced CA1 damage, this experimental study was designed to evaluate the therapeutic effects of CUM against NIC injury in the hippocampus CA1 region of rats.

Materials and Methods

Animals

This experimental study was conducted from December 2017 to April 2018 in the Medical School Department of Anatomy in Kermanshah University of Medical Sciences, Iran. A total of 48 male Wistar rats) 220–250 g (were purchased from the Pasteur Institute and transferred to the animal house in the medical school. During the study, the animals were kept under standard conditions including 12/12 h light-dark cycles and 22°C ± 2°C in special cages and on a straw bed.

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Treated municipal water and plate food were available to the animals ad libitum. All investigations conformed to the ethical and human principles of research and were approved by the Ethics Committee of Kermanshah University of Medical Sciences (ethics certificate no. 1396.562).

Chemicals, study groups, and treatment of animals

CUM is a bright yellow chemical present in Curcuma longa. CUM is a diarylheptanoid, belonging to the group of curcuminoids which are natural phenols responsible for turmeric's yellow color. It is a tautomeric compound existing in enolic form in organic solvents and as a keto form in water. A total of 15 mg of CUM powder (Sigma, USA) with a chemical formula of 4-(OH)-3-(CH3 O) was first dissolved in 1.5 cc vehicle solution (carboxymethylcellulose + normal saline [0.9%] 50/50 ratio), to obtain CUM with a dose of 10 mg/kg, whereas the other doses were made similarly and immediately injected to animals after being prepared. Avial of NIC (Sigma, USA) with a dose of 0.5 mg/kg of body weight was dissolved in vehicle solution. Next, 48 male rats were randomly divided into eight groups with six rats in each. The first group (normal group) received vehicle solution through i. P injection equal to the amount received by the experimental groups. In the second group (NIC group), each animal received 0.5 mg/kg single dose of NIC i. p. The solvent of NIC was vehicle solution. The third to fifth groups consisted of CUM administration groups. In these groups, each animal received 10, 30, and 60 mg/kg of CUM i. P for 28 days at 10 A. M. Finally, the sixth to eighth groups were NIC + CUM administration groups, wherein each animal received a single dose of 0.5 mg/kg NIC for induction of damage. Then, they received 10, 30, and 60 mg/kg of CUM i. P for 28 days at 10 A. M.

Transcardiac perfusion

In this part, 24 h after the last injection of the drug, animals were anesthetized with ketamine 70 mg/kg and diazepam 10 mg/kg i. p. Following the completion of thoracotomy, the apex of the left ventricle was pierced with a 1-mm diameter of glassy cannula, and then fixed to the ascending aorta. The ascending aorta was connected to a plastic tube by the glassy cannula, whereas the descending aorta was clamped. The cannula linked to the normal saline solution was implanted into the aorta. The descending aorta was fastened, and the solution was removed through the incision made on the right atrium. Formalin 5% and buffer phosphate 7% were inoculated into the brain by the cannula, and the brain was fixed within 15 min. After perfusion, the brains were removed from the skull and stored in the same perfusion solution for 3 days.

The tissue preparation and Golgi methods

The Golgi method was used to observe the neuron dendrites in the hippocampus CA1 region. After brain fixation, tissue blocks were put inside 3% potassium dichromate solution for 48 h. The blocks were washed in 0.75% silver nitrate solution. Then, paraffin-embedded blocks were prepared using Automatic Tissue Processor. Next, 5-µm coronal histological thin sections were cut from paraffin-embedded blocks with five sections per animal chosen. To unify the section selection, the first section was the 4th and the last was the 24th (5-section interval), and finally, the routine protocol for Golgi methods was implemented. At the end of the tissue processing, the stained sections were assessed under microscope Olympus BX-51T-32E01 connected to a DP12 Camera with 3.34-million pixel resolution plus Olysia Bio software (Olympus Optical Co. Ltd., Tokyo, Japan).

Cresyl violet method

The cresyl violet staining method was used to determine the number of living cells in the hippocampus CA1 region. Six rat heads from each group and five slides from each rat were selected for staining. After preparation of 5-µm cuts by microtome and performing tissue processing, the left hemispheres were stained using cresyl violet staining technique. Once the photo was prepared, the number of cells was

counted in 1 mm2 . In the slides stained through cresyl violet technique, the round cells without peak nose were considered as living cells.

Morphometrical technique

The dendritic tree of pyramidal neurons was revealed through camera lucida with magnification of ×750 with the dendritic exclusion order from the cell body used for counting the dendritic sections. In addition, the Sholl procedure was applied to assess the concentration of dendritic divisions. In the slips marked through cresyl violet method, the round cells without peak nose were considered as living cells. The slides were then imaged by Motic microscope, and cells were counted byImageJ software 1.45 (Olympus Optical Co. Ltd., Tokyo, Japan).

Griess technique

The samples were centrifuged (12,000 rpm) at 4°C for 10min, and the supernatant was used to measure the NO level. In this study, zinc sulfate powder (6 mg) was mixed with serum samples (400 µl) which vortexed for 1 min. 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. Sodium nitrite (0.1 M) was employed for the standard curve, where the increasing concentrations of sodium nitrite (5, 10, 25, 50, 75, and 100 µM) were also prepared. The Griess solution was read through an enzyme-linked immunosorbent assay reader (stat fax 100; USA) at the wavelength of 540 nm.

Ferric reducing/antioxidant power

Ferric reducing/antioxidant power method was used to measure the serum total antioxidant capacity (TAC) level. In this technique, the ability of the blood plasma to retain ferric ions was measured. This process required a great quantity of FeIII. A blue stain was formed when the compound of ferric tripyridyl triazine in acidic pH returned to FeII; moreover, the absorption was measured at the maximum wavelength of 600 nm. The factor defining the speed of the FeII-TPTZ and the blue color was the only vitalizing power of the sample. TAC values were strategized through the standard curve with diverse concentrations of iron sulfate

Measurement of hippocampal malondialdehyde

Malondialdehyde (MDA) levels were evaluated as an index of lipid peroxidation. In this regard, homogenizing of the samples was carried out by homogenization buffer containing 1.15% KCl solution and the specimens centrifuged at 5000 rpm for 10 min, respectively. Then, the homogenated samples were added to a reaction mixture containing sodium dodecyl sulfate, acetic acid (pH 3.5), thiobarbituric acid, and distilled water. The reaction mixture was heated at 95°C for 1 h and centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured by a spectrophotometer at 550 nm.

Statistical analysis

After extracting the information, the Shapiro test was first conducted to confirm the normality of data distribution. One-way analysis of variance (ANOVA) was used for statistical analysis, and Tukey's post hoc test was utilized to determine the differences among the groups. SPSS (New York: IBM, SPSS version 16.0) was used for data analysis; the results were expressed as mean ± standard error, and $P < 0.05$ was considered statistically significant.

Results

Number of neurons

The results of the Shapiro test showed that all data had a normal distribution, and parametric tests were used accordingly at any significant difference between the groups ($P > 0.05$). Further, the results of ANOVA test indicated that the mean number of neuronal dendritic spines decreased significantly in all the NIC + CUM groups (doses: 10 mg/kg = 11.50%, 30 mg/kg = 11.66%, and 60 mg/kg = 11.83%) compared to the normal group ($P < 0.01$) [Figures 3 and 4]. On the

other hand, the results of Tukey's post hoc test did not reveal any significant difference between the groups ($P > 0.05$). Nitrite oxide The results of independent t-test indicated that the mean level of blood serum NO had a significant fall in the NIC group compared to the normal group (404.285 µm)($P < 0.01$), whereas the mean level of this value was not significant in all the CUM groups compared to the normal group ($P > 0.05$). Further, the results of ANOVA showed that the mean level of NO in blood serum declined significantly in the CUM (doses: 10mg/kg=190.61 µM, 30 mg/kg = 193.83 µM, and 60 mg/kg = 190.69 µM) and NIC+CUM (doses: 10mg/kg=309.69 µM, 30mg/kg=295.88 µM, 60 mg/kg = 299.58 µM) groups in all doses compared to the NIC group ($P < 0.01$) [Figure 5]. Finally, the results of Tukey's post hoc test did not show any significant difference between the groups ($P > 0.05$). Total antioxidant capacity The results of independent t-test revealed that the TAC serum level diminished significantly in the NIC control group compared to the normal group ($P < 0.01$). The results of ANOVA showed that the TAC level grows significantly in all the CUM groups (doses: 10 mg/kg = 2.09 mmol/l, 30 mg/kg = 2.03 mmol/l, 60 mg/kg = 2.04 mmol/l) and NIC + CUM groups (doses: 10 mg/kg = 1.24 mmol/l, 30 mg/kg = 1.28 mmol/l, 60 mg/kg = 1.31 mmol/l) compared to the NIC group ($P < 0.01$) [Figure 6]. Finally, the results of Tukey's post hoc test did not show any significant difference between the groups ($P > 0.05$). Malondialdehyde levels Serum levels of MDA showed a significant increase in the NIC group compared to the normal group ($P < 0.05$). Furthermore, a significant decrease in MDA levels was showed in all the CUM and CUM + NIC groups compared to the NIC group ($P < 0.05$), whereas it had no significant effect on the levels of MDA in all the NIC groups compared to the normal group ($P > 0.05$). Discussion NIC causes the production of many side effects, affecting the central and peripheral nervous system. In this regard, ventral hippocampal α4-β2 blockade-induced working memory deficits are reversed by chronic systemic NIC treatment, whereas ventral hippocampal α7 blockade-induced working memory deficits have not been found to be reversed by the same NIC regimen. Temporal lobe and hippocampal organization are involved in transferring the short-term memory to long term. The present study aimed to investigate the effects of CUM on NIC-induced disorders in the hippocampus CA1 region. The results suggest that the number of neurons and dendritic thorns decreases significantly in the NIC group compared to the normal group. In NIC + CUM groups, there is a significant increase in the number of dendritic thorns compared to the NIC group. The results may indicate the control of apoptosis and neurodegeneration by different administration doses of CUM. The results of Tewari and Misra are consistent with those of the present study indicating that NIC could damage the cells in the hippocampus through increased protein accumulation in the cell membrane. Similarly, NIC could significantly reduce the number of neurons in the hippocampus through cell destruction. It seems that NIC induces the oxidative stress; consequently, the production of free radicals and hydroxyl radicals can cause cell damage. The generated free radicals following oxidative stress induction may have the potential to damage cellular compositions, including proteins, lipids, and DNA. Similarly, in the current study, the number of neurons and dendritic thorns in the hippocampus decreases due to the oxidative stress caused by administration of NIC. In this regard, the lipid in the membrane of the neural cells has a high content of oxidized unsaturated fatty acids. Therefore, it seems that NIC can produce ROS through P-450 enzyme which causes the degradation of the nucleus in neurons. Unsurprisingly, many brain diseases are associated with the changes in the morphology and density of dendritic thorns. Dendrite thorns are likely to be involved in memory. NIC can reduce the length and the number of dendritic thorns in nucleus accumbens by affecting the neurotrophic factors in the striatum. The results of a study by Brown and Kolb suggested that NIC injections could reduce the length and the number of dendritic thorns, which are consistent with the results of our study. Similar to the current study, by the

presence of oxidative stress in hippocampus horns, the NIC could reduce the number of neurons and dendritic thorns by the means of β2-nAChR deactivation in postsynaptic cells. Further, the NIC can reduce the number of thorns by deactivating α4-β2-nAChRs in the presynaptic membrane. It seems that NIC reduces the number of dendritic thorns in two ways: the regulation of glutamatergic synapses in pyramidal neurons and the activation of Gamma-Aminobutyric acid in the internal neurons or interneurons. CUM is a purifier of ROS, which seems to have the potential to offset the oxidative stress. The results of the study by Shin et al. confirm those of the present study where the CUM could prevent the cell death caused by kainic acid due to the presence of oxidative stress in the hippocampus. CUM seems to control the production of cyanide-induced superoxide in the brain, suggesting its protective properties. In addition, the NIC treatment increases lipid peroxidation and the levels of Glutathione, interleukin-1 beta, tumor necrosis factor alpha, and Bcl-2-associated X protein, whereas it reduces the B-cell lymphoma 2, cAMP Phospho-Response element-binding protein, and Brain-derived neurotrophic factor levels in the hippocampus. CUM has also proved to significantly improve the spatial memory impairment induced by HIV-1 gp120 V3 in rats. The results of a study by Pan et al., in line with the findings of the present study, show that CUM improves learning and memory in mice.[31] CUM can reduce neuropathological alterations in the hippocampus and control the apoptosis by increasing the density of Bcl-2 protein. The present study revealed a significant increase in the serum NO level in the NIC group compared to the normal group. In all the NIC + CUM groups, there is a significant drop in serum NO level in comparison with the NIC group. NO as a free radical can regulate the angiogenesis, apoptosis, and cell cycle. NO seems to play a key role in the destruction of myelin in the central nervous system. NIC can enhance the glutamate release and N-methyl-D-aspartate receptor activation. The activation of NMDA may increase the formation of NO in the hippocampus. The results of a study by Keser et al. indicate that cell exposure to NIC increases the activity of NO in the mice frontal cortex. According to the results of this study, a significant decline occurred in total antioxidant levels in the NIC group compared to the normal group. In all the NIC + CUM groups, there is a significant decrease in the serum total antioxidant levels in comparison with the NIC group. The reduction of TAC level in this study implies the effects of oxidative stress caused by NIC in the hippocampal neurons. This result expresses as an increment in the levels of ROS and a reduction in the activity of antioxidant enzymes. Further, through activation of P-CREB/BDNF signaling pathway, the CUM confers neuroprotection against NIC-induced inflammation, apoptosis, and oxidative stress. In the present study, improved levels of TAC in rats treated with CUM highlight the antioxidant effects of CUM. CUM can also inhibit NO production by reducing the activity of the nuclear factor-kappa β. Our study had certain limitations which are as follows: first, there were no recognition methods of antioxidant levels in the CUM; second, there was also absence of references about the effect of plants or extracts in the CA1 region. Further, the death of some animals due to NIC administration was another important limitation. Hence, prospective studies should be performed to evaluate the molecular interactions between CUM and CA1 region in rats. The current study indicates that CUM could significantly reverse some CA1 injuries against the destructive properties of NIC in rats. It appears that CUM provides protection against oxidative stress resulting from NIC. Such an ability of the CUM may be due to its strong potential antioxidant attributes. Accordingly, it leads to CA1 tissue recovery and prevention of NIC adverse effects on TAC, NO, number of neurons, and dendritic spines as evidenced in the abovementioned examination of male rats. However, supplementary studies are essential to describe its molecular mechanism.

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Conflicts of interest

There are no conflicts of interest.

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