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Khaksar Zabihollah
Department of Anatomical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Azhdari Sara
Department of Anatomical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Correspondence:
Khaksar Zabihollah
Department of Anatomical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Histomorphometric Study of Lycopene Effect on Dopamine Receptors and GABA Neurons in Rat Fetuses from Mothers with Parkinson's disease

Khaksar Zabihollah, Azhdari Sara

Abstract
Introduction: After Alzheimer, Parkinson's disease is a common damaging age-related disorder for the central nervous system and nerves. Till now, any research has not been performed associated with the effect of this disease on rat fetuses born to mothers with Parkinson's disease as well as using of herbal extracts to improve the disease. So the aim of this study is histomorphometric study of lycopene effect on dopamine receptors and GABA neurons in fetuses born to mothers with Parkinson's disease.
Methods: In this study, a number of 65 timed-pregnant Sprague-Dawley rats both healthy and parkinsonism were randomly divided into five groups including control group (without getting any material), lycopene control group (0.5 ml/kg lycopene via gavage), patient group (unilateral injection (6-hydroxy dopaminergic to substantia nigra area by Hamilton needle)), sham group (induction of Parkinson's disease + 0.02% of ascorbic acid by unilateral injection into substantia nigra area), and treated group with lycopene (induction of Parkinson's disease + 0.5 ml/kg lycopene). Following the 15th and 19th day of pregnancy, the fetuses under general anaesthesia were extracted and their brains exited for additional process. The density of D1, D2, and GABA receptors were investigated by hematoxylin and eosin (H&E) staining and optical microscopy.
Results: The number of receptors (D1, D2, and GABA) in substantia nigra areas and brain cortex of 15 and 19-day-old fetuses in sham and patient groups had a significant reduction compared to control and lycopene groups. And the experimental group treated by lycopene had a significant increase at level of 5% in the number of receptors compared to patient group.
Conclusion: The results show that the number of dopamine and GABA receptors in the substantia nigra areas and the brain cortex of fetuses on 15th and 19th day of embryonic period is affected by Parkinson's disease induced by mothers. By increasing the number of days, the number of receptors increased. However the number was less than the control group, and the number of receptors increased in groups receiving lycopene, due to its antioxidant and neuroprotective properties.

Keywords: Histomorphometric, Lycopene, Dopamine Receptor, GABA Receptor, Parkinson, Rat Fetuses.

1. Introduction
After Alzheimer, Parkinson's disease is a common damaging age-related disorder for the central nervous system and nerves. In most of epidemiological studies found that more than one million people in the United States show signs of the disease and approximately 50,000 people will be added to this statistics per year [1]. Parkinson's disease is clinically diagnosed by high tremors at rest, reduction of voluntary movements (imbalance) and progressive muscle stiffness, especially in hands and feet [2]. The important and main cause of Parkinson's disease is decline and loss of brain dopamine (due to death of dopamine-secreting cells in substantia nigra). Although it is not the only factor and the disease is also attributed to the loss of neurons at nigrostriatal dopaminergic pathways [3].
However, neuropathology of Parkinson's disease is limited to the nigrostriatal pathway. And tissue abnormalities are also found in the other dopaminergic and nondopaminergic cellular groups [3]. However, there is also a major ambiguity in understanding of cellular and molecular biology of Parkinson's disease. As a result, studies now widely are relying on experimental models of Parkinson's disease to get a larger view of its cause. However, recent genetic discoveries are associated with a number of different Parkinson's disease models [1].
Dopamine receptors are associated with most of cellular functions. Dopamine receptors activity in mammalian central nervous system is essential for regulation of mood, motivation and dynamic functions [4]. Dopamine receptors are G protein-coupled receptors that are divided

by type of G protein into two types of D1 receptor (which is attached to Gαs protein) and D2 receptor (which is attached to Gαi protein) [5, 6]. Typically, the activity of dopamine receptors leads to changes of intracellular cAMP levels as well as stimulation of signaling cascades that increase gene transcription [6].

However dopaminergic pathways in adults' brain have been studied in more details, while function and role of dopamine system has not been well studied during the course of evolution of rats. Dopamine receptors in rodents' brain in mid and late embryonic development in middle frontal cortex are involved with wide range of connections of brain regions in attention, cognition and working memory [7]. It is still not well understood that at what age dopamine receptors become functional and what signaling cascade in fetus brain reflects the characteristics of these receptors in adults [7]. Our knowledge from expression and application of D1 and D2 dopamine receptors during early embryonic development is critical to know quality of dopaminergic system portion in organization of cortical and subcortical regions of cerebrum, since it may be involved in aspects of evolution of neurological-mental disorders such as schizophrenia [7]. However, Crandall et al., in 2007 showed that the migration of GABAergic neurons from ganglionic eminences to cerebral cortex is changed with D1 and D2 receptors. The activation of D1 receptors leads to increase the migration of neurons to cerebral cortex, while D2 receptors reduce the migration [8]. Due to the connections between dopamine receptors and GABA neurons can be predicted that effective factors on dopaminergic receptors will also affect on GABAergic neurons behaviors. Nowadays, medications and herbal extracts are used to improve most of diseases. Lycopene is one of 600 carotenoids which are found in high levels in tomatoes [9] and have antioxidant and neuroprotective properties [10].

So, according to the above, and since that Parkinson's disease impairs GABAergic and dopaminergic neurons as well as unknown potential effects of the disease on fetuses born to mothers with Parkinson's disease and effective properties of lycopene in treatment of many diseases, it is tried in this study to evaluate lycopene effects on dopamine receptors and GABA neurons in rat fetuses born to mothers with Parkinson's disease.

2. Materials and Methods

A number of 65 Sprague-Dawely female rats with an average weight of 200 ± 20 g and approximate age of 3-3.5 months were prepared This experiment was accomplished under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran. Also, the recommendations of European Council Directive (86/609/EC) of November 24, 1986, were used regarding the standards in the protection of animals used for experimental purposes.

The animals were kept for 10 days in Animal House of Science Department of Veterinary Medicine at Shiraz University, Iran, in order to deal with environmental conditions (12 hours of light and 12 hours of darkness, at 23 ˚C). All rats were provided tap water and rat food ad libitum. Females in estrus were placed with males for 16 hours. Then, vaginal smears were taken the following morning day. The day on which sperm were present was designated as the day 0 of gestation (GD 0). For parkinson's groups, first, in number of rats Parkinson were induced then used for mating.

Afterwards, the pregnant rats were randomly divided into the following groups:

1. The control group: this group was consisted of 5 rats, without any prescriptions.
2. Lycopene control group: this group was consisted of 15 healthy rats. In this group, lycopene was solved in 0.5% of sodium carboxy-methyl-cellulose and they daily and orally received 0.5 ml of solution per100 g of body weight until sampling [11].
3. Sham group: this group was consisted of 15 rats. This group is similar to the patient group, but only 0.02% of ascorbic acid in 0.9% solution of sodium chloride (normal saline) was injected into their right side of substantia nigra areas.
4. Patient group: this group was consisted of 15 rats. Parkinson's disease was induced in this group, with unilateral injection of 6-hydroxy dopaminergic into substantia nigra areas [12].
5. Lycopene treated group: this group was consisted of 15 rats with Parkinson. These rats received lycopene like group 2 until sampling.

Parkinson's disease, Method of Induction

For this purpose, rats anesthetized with a mixture of ketamine (87 mg/kg) + xylazine (13 mg/kg) [13]. An amount of 5 microgram of 6-hydroxy dopaminergic was dissolved in 0.02% of ascorbic acid in 0.9% solution of sodium chloride (normal saline) and then received to 2 microliter volume (this amount was doubled when injected into both sides of brain). The resultant solution at a rate of 0.5 microliter per minute and by the stereotaxic device (Made in Germany) and using a 5µl Hamilton syringe was injected according to paxinos atlas into right side of brain substantia nigra areas [12, 13].

2 dams were anesthetized with 60mg/kg of sodium thiopenthal each day and after withdrawal of the fetuses, the mothers were killed with a high dose of sodium thiopenthal. The number of samples involved in the study was at least 5 in each age. The skulls of fetuses and neonates were immediately opened, and the brains were collected and fixed in 4% buffered formalin at least for 24 h. These specimens were then processed through graded alcohols and xylene, and embedded in paraffin blocks. The coronal brain sections at 5-6 micron thick were prepared serially using a microtome (Sigma Company). The sections were stained with Hematoxylin-Eosin (H&E). Histomorphometric studies were done on substantia nigra and brain cortex areas and the obtained results were analyzed with SPSS software version 18. D1, D2 and GABA primary antibodies purchased from Sigma Aldrich Company for immunohistochemical staining.

3. Results

According to Chart 1, the number of D1 receptors in substantia nigra area of 15-day old fetus was more than D2, and GABA receptors. The number of receptors (D1, D2, and GABA) in patient and sham groups was significantly decreased compared to control and lycopene control groups. The number of receptors in experimental group treated with lycopene was significantly increased at level of 5% compared to patient group.

According to figure 2, the number of D2 receptors in brain cortex of 15-day old fetus was more than D1, and GABA receptors. The number of receptors (D1, D2, and GABA) in patient and sham groups was also significantly decreased compared to control and lycopene control groups. The number of receptors in experimental group treated with lycopene was significantly increased at level of 5% compared to patient group.

Chart 1: The Average number of receptors in substantia nigra area of 15-day old fetus

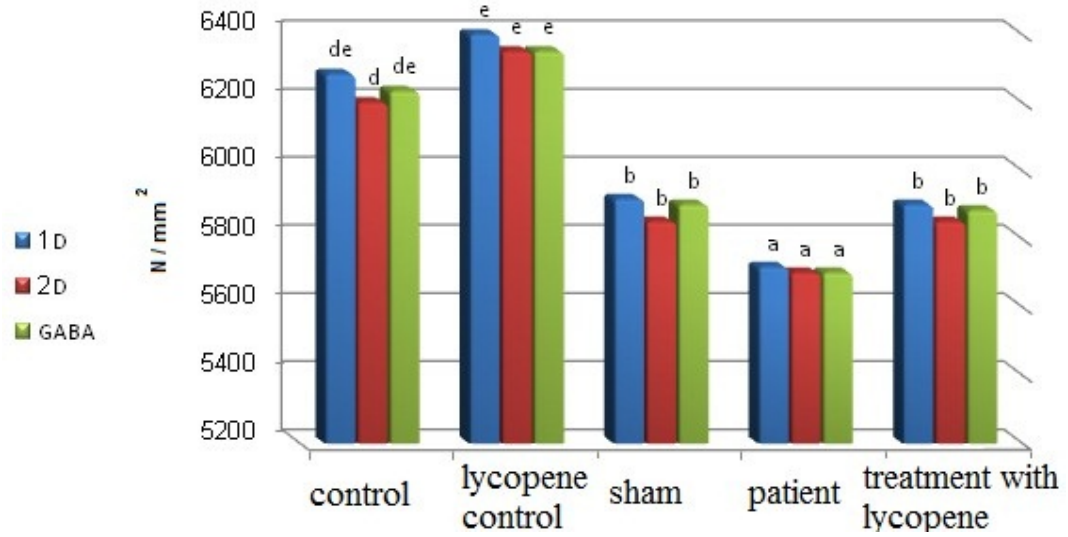
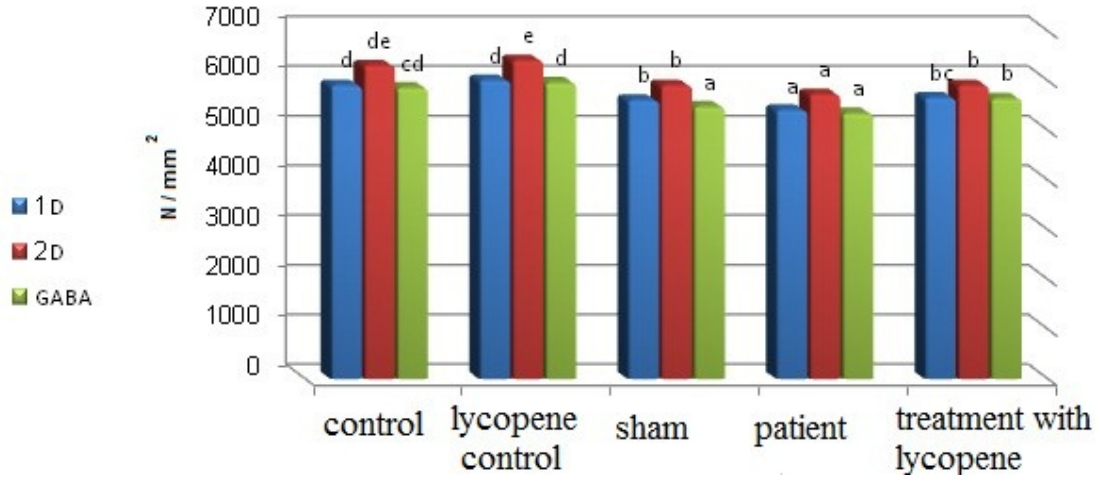
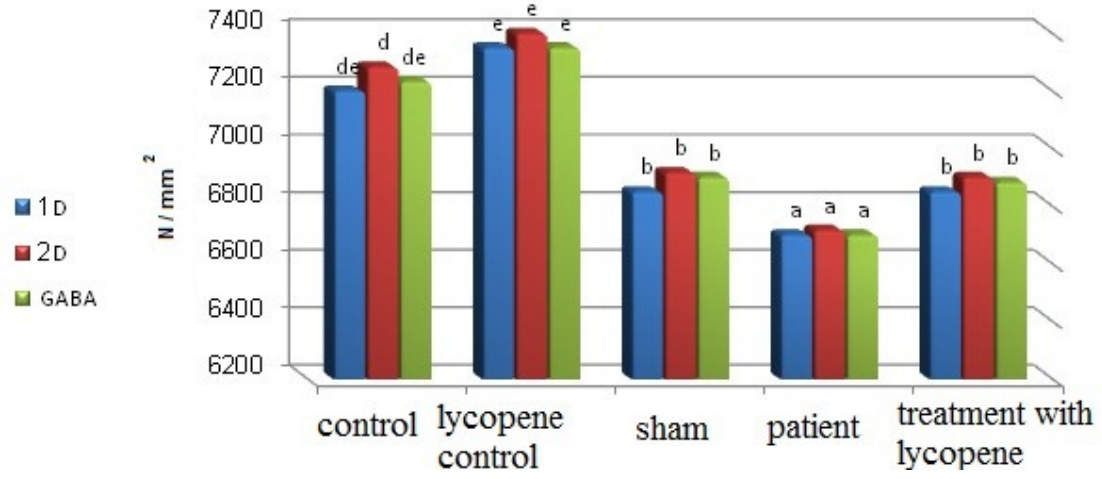


Chart 2: The Average number of receptors in brain cortex area of 15-day old fetus



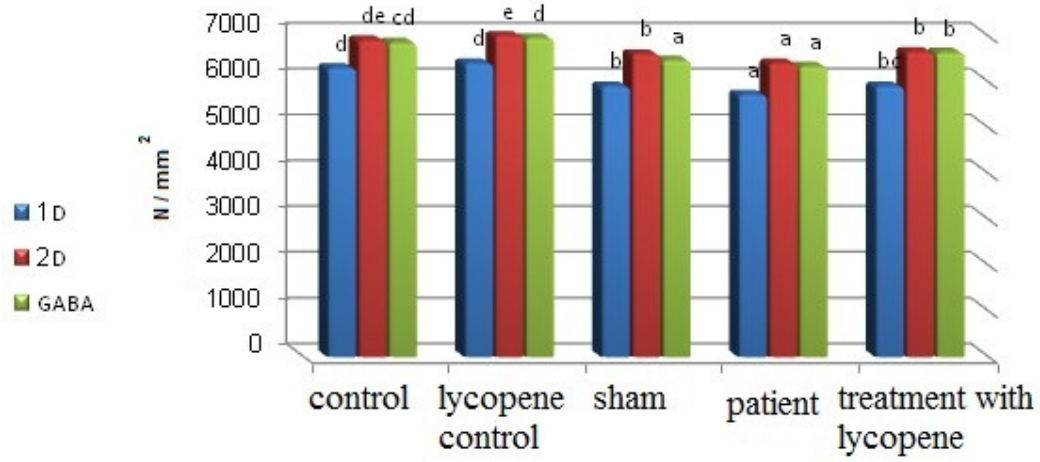
According to figure 3, the number of D2 receptors in substantia nigra area of 19-day old fetus was more than D1, and GABA receptors. The number of receptors (D1, D2, and GABA) in patient and sham groups was significantly decreased compared to control and lycopene control groups. The number of receptors in experimental group treated with lycopene was significantly increased at level of 5% compared to patient group.

Chart 3: The Average number of receptors in substantianigra area of 19-day old fetus



According to figure 4, the number of D2 receptors in brain cortex of 19-day old fetus was more than D1 receptors. The number of receptors (D1, D2, and GABA) in patient and sham groups was also significantly decreased compared to control and lycopene control groups. The number of receptors in experimental group treated with lycopene was significantly increased at level of 5% compared to patient group.

Chart 4: the average number of receptors in brain cortex area of 19-day old fetus



4. Discussion

Brain development requires a series of complex interactions and environmental factors. Impairments in these components can influence on neuronal structure, function or connections and can lead to a change in development pathway. Massages related to dopamine and GABA receptors play a fundamental role in neuronal growth, differentiation of brain and formation of nervous currents [14]. The results show that D1 receptors were more than D2, and GABA receptors in substantianigra area of 15-day fetus of mothers with Parkinson's disease.

A review of the literature shows that there is no comprehensive study regarding dopamine and GABA signaling pathways in fetus brain [15]; however, it is stated that dopamine is one of the neurotransmitters that appears during early development of brain [16]. Tyrosine hydroxylase expressing neurons in mice appear in ventral midbrain on 11th day of embryonic period [17]. Dopaminergic axons of tyrosine hydroxylase expressing neurons enter to growing striatum on 12th to 13th day of embryonic period and reach to cerebral cortex on about two days later, which suggest that dopamine effects on neurogenesis. However, its concentration is different depending on where it is most needed [18]. mRNA is expressed in the cerebral cortex of mouse fetus for all dopamine receptors in ventricular area and cortical plate, so that the amount of mRNA expression for D2-like receptors (D2, D3, D4) is often more than D1-like receptors (D1, D5)in reproduced areas of brain cortex [4]. As if in the present study, the number of D2 receptors in brain cortex of 15 and 19 day-old fetus was more than D1 receptors. This agrees with previous studies. So, the process of receptors changes in brain in fetus rats of mothers with Parkinson's disease is similar to normal and healthy fetus. This agrees with previous studies.

Performed studies on distribution of D1 and D2 receptors in transgenic mice with a neurological disorder stated that the concentration of dopamine in these animals is less than healthy ones [19]. What is certain is that the average number of receptors in fetus brains in this study is less than in control and lycopene groups. This reflects the negative effects of Parkinson's disease on changes of dopamine and GABA receptors in development pathway of fetus in substantianigra and brain cortex. It was stated in previous studies about changes of dopamine receptors in brain of (with disorder in dopamine and GABA receptors) consuming drugs that the disorder could spread to fetus in the future and babies born to addicted mothers had abnormalities in dopamine receptors. It was also reported that probably a kind of disorder in fetus observes in diseases with abnormality in dopamine receptors [20]. As if in the present study, the disorder related to Parkinson's disease was partly observed in substantianigra area of fetus rats' brain in patient control. This confirms previous studies.

Recent studies have shown that monoamines and their signaling pathways can regulate axons growth in embryonic period by changes in the level of cyclic nucleotides [21, 22]. In addition, it has been shown that stimulation of dopaminergic systems in adults' brain by external factors such as medicines regulates axons guidance molecules in various areas of brain [6, 23]. It is likely that the above mentioned mechanisms also occur in association with fetus brain and as a result, fetus brain is experiencing some problems in its various areas during development.

The results of present study showed that an increase in the number of D1, D2, and GABA receptors was observed in treated groups with lycopene and lycopene control group compared to the patient group. This reflects the positive effects of lycopene extract on improvement the number of receptors in Parkinson's disease. Studies suggest that lycopene is not an essential nutrient for human, however normally found in diets, and is transported in blood by various lipoproteins after it is absorbed in intestine. Lycopene first accumulates in blood, lipids, skin, liver, adrenal glands, prostate, and testicle, however is found in many tissues [3]. Lycopene has recently been considered by researchers due to its high antioxidant properties. It was reported that lycopene has neuroprotective properties [10] and increases cognitive functions [24]. Also, it was found that the amount of serum levels of lycopene reduced in neurodegenerative disorders and Parkinson's disease. The amenability of lycopene in management of diseases and neuroprotective properties has been proven in studies [25, 26].So increase of the number of receptors in various days is quite logical compared to patient group that did not receive lycopene.

5. Conclusion

The results show that the number of dopamine and GABA receptors in substantianigra area and brain cortex in 15th and 19th days of embryonic period was affected with Parkinson's disease-induced mothers. The number of receptors increased with increase of days but their numbers were less than control group. Due to antioxidant and neuroprotective properties of lycopene, an increase in the number of receptors was observed in the groups that received the extract. So, lycopene can be recommended to pregnant women to reduce the risks of the disease and improvement of its symptoms.

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