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The Effect of Lycopene on Dopaminergic Receptors and GABA Neurons in Hippocampus and Substantia Nigra Areas in Rat with Parkinson's Disease

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Abstract

Parkinson's disease is a progressive degenerative neurological disorder and is associated with reduction of dopamine-secreting neurons and maybe GABA neurons in substantia nigra and basal ganglia pathways. At present, there are various methods of treatment for these patients that the most important one is treatment methods with chemical medicines; however these drugs have many side effects. Thus, researchers turn toward using of medicinal plants such as lycopene which is an antioxidant and neuroprotective reagent to minimize the side effects of these medicines. The main objective of this study is investigation of lycopene effects on density of dopaminergic receptors and GABA neurons in hippocampus and substantianigra areas of Parkinson's disease modelrats.

Methods: In this study, a number of 65 adult male rats 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 substantianigra area), and treated group with lycopene (induction of Parkinson's disease + 0.5 ml/kg lycopene); and 15, 30 and 60 days were considered for treatment periods. Then the rats were euthanized in every single day and their brains removed for further process and density of D1, D2, and GABA receptors were investigated by hematoxylin and eosin (H&E) staining and optical microscopy.

Results: The number of D1, D2, and GABA receptors in sham and patient groups in 15, 30 and 60 days of treatment period had a significant reduction in hippocampus and substantianigra areas compared to control and lycopene groups. Also, a significant increase in hippocampus and substantia nigra areas in various days of treatment was observed in the experimental group treated with lycopene compared to patient group. The receptors density in various days of treatment in hippocampus area of the patient group was more than substantia nigra area.

Conclusion: according to the above, Parkinson's disease reduces the density of D1, D2, and GABA receptors in hippocampus and substantianigra areas and lycopene extract causes reduction of side effects and somewhat improvement of Parkinson's disease, due to its neuroprotective and antioxidant properties. The obtained results can be somewhat generalized to human.

Keywords: Lycopene, Dopaminergic, GABA neurons, Parkinson, Rats.

1. Introduction

Parkinson's disease is a progressive and degenerative neurological disorder and is associated with reduction of dopamine-secreting neurons in the pathway of substantia nigra and basal ganglia. The disease occurs in all races and with almost equal gender distribution. Its prevalence is 1-2 per thousand of population and this disorder becomes more common with aging. The most common cause of the disease is idiopathic; however it can be seen as a result of consumption of medicines or toxins or in the context of other neurological diseases^[1].

The natural balances between dopamine and acetylcholine which both exist in striatum have been disturbed in Parkinson's disease. Major clinical symptoms are as following: tremor at rest which increases at times of mental stress and improves with voluntary activities, rigidity or enhancement of resistance as gear, which are sensible characteristics of the disease, against passive movements, impairment in power which is cause with curved position of patients^[2, 3], slow movements including slow motions and difficulty in starting movements and loss of automatic movements^[1].

As mentioned above, the main factor of Parkinson's disease is a decline and loss of brain dopamine (caused by death of dopamine-secreting cells in substantia nigra area), however this is not the only factor but is also attributed to nigrostriatal dopaminergic pathway^[4]. This pathway is consisted of dopaminergic neurons which their cell bodies are located in substantia nigra and their axons and neuronal endings (or terminations) reach to the striatum^[4].

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However, neuropathology of Parkinson's disease is limited to nigrostriatal pathway and also tissue abnormalities have been found in dopaminergic and non-dopaminergic cells [4]. GABAergic neurons are also consisted of 20 to 30 percent of total neurons of brain cortex and have a critical role in regulation of performance [5]. The development of GABAergic neurons regulates a new class of neural epithelial cell proliferation, neural migration and formation of flow, during the period [6].

Abnormal function of GABAergic neurons occurs in the most of developmental disorders such as epilepsy, schizophrenia, anxiety and autism [7, 8].

One of the symptoms of developmental disorders such as schizophrenia, epilepsy, and autism is involvement of multi-neurotransmitter systems especially dopaminergic and GABAergic systems [5].

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 increases the migration of neurons to cerebral cortex, while D2 receptors reduce the migration [5]. Due to the connections between dopamine receptors and GABAergic neurons can be predicted that effective factors on dopaminergic receptors will also affect on GABAergic neurons behaviors.

At present, there are various methods of treatment for these patients, that the most important one is the rapoetics. Based on pathogenic mechanisms which are destruction of dopamine-secreting neurons in substantia nigra and lack of dopamine in basal ganglions of brain, medications with dopamine agonists (dopaminergic) and anticholinergic medications are used for treatment. But despite of existing treatments, first, these treatment methods do not completely remove patients' disabilities and these patients are always exposed to risk induced by these movement problems such as trauma caused by falls, nutritional problems and etc. And secondly, these drugs have early and late complications which compounded the problems of these patients [9, 10].

Lycopene is one of 600 carotenoids which are found in high levels in tomatoes [11]. Lycopene is an essential nutrient for human. It can normally be found in diet and is transported in blood by various lipoproteins when is absorbed in intestine. It primarily accumulates in blood, lipids, skin, adrenal glands, prostate and testicle, however it is found in most of tissues [4]. Due to the high antioxidant properties, Lycopene presently is favoured by researchers [12].

Because dopaminergic receptors are involved in many mental disorders and have influence on mental developments such as GABAergic neurons migration and neuronal damage diseases such as Parkinson affects on dopaminergic receptors and due to the nature of lycopene on cognitive behavioral as well as antioxidant and neuroprotective, the current study was performed to investigate lycopene effects on dopaminergic receptors and GABA neurons in hippocampus and substantianigra areas of Parkinson's model mice to provide therapeutic strategies for improvement of this disease.

Materials and Methods.

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. A number of 65 healthy adult male rats

with an average weight of 200 ± 20 g and approximate age of 3-3.5 months were prepared. The animals under a standard condition were kept for a week in Animal House of Anatomy and Histology Department of Veterinary Medicine at Shiraz University, Iran in order to deal with environmental conditions. Then the 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 per 100 g of body weight until sampling [13].
3. **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 ubstantianigra areas.
4. **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 to their right side of substantia nigra areas [14].
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) (15). An amount of 5microgram of 6-hydroxy dopaminergic was dissolved in 0.02% of ascorbic acid in 0.9% solution of sodium chloride (normal saline) and then the volume was increased to 2 microliter (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 stereotaxic device (made in Germany) and using a 5 μ l Hamilton syringe was injected into right side of brain into substantia nigra areas.

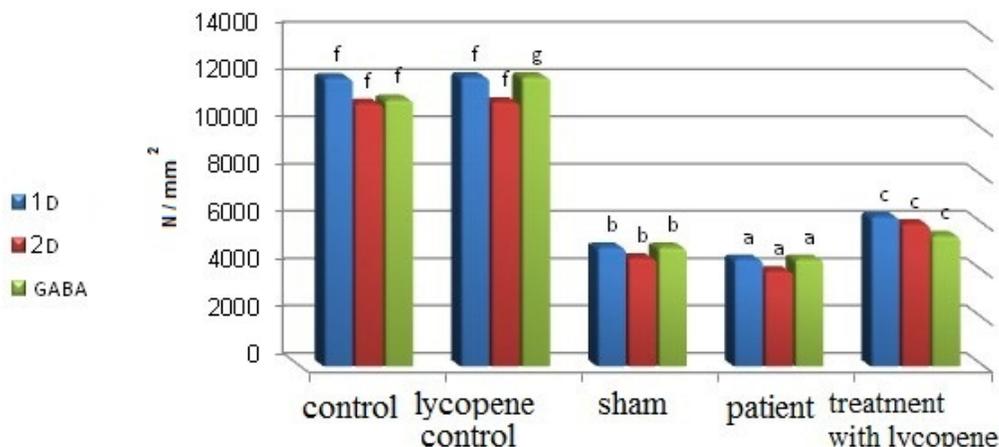
Sampling was performed on 15, 30 and 60 days in different groups (5 rats per day were chosen) with the exception of group 1. Animals were euthanized with high dose of sodium thiopental. Their skulls immediately opened, the brains were removed and transferred into 4% buffer formalin for at least 24 h. The brain tissues were processed through graded alcohol and xylene and infiltrated and embedded in paraffin.

The coronal sections at 5-6 micrometers thick were prepared serially using a microtome device (made in). The sections were stained with Hematoxylin-Eosin (H&E). Histomorphometric studies were done on hippocampus and substantia nigra 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.

2. Results

According to figure 1, the number of D1, D2, and GABA receptors had a significant reduction in hippocampus area of sham and patient groups compared to control and lycopene groups. There was also a significant increase in the number of receptors of hippocampus area in lycopene group compared to patient and sham groups. The number of GABA receptors in lycopene group had a significant increase compared to these receptors in control groups.

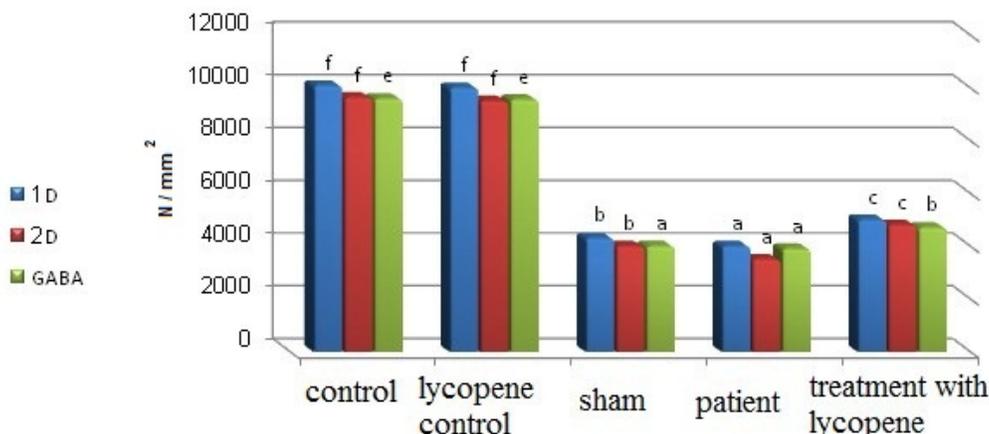
Chart 1: The Average number of receptors in hippocampus area of rats that treated on 15th day



According to Chart 2, the number of D1, D2, and GABA receptors in substantianigra area of sham and patient groups had a significant reduction compared to lycopene and control

groups. There was also a significant increase in the number of receptors at 15th day of treatment in substantianigra area of lycopene group compared to patient group.

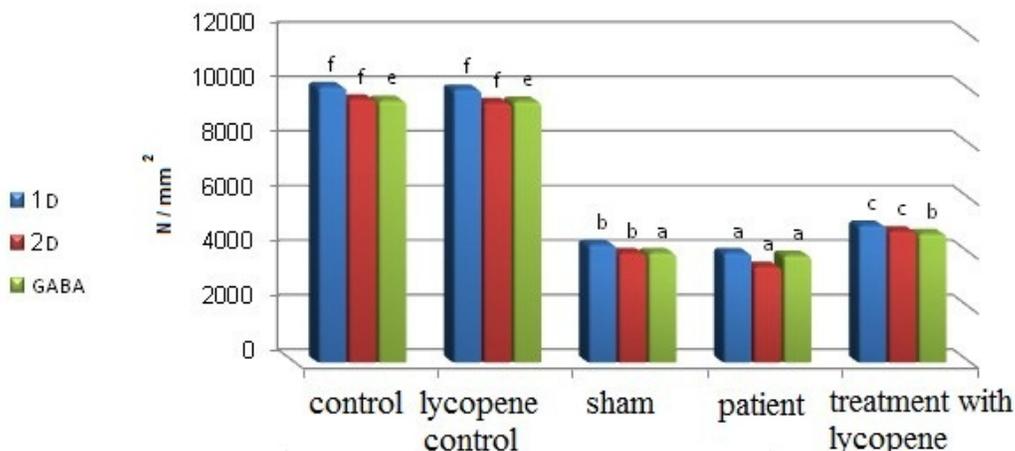
Chart 2: The Average number of receptors in substantianigra area of rats that treated on 15th day



According to Chart 3, the number of D1, D2, and GABA receptors in hippocampus area of sham and patient groups had a significant reduction compared to lycopene and control

groups. There was also a significant increase in the number of receptors at 30th day of treatment in hippocampus area of lycopene group compared to patient group.

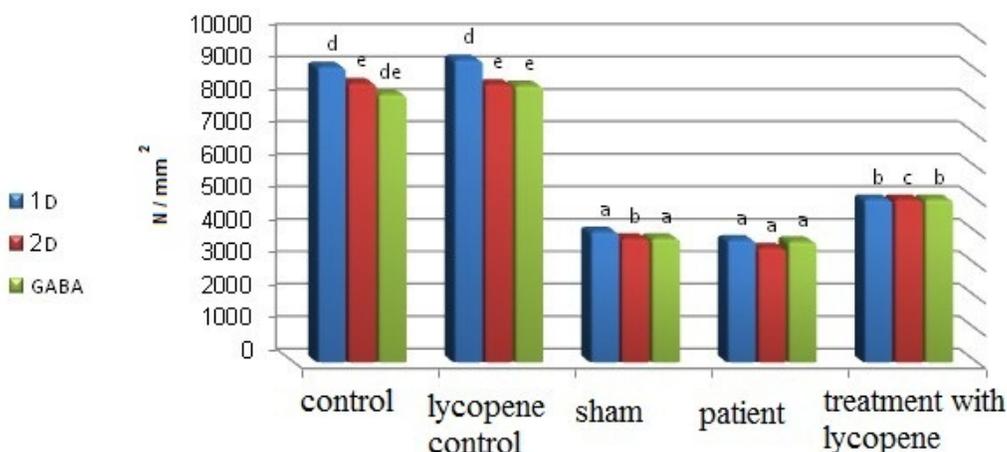
Chart 3: The Average number of receptors in hippocampus area of rats that treated on 30th day



According to Chart 4, the number of D1, D2, and GABA receptors in substantia nigra area of sham and patient groups had a significant reduction compared to lycopene and control groups, at 30th days of treatment. There was also a significant

increase in the number of receptors at 30th days of treatment in substantianigra area of lycopene group compared to patient group.

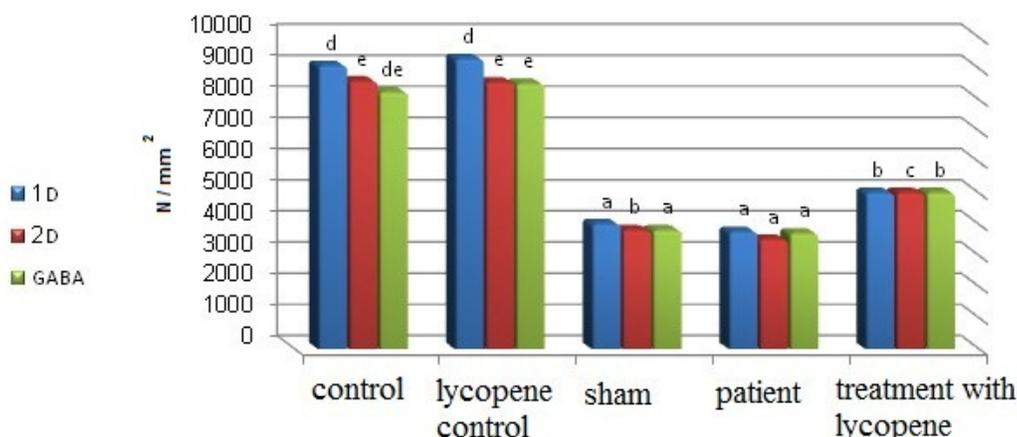
Chart 4: the average number of receptors in substantianigra area of rats at the 30th days of treatment period



According to Chart 5, the number of D1, D2, and GABA receptors in hippocampus area of sham and patient groups had a significant reduction compared to lycopene and control

groups. There was also a significant increase in the number of receptors at 60th days of treatment in hippocampus area of lycopene group compared to patient group.

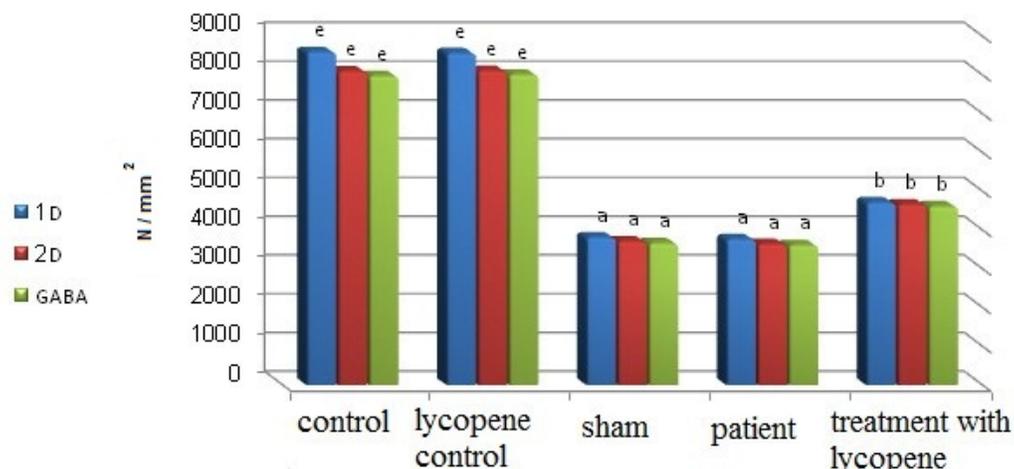
Chart 5: the average number of receptors in hippocampus area of rats at the 60th day of treatment period



According to Chart 6, the number of D1, D2, and GABA receptors in substantianigra area of sham and patient groups had a significant reduction compared to lycopene and control groups, at 60th day of treatment. There was also a significant

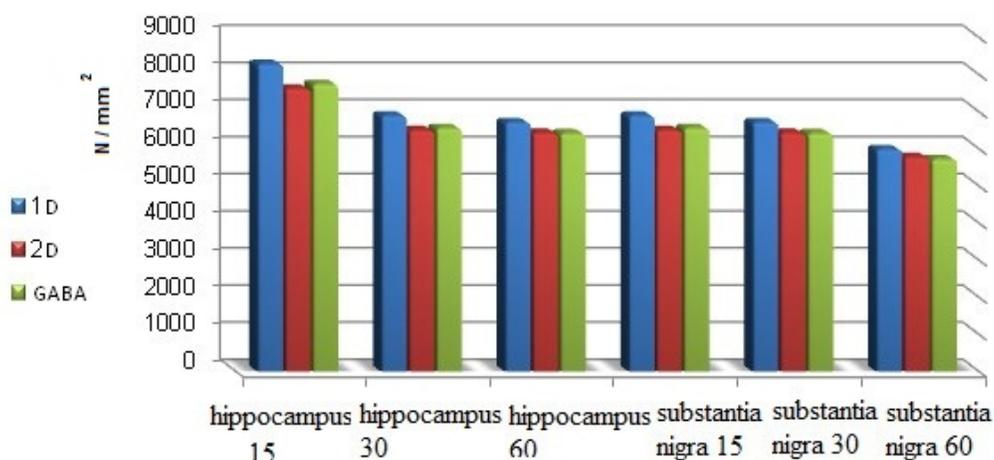
increase in the number of receptors at 60th day of treatment in substantianigra area of lycopene group compared to patient and sham groups.

Chart 6: the average number of receptors in substantianigra area of rats at the 60th day of treatment period



According to Chart 7, the number of receptors in hippocampus was more than in substantianigra area of patient groups and

what is certain is that the density of these receptors was reduced by increase of treatment days.

Chart 7: the average number of receptors in various parts of brain of rats at the 15th day of treatment period

3. Discussion

It was stated in the past about investigation of dopamine receptors' density using of agonists in various areas of brain that these receptors are spread in substantianigra area as well as in ventral tegmental area, cortical region of hippocampus area, olfactory bulb, and most areas of brain. It was also stated according to radiography results that receptors density is high in hippocampus area and then in substantianigra area. This kind of distribution is depending on agonist's type which is used for identification [16].

Density of dopamine receptors as well as GABA receptors in treated mice in the present study was high in hippocampus area compared to substantianigra area. This agrees with the previous researches.

Density of receptors on 15th day of treatment was higher than 30th day, and the density in 30th day was higher than 60th day; on the other hand, as stated before GABA receptors act contrary to D2 and similar to D1 [5] that had been proved in the present study. By increase of GABA receptors, the number of D2 receptors reduced and D1 receptors increased compared to GABA; the density of receptors (dopamine and GABA) in hippocampus and substantianigra areas of investigated groups increased by increase of treatment periods, indicting the negative effect of Parkinson's disease on dopamine and GABA receptors.

Studies in the past on investigation of dopamine receptors effect in mice that their brains physically suffered showed that these receptors play an important role in improvement of patient's brain and the ratio of activity of these receptors in brain of healthy mice was more than in patient mice [17]. The reduction in dopamine receptors in rats which received 6-hydroxy dopamine was also higher in the present study compared to control and healthy groups, indicating the negative effects of Parkinson disease on changes of these receptors. This agrees with earlier researches. It was stated in the past about the role of aminobutyric acid receptors subunits on mental disorders (e.g., Parkinson's disease) that GABA is the most important inhibitory neurotransmitters in central nervous system and plays an important role in coordination of local neural networks and performance of mental areas [18]. Studies have found that density of GABA receptors in brain regions different in various diseases related to the nervous system; for example, GABA receptors in anxiety disorders in frontal cortex areas and temporal cortex has a greater reduction [19], also density of GABA receptors in epilepsy disease of hippocampus and Parahippocampus regions has a greater reduction (20-22). Density of GABA receptors in the lateral

cortex and purkinje cells of cerebellum in autism disease is declined more than the other areas [19, 23].

So according to the above, the density of receptors changes in various diseases, as if in the present study it was found that the density of receptors in Parkinson's disease in substantianigra area was less than in hippocampus. As noted before, Parkinson's disease is a degenerative neurological progressive disorder which associates with reduction of dopamine and GABA- secreting neurons in substantianigra and basal ganglia pathways [1, 4]. Therefore, it is predicted that density of receptors in substantianigra area is less than in hippocampus area. This agrees with results of the present study. In addition to the above, the decline procedure (in receptors) in patient groups that did not receive any extract increased over time; however, the number of receptors in hippocampus and substantianigra in lycopene groups increased in compared to patient groups, on various days; that shows the positive effects of lycopene extracts on treatment and increase of receptors in people with Parkinson's disease.

Some studies stated that lycopene is an essential nutrient for human. It is normally found in diet, 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 [4]. Lycopene has recently been considered by researchers due to its high antioxidant properties. It was reported that lycopene has neuroprotective properties [12] 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. The increase of receptors' numbers in hippocampus and substantianigra areas was also observed in lycopene group compared to patient group. This shows useful function of lycopene in brief improvement of the disease.

4. Conclusion

Given the above, it was found that the number of dopamine (D1 and D2) and GABA receptors in substantianigra area in Parkinson's disease was less than in hippocampus area. This reduction in the studied receptors was seen more over time. Lycopene, due to its antioxidant and neuroprotective properties, reduces the number of brain lesions and thus increases the number of receptors in hippocampus and substantia nigra areas in Parkinson model rats. So, the use of

lycopene is recommended to reduce the side effects of Parkinson's disease, and use of higher doses of lycopene is also recommended for more effective treatments.

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