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Curative Effect of L-Arginine on Neurotoxicity Mice Model

Running title: Effect of L-Arginine on Neurotoxicity Model

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Abstract: ***Purpose: Our study aimed to evaluate the therapeutic effect of L-Arginine onneuron damage induced byhigh dose of monosodium glutamate in brain of Swiss albino mice using histopathological and histochemical examinations. Design/ Methodology/ Approach: Twenty animals were divided into four groups: control group; neurotoxicity model group, arginine treated group and neurotoxicity model- arginine treated group. The experimental period was ten successive days. Findings: Administration of L-Arginine in neurotoxicity model animals resulted in marked ameliorations of cerebellum damage observed in neurotoxicity model group as evidenced by reappearance of normal histological structure and Nissl's granules in Purkinje cells and restore lost cells of granular layers. In conclusions, the administration of L-Arginine could suppress the neurotoxicity induced by high dose of monosodium glutamate. We suggest that L-Arginine may be useful in combating neuron damage.***

Keywords: Neurotoxicity, L-Arginine, Histopathologicalstudy, Nissl's granules, brain, Mice

1. Introduction

Neurodegenerative diseases (as amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, fatal familial insomnia, and Huntington's disease) affect millions of people worldwide. It is characterized by degeneration and/or death of neurons**[1-2]**.Excess monosodium glutamate cause sudden neuron death through release of excitatory amino acids mainly glutamate in process called exitotoxicity process**[3-4]**. Therefore, it has been used in both acute and chronic degenerative diseases, as focal and global ischemia, hypoxia or traumatic brain injury, Parkinson's disease, Alzheimer's disease, Huntington's chorea, spinocerebellarde generations, amyotrophic lateral sclerosis **[3,5, 6]**. This confirmed also by **Zhang, et al., [7],** who stated that excess monosodium glutamate administration (4.0 g/kg/d, ig, ten days) induced behavioral disorders (hyperactivity, disturbance of cooperation movement ability, and lesions of learning and memory), neuronal degeneration and necrosis as investigation histological.

Negative effects of excess uses of monosodium glutamate was investigated in various organs as thymus **[8]**, brain **[9]**, pancreas **[10]**, testis **[11]**, liver and kidney **[12-13]**, and also its extensive uses was linked with several diseases as obesity, hypertension, headaches, asthma exacerbation, neurotoxic effects and detrimental effects on the reproductive organs **[14]**. Recently, **Hajihasan et al.,[15],** reported ingestion of foodstuffs rich in in monosodium glutamate can result in the outbreak of several health disorders such as neurotoxicity, hepatotoxicity, obesity and diabetes.

Cerebellum is represented about ten percent of the total weight of brain, and is contained approximately half of the brain's neurons, specialized cells that transmit information via electrical signals **[16]**. It receives information from the sensory systems (spinal cord, and other parts of the brain), and then regulates motor movements. The cerebellum also coordinates voluntary movements such as posture, balance, coordination, and speech, resulting in smooth and balanced muscular activity **[17-18].** Newer findings shown that cerebellum has been implicated in the regulation of differing functional such as affection, emotion and behavior as well as language, attention, and mental imagery **[19-20]**.

L-Arginine (2-amino-5-guanidino-pentanoic acid) is one of essential amino acid, considered as a normal constituent of the body and is found rich in natural foods as dairy products, nuts, sea foods, wheat flour, and seeds**[21]** that participates in multiple biochemical processes in mammals as protein synthesis, urea cycle, synthesis of amino ,nitric oxide (NO), polyamines, creatine, agmatine, and other guanidino compounds acids **[22].** It considered as precursor of NO, enhances memory development **[23-24]**. Nitric oxide is considered as a vital neuromodulator and is involved in learning, synaptic plasticity, long-term potentiation and the consolidation of long-term memory **[25].** This confirm by **Law**, **et al., [26], who** suggested that depletion of NO generation which observed in aging may have a role in senile memory impairment. Moreover, **Hami etal., [27]**, revealed that pretreatment with L-arginine (300 mg/kg, i.p.) during 7 consecutive days prevent Parkinson's diseases in Balb/c mice induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine**.**Likewise, **Lundblad et al., [28] & Garry et al., [29]**, recorded that L-arginine administration increase cerebral blood flow and reduce neurological damage after experimental traumatic brain injury.

Therefore, the aim of this paper was designed to study the amelioration effect of L-arginine on brain damage induced by excess monosodium glutamate administration in Swiss albino mice using histopathological and histochemical studies.

2. Materials and methods

Animals

Twenty Male Swiss albino mice aged 9 – 12 weeks and weighing 25 -30 gm were used in this study. Animals were obtained from animal house of National Organization for Drug Control and Research (NODCAR) in Giza, Egypt. Animals were supplied with standard commercial diet pellets and water that *Ad-labium*, kept in plastic cages for 7 days to be accommodated with our laboratory conditions before treatment. All male Swiss albino mice were grouped and housed according to the guidelines of the institutional animal's ethics committee of NODCAR. All the experimental procedures were carried out accordance with international guidelines for the care and use of laboratory animals

Chemicals

Monosodium Glutamate (MSG) salt (Shanghai Bio Life Science & Technology Co., Ltd. (China)) was fresh prepared by dissolved in distilled water to prepare desired dose 4gm/kg.bw according to **Zhang., et al., [7],** for oral administration by oral gavage via metal intragastric tube for 10 days. L-Arginine (Cayman chemical company, USA) was fresh prepared by dissolved in distilled water to prepare desired dose 100mg/kg.bw according to **Sánchez-Fidalgo, et al., [30],** to administrate orally by oral gavage via metal intragastric tube for 10 days.

Experimental Groups

Animals were divided randomly into equal four groups as follows: Control group: in this group animals treated orally with distil water (1ml/kg.bw); **Neurotoxicity model group**: animals orally administrated monosodium glutamate (4gm/kg.bw) according to **Zhang., et al., [7].; Arginine treated group**: mice treated orally with L-Arginine at dose 100mg/kg.bw according to **Sánchez-Fidalgo, et al., [30]**; and **Neurotoxicity model-arginine treated group**: animals treated with high dose of monosodium glutamate (4gm/kg.bw) simultaneous with L-Arginine (100mg/kg.bw). The experimental period was 10days.At end of experimental, the animals were subjected to cervical dislocation; dissection and brain tissues were freshly collected directly and immediately transferred to 10% formalin to use in histopathological and histochemical examinations.

Histopathological and histochemical investigations

After 24 hours, the specimens were washed, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Five micron thick paraffin sections were prepared, mounted on clean slides and stained with Ehrlich's haematoxylin-eosin for histological examination **[31] and** toluidine blue stain for demonstration of Nissl's granules.

3. Results

H&E examination of cerebellum in brain:

Light microscopic examination of H&E stained sections from control group showed that cerebellum formed of successive three layers: molecular layer; Purkinje cell layer, and granular layer was the innermost. Purkinje layer appeared as one row of Purkinje cells that have flask shaped with apical dendrites and have pale basophilic cytoplasm and a central vesicular nuclei with prominent nucleoli. Closely packed granule cells formed granular layer **(Figure 1a).**on another hand, cerebellum section from Neurotoxicity model group revealed disorder of Purkinje layer with severe degenerated Purkinje cells with pyknotic nuclei as well as wide area of empty spaces. In addition to, cells of the granular layer showed markedly loss **(Figure 1b)** compared to control group **(Figure 1a)**.

While **L-Arginine treated group** showed normal histological structure of three layers (molecular, Purkinje and granular layers. When administration excess monosodium glutamate simultaneous with L-Arginine revealed reappearance of intact histological structure of Purkinje cells and restores lost cells of granular layers compared to neurotoxicity model group **(Figure 1b)**.

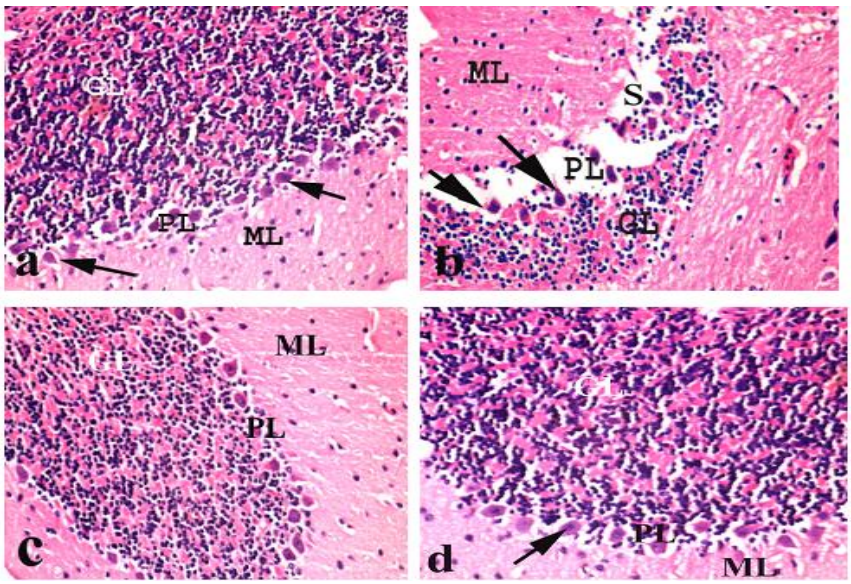

Figure 1: A photomicrograph of cerebellum section staining with H&E, X 200), from: (a) control group showing normal appearance of three layers: molecular (M), Purkinje (P) and granular (G) layers**.** Purkinje layer consisted from one

row of Purkinje cells with flask shaped and apical dendrites (arrow).**, (b) Neurotoxicity model group** showing distribution degenerated Purkinje cells with pyknotic nuclei (arrow) and empty spaces (s) in Purkinje layer (P). Granular layers (G) revealed marked loss of its cells., **(c)Arginine treated group** showing normal histological structure of three layers (molecular (M), Purkinje (P) and granular (G) layers)., **and (d)Neurotoxicity model-arginine treated group** showing reappearance of intact Purkinje cells (arrow) and restore lost cells of granular (G) layers (G).

Toluidine blue staining (demonstration Nissl's granules)
Cerebellum section from control group stained with toluidine blue showed Nissl's granules in cytoplasm of Purkinje cells that surrounded vesicular nuclei **(Figure 1a)**. In contrast, Neurotoxicity model group showed some Purkinje cells pale with pyknotic nuclei and faintly appearance cytoplasm (few Nissl's granules) and others show shrunken deeply stained **(Figure 1b)** compared to control group **(Figure 1a)**. When animals treatment with excess monosodium glutamate and L-Arginine showed reappearance of normal Purkinje cells with restore Nissl's granules in their cytoplasm **(Figure 1c)**compared to neurotoxicity model group **(Figure 1b).**

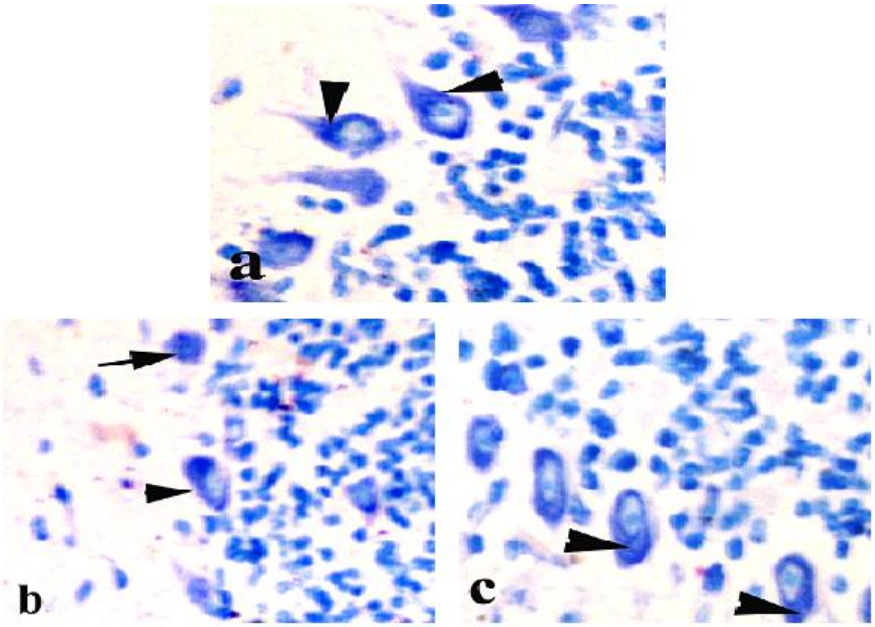

Figure 2: A photomicrograph of cerebellum section staining with toluidine blue, X 1000 from: (a) control group showing Nissl's granules surrounded vesicular centrally nuclei of Purkinje cells (arrow head)., (b) Neurotoxicity model group showing some Purkinje cells appears pale with pyknotic nuclei and faintly appearance (few Nissl's granules) of their cytoplasm (arrow head) and others show shrunken deeply stained (arrow), and (c) Neurotoxicity model-arginine treated group showing reappearance of Nissl's granules in cytoplasm of Purkinje cells surrounding the centrally nuclei (arrow head).

4. Discussion

Aim of this current study is to evaluate the amelioration effect of L-Arginine (100mg/kg.bw) on a model of neurotoxicity induced by excess monosodium glutamate administration (4gm/kg.bw) for ten days. Our results demonstrated that administration of excess monosodium glutamate can caused neuron damage, severe degenerated Purkinje cells with pyknotic nuclei and loss of granular layer cells compared to control group. It also caused loss of Nissl's granules in cytoplasm of Purkinje cells as evidenced by toluidine blue staining. These observed neurotoxicity were agreement with work done by **Zhang, et al., [7]**; **Yu, et al., [32], Zhang, et al., [33]**and could be attributed to excessive activation of glutamate receptor which causes enhanced increasing Ca2+ and Na+ influx that triggers a cascade of enzymatic activities which resulting in neuronal cell death and therefore dramatically changing the normal cellular physiology **[7, 34].** Excessive Ca2+ induced from abnormal stimulation of glutamate receptor caused activation of number of intracellular mechanisms that are potential sources of reactive oxygen species (ROS) **[35-36].** Production of reactive oxygen species resulted in a series of intracellular events such as lipid peroxidation, protein oxidation and protein cross-linking that cause cell death **[37]**

Another reason for observed neurotoxicity of excess monosodium glutamate in current study is enhancing reactive oxygen species, malonaldehyde, and oxidative stress **[13,15, 38]**. Reactive oxygen species caused disruption of cellular metabolism and lipids, carbohydrates, proteins and nucleic acids damage. Oxidative stress is associated with many human diseases as neurodegenerative diseases, diabetes, cardiovascular diseases, atherosclerosis, inflammatory bowel disease, osteoporosis and carcinogenesis **[39-40]**.

A neurotoxic effect of monosodium glutamate administration on neonatal was recorded by **Bodnár et al., [41]**. Administration of monosodium glutamate resulted in distortion of hypothalamus neurons in rats that lead to several metabolic abnormalities as growth disturbances, self mutilation, pseudo-obesity and hypogonadism **[42-43]**.

On another hand, treatment neurotoxicity model animals with L-Arginine resulted in marked amelioration of the histological alterations observed in cerebellum of brain that induced by excess monosodium glutamate administration as evidenced by reappearance of intact Purkinje cells and restore cells of granular layers as well as return appearance of Nissl's granules in Purkinje cells that disappearance in animals treated with excess monosodium glutamate. These were confirmed by work done by **Hami et al.,[27]&Hosseini, et al., [44]**.These observed curative effects of L-Arginine on neurotoxicity of excess monosodium glutamate could be attributed to increase anti-oxidative enzymes **[45]**and attenuation of oxidative stress and malonaldehyde in brain tissues **[27]** as well as scavenge of free radicals **[46]**. L-arginine has ability to interact with superoxide anion, directly or indirectly through NO **[46, 47]**. Malondialdehyde is the end-products of lipid peroxidation **[48]**. Its level was increase after ovariectomy and associated with declination activity of antioxidative enzymes **[49].**

Another explanation of modulation role of L-arginine against neurotoxicity induced by excess monosodium glutamatein the present study is production of nitric oxide and modulation of glutamate reuptake into neural cells so decreasing extracellular glutamate levels, and attenuating glutamate neurotoxicity **[27]**.L-arginine is a semi-essential amino acid and has important roles in the function of normal brain. It is oxidized to nitric oxide. L-arginine and nitric oxide play a modulatory role in the brain, and are involved in synaptogenesis, synaptic plasticity, neurogenesis, neuroprotection, memory and learning function, and neuroendocrine secretion **[50].** Nitric oxide also promotes easy and efficient flow of blood through the blood vessels going to the brain **[29].**

In conclusion, the results of the present study showed that L-arginine prevent neurotoxicity induced by excess monosodium glutamate through production nitric oxide and amelioration histological alterations in brain tissues.

Conflicts of interest

The authors have no conflicts of interest to declare.

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