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Potential Theraputic Effect of Liraglutide in Male Rats Parkinsonn'Disease Model

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Abstract:

Parkinson's disease (PD) is a progressive neurodegenerative, chronic disease presented by both nonmotor and motor features. The neuronal loss which rich by dopamine in striatum was the main cause for the PD motor symptoms. While neuronal loss in non-dopaminergic areas support PD nonmotor. liraglutide (Glucagon like peptide-1 mimetic), have effect on CNS by decreasing the number of neutrophils infiltration within CNS which reflect anti-inflammatory characteristics in model of intracerebral haemorrhage in mice. This study represent an attempt o investigate the potential therapeutic effect of liraglutide in male rats Parkinson's disease model. Thirty six adult male rats were divided into three equal groups. Rats in group A were treated with saline intraperitoneally for 30 days. Meanwhile these rats in group B and group C were treated with 6- hydroxydopamine (6-OHDA) toxicant unilaterally intrathecal injection in a dose of 8 µg/per rat in 2 µl distal water, to introduce Parkinson model in rats. Group B were treated similar to that of group A with saline intraperitoneally for 30 days, while group C were treated with liraglutide intraperitoneally for 30 days in a dose 25nmol/kg. Histological changes were observed by light microscopy. The histopathological study showed that neurotoxins, that is, 6-OHDA, caused marked hypertrophic changes, infiltration of neutrophils, alterations of architecture, and even cell death in group B. Furthermore, many neurons were shrunken, and darkly stained with small nuclei compared with normal vehicle treated rats of group A. There is marked reversal of neuronal damage or neuronal alterations observed with liraglutide (25 nmol/ kg) treated rats in group C.

التأثير العلاجي المحتمل لليراجلوتايد على نموذج مرض الباركنسون في ذكور الجرذان

الخلاصة:

مرض الباركنسون هو مرض مزمن، مرض الأعصاب التدريجي يتميز بمميزات حركية وبميزات لا حركية. الأعراض الحركية لمرض الباركنسون سببها فقدان خلايا الدوبامين العصبية . والأعراض الغير حركية لمرض الباركنسون سببها فقدان الخلايا العصبية في مناطق لا تحتوي على الدوبامين. الجلوكاجون مثل الببتيد (GLP-1) يعزز نمو الخلايا العصبية وتكاثرها. ويحد من موت الخلايا العصبية المبرمج. دواء ليراجلوتايد (مماثل الجلوكاجون مثل الببتيد) يظهر خصائص مضادة للالتهابات عن طريق خفض نضوح الخلايا العدلة في الجهاز العصبي المركزي في الفئران التي لها نزيف داخل المخ. الهدف من الدراسة هو التحقق من ألتأثيرات العلاجية المحتملة لليراجلوتايد في ذكور الجرذان ذات نموذج من مرض الباركنسون. تم تقسيم ستة وثلاثين من ذكور الجرذان البالغين إلى ثلاث مجموعات متساوية. تم علاج الجرذان في المجموعة ألاولى مع المياه المالحة بالحقن البريتونى لمدة 30 يوما . وفي الوقت نفسه تم علاج هذه الجرذان في المجموعة الثانية والمجموعة الثالثة مع هايدروكسي دوبامين السمي والذي تم حقنه داخل السائل الشوكي من جانب واحد في جرعة 8 ميكروغرام / لكل جرذ في 2 ميكرولتر من الماء المقطر، ليستحث نموذج باركنسون في الجرذان. وعولجت المجموعة الثانية مماثلة لتلك التي من المجموعة ألاولى مع المياه المالحة بالحقن البريتونى لمدة 30

يومـا، فـي حـين تـم التعامـل مـع المجموعـة الثالثـة مـع ليراجلوتايـد بـالحقن البريتـونى لمـدة 30 يومـا فـي جرعـة 25 نـانومول / كيلـوغرام. أظهـرت الدراسـة النسـيجية أن هايدروكسـي دوبـامين تسـبب بتغيـرات ملحوظـة فـي المجموعـة الثانيـة كزيـادة حجـم الخلايـا، نضـوح الخلايـا العدلـة، التغيـر فـي شـكل الخليـة، تحطـم الاعصـاب وحتـى مـوت الخليـة. وعـلاوة علـى ذلـك، فـإن العديـد مـن الخلايـا العصـبية انكمشـت، وأصـطبغت بعمـق مـع نـواة صـغيرة بالمقارنـة مـع الجـرذان الغيـر المعالجـة العاديـة للمجموعـة الاولـى. وامـا بالنسـبة الـى المجموعـة الثالثـة فالنتـائج اظهـرت انعكـاس كبيـر مـن تلـف الخلايـا العصـبية الحاصـل نتيجـة هايدروكسـي دوبـامين والتـي لوحظـت بعـد حقـن ليراجلوتايد (25 نانومول / كيلوغرام) لمدة شهر للمجوعة.

Introduction:

Parkinson's disease (PD) was first discovered in 1817 by Dr. James Parkinson which describe as a shaking palsy. It was a progressive neurodegenerative, chronic disease which characterized by features as nonmotor and motor. Because the Parkinson's disease was a progressive degenerative effects by its action on mobility and muscle control; therefore, it had a significant clinical impact on patient itself, his family, and on his caregiver. The loss of striatal neuron that dopamine abundant lead to PD motor symptoms; meanwhile, a PD nonmotor symptom's presence that support neuronal loss in non-dopaminergic areas[1].

Parkinson's disease prevalence in developed countries is speculated between 100 and 300 /100,000. The incidence of PD is stated in the studies that conducted in Europe, were between 9 and 22 /100,000[2].

Management of PD could be divided into pharmacologic, nonpharmacological, and surgical therapy. The pharmacologic treatment of PD could be further divided into neuroprotective and symptomatic treatment. Practically all the treatments available for PD are symptomatic in nature and do not reverse or slow the causes of PD. However, there are many investigation about agent which discovered to have a promise neuroprotective effects in PD model in animal or in patient with PD [3].

The nonpharmacological management of PD include education, support, nutrition and exercise [4].

There are many symptomatic drugs for treatment of PD motor symptoms which includes: Levodopa, Monoamine oxidase (MAO) B inhibitors, Dopamine agonists, Catechol-O-methyl transferase (COMT) inhibitors, Amantadine and Anticholinergic agents[4].

There were many neuroprotective drugs or potential disease modifying therapies which are evaluated by the Neurological Disorders National Institute to show its effects on PD patients[5,6].

Exenatide is a glucagon-like peptide-1 mimetic that's receptor agonist of glucagon like peptide (GLP-1) used in patients with type 2 diabetes to medicate them. Glucagon like peptide (GLP-1) and glucose-dependent insulinotrophic polypeptide (GIP) are secreted from intestinal cells as incretin hormones when the nutrients incoming. After feeding, Glucagon like peptide (GLP-1) and GIP are secreted to blood stream. The two major incretin hormones are secreted from different cell in small intestinal lumen. Glucose-dependent insulinotrophic polypeptide (GIP) and GLP-1 are excreted from K cells and L cells of the small intestine, respectively [7].

In the brain, GLP-1 prove to pass the BBB through simple diffusion, so GLP-1 could induce neurite outgrowth, promotes proliferation and neuronal growth and deprives neuronal apoptosis [8]. Glucagon like peptide-1 receptor (GLP-1R) is a member of 7-transmembrane-spanning, heterotrimeric G protein- coupled receptors of the class B family [9]. There is a study demonstrate that GLP-1 receptors widely expressed in humans and rodents in all parts of the brain [10]. Glucagon like peptide-1 receptor consist from a subunit, G subunit activated by GLP-1 leads to activation of the adenylyl cyclase which lead to enhance production of intracellular cyclic adenosine monophosphate (cAMP) [11]. Glucagon like peptide-1 promote

cAMP-mediated pathways which lead to a central effect as inhibition of apoptotic actions in β-cells[9], also the agents which elevate the cAMP appear to have neuroprotective effects in many neuronal cells[12]. The neuroprotective effect of GLP-1 may be mediate by another signaling pathway, like phospho inositol 3 kinase (PI3K) and Mitogen-activated protein kinases (MAPK) pathways[13].

Materials and Methods:

Study design:

Thirty six of adult male Wister rats, weighing (200 – 250) mg have been used in an experiment after getting approval from ethical committee at College of Pharmacy/ Al- Mustansiriyah University, which were obtained from animal house, divided into 3 groups, each group consists of 12 animals.

Rats in group A were treated with saline intraperitoneally for 30 days. Meanwhile, the rats in group B also treated with saline intraperitoneally for 30 days but after unilateral intrathecal injection of 6-hydroxydopamine (6-OHDA) toxicant in a dose of 8 µg/per rat in 2 µl distal water, to introduce Parkinson model in rats. While group C were treated with intraperitoneally liraglutide in a dose 25nmol/kg for 30 days but after unilateral intrathecal injection of 6- hydroxydopamine (6-OHDA) toxicant in a dose of 8 µg/per rat in 2 µl distal water, to introduce Parkinson model in rats. For keeping three animals / cage, they used plastic cages of (20x25x35 cm) dimension.

This research completed in Baghdad at College of Pharmacy/ Al- Mustansiriyah University on 2016.

Animal model:

There are different methods to include Parkinson's model. In the present study, 6 –hydroxydopamine (6- OHDA) was selected as toxin given as unilateral intrathecal injection in stereotaxic coordinate in substintia nigra pars combacta to induce Parkinson[14].

In the current study, the rats were fixed in the flat position and then anaesthetized with I.P injection of xylazine (10mg/ kg) and ketamine (80 mg/ kg).

Then rat's hair was shaved and cleared with povidone iodine 10 %, then an incision was made centrally. Second stage, three dimension (−5.0 mm anteroposterior(AP) from bregma , 2.1 mm mediolateral (ML) from the midline and -7.7 mm dorsoventral (DV) from the skull) were used to determine the coordinate as stereotaxic position of hole[15]. After making hole by drill, the 6-OHDA (8 µg/per rat in 2 µl distal water) was infused into the right substantia nigra. It is important to keep syringe for an additional 2 min and withdraw it slowly[16]. Each day of the experiment, Liraglutide 25nmol/kg given I.P to group C[17].

Chemicals

6- Hydroxydopamine purchased from hyper chem – China, while liraglutide purchased from Novo nordisk-Denamark.

Histopathology

Brains were collected from the rats and fixed with 10% formalin for 24 hr and then embedded into paraffin, then sectioning into 5um by slide microtome and finally stained with haematoxylin and eosin (H&E). The sections were examined under light microscope and photomicrograph were obtained.

Results:

At sacrifice, microscopic finding of control group of rat's brain show a normal architecture, normal brain tissue cells, no degeneration or necrosis present and infiltrating inflammatory cells, as in figure 1. Parkinson's model by 6-OHDA in group B cause marked hypertrophic changes, infiltration of neutrophils, alteration of architectures, neuronal damage and even cell death. Furthermore, many neurons were shrunken and darkly stained with small nuclei, as shown in figure 2. The tissue section from treated group (6-OHDA + liraglutide) as in figure-3 showing the majority of substantia nigra cells were preserved and look like normal

with a little of necrosis and inflammatory cells.

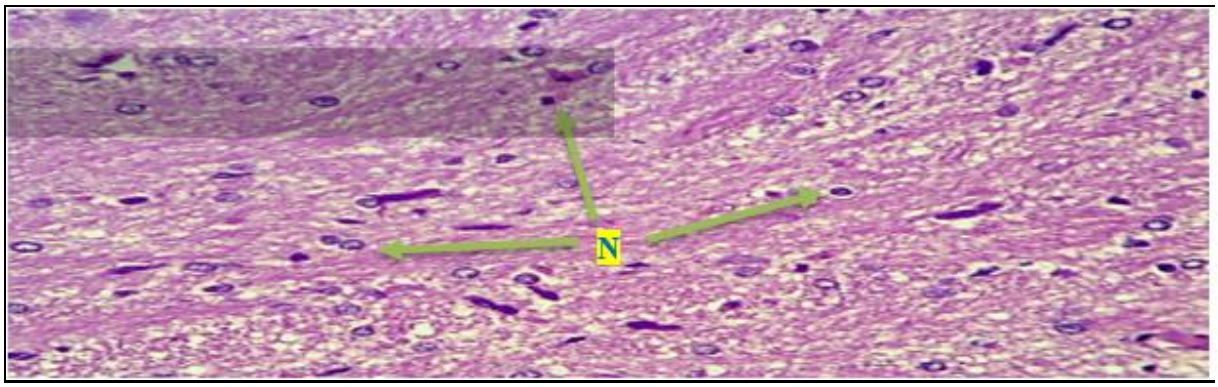

Figure-1: Light microscopic section of rat substantia nigra of H&E stain of group A which received I.P saline for 30 days showing normal substantia nigra cells, no necrosis or degeneration and no inflammatory cells, (N: normal cells).(400X).

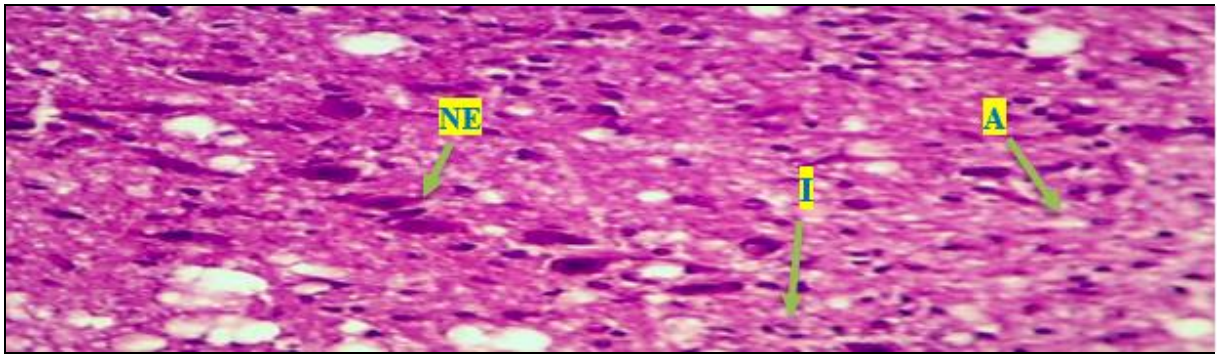

Figure-2: Light microscopic section of rat substantia nigra of H&E stain of group B which received 6- OHDA (8µg) in the right substantia nigra showing degeneration and necrosis of substantia nigra cells with mononuclear inflammatory cells, (NE :Necrotic cells, I: Inflammatory cell, A: Apoptotic body). (400X).

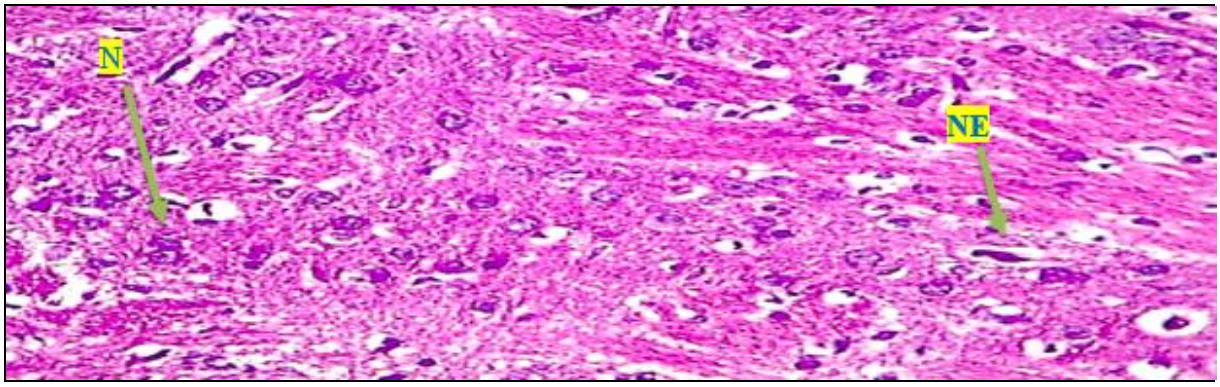

Figure-3: Light microscopic section of rat substantia nigra of H&E stain of group C received liraglutide (25 nmol/kg) intraperitoneally after unilateral intrathecal injection of 6-hydroxydopamine toxin showing that majority of substantia nigra cells preserved and living look – like normal with rare degenerated cells, (N: normal cells, NE :Necrotic cells).(400X).

Discussion:

Histological results of brain tissue obtained from the group B injected with 6-OHDA unilateral intrathecal injection at first day and I.P saline continuously for 30 days to some

extent is identical with prior studies as Sotelo and Blandini said that Stereotaxic injections of 6-OHDA in the substantia nigra of rats cause severe local pathological patterns represented by an unselective necrotic lesion, in which the oedema (as a part of inflammatory process) is the most prominent neuronal and glial elements show clear degenerative changes. As a consequence of inflammation at subcellular level primarily, the 6-OHDA destroys the rough endoplasmic reticular system, thus inducing a necrosis of the whole neuron as a result of decreasing ATP production, increasing ROS production, and increasing apoptosis of dopaminergic cells [14,18]. The present study showed that both of necrosis and apoptotic bodies present in group B as both mechanisms of cell death (necrotic and apoptotic) occur in response to 6-OHDA as there are many attempts to explain which apoptotic pathways are activated, several studies have pinpointed a role for the mitochondrial-caspase cascade in 6-OHDA-induced apoptosis, which initiates the activation of the main effector caspases 3 and 7[19].

The dopaminergic damage can occur by another means, like the 6- OHDA which cause microglia activation, once activated, morphological changes occur on microglia as well as phenotypic alterations in gene expression and generation of signaling molecules. The most important thing is that the microglia will mount a series of responses, starting from the oxidative stress response, often linked with the product ROS which are oxygen-containing molecules that react with and oxidize vulnerable cellular constituents, including proteins, nucleic acids, and lipids. Particularly, the brain is vulnerable to the excess generation of ROS and RNS[20].

There is another way of causing degeneration of the toxic substance by microglia cause , like TNF-α, which secreted from activated microglia that is induced by 6- OHDA as *in vitro*, TNF-α administration or expression has been shown to be toxic to dopaminergic neurons[20].

Meanwhile, the histopathological findings in treated group with liraglutide (25nmol/kg) I.P for 30 days after 6-OHDA neurotoxin, showed that majority of brain tissue cell preserved and appearantly normal with some degenerated cells, similarly to the extent of not quite what Salcedo and coworkers findings in their research, decreased infiltration of neutrophils, reduced intracellular space, and regained normal architecture and moderate necrosis in the region of brain by ability of GLP-1R stimulation to induce beneficial physiological effects on neural cell proliferation, neural stem cell differentiation with neurite outgrowth and improved synaptic plasticity[21].

Stimulation of the GLP-1R has been shown to protect neurons from 6-OHDA exposure as GLP-1 agonist protected SH-SY5Y cells against 6-OHDA-induced cell lesion. When cultures are made of mesencephalic neuron, that are rich in dopaminergic neurons, the number of tyrosine hydroxylase-positive cells lowered by 6-OHDA, indicating dopaminergic neuronal death so treatment with GLP-1 agonist was not only shown to save dopaminergic neurons but to induce a 60% increase in tyrosine hydroxylase-positive cells over control values[22]. Likewise, the normal architecture of brain tissue can be maintained by liraglutide which has antiapoptotic action and neuroprotective effect by mediated actions through the G protein-coupled GLP-1R. Glucagon like peptide recptor (GLP-1R) which is a member of the class B family of 7-

transmembrane-spanning, heterotrimeric G protein-coupled receptors. In humans and rodents, a single structure which is identical to GLP-1R has been identified and expressed in a wide range of tissues, including the brain. Ligand activation of the Gα subunit of GLP-1R on cells leads to activate the adenylate cyclase activity and production of cAMP[11].

Cyclic adenosine monophosphate-mediated pathways are central to the antiapoptotic actions of GLP-1 in neuronal cells by the activation of PI3K lead to phosphorylation of PKB and inhibition to bax, bad and caspase 3,9[9].

In addition, neuroprotective activity of liraglutide by cAMP/PKA/CREB pathway was shown in neuronal cultures and in a mouse model of traumatic brain injury, where liraglutide rescued neuronal cells from oxidative stress and glutamate excitotoxicity-induced cell death and ameliorated memory impairment in mice caused by traumatic brain injury via cAMP/PKA signalling[23].

Liraglutide neuroprotective effect can be explained by the ability of liraglutide to activate the PI3K/AKT and MAPK pathways, and phosphorylated Akt and ERK up-regulate the expression level of Bcl-2 and Bcl-xl. The Bcl-2 and Bcl-xl can supress ROS generation, and the reduced level of ROS in turn decreases the inhibition of AKT and ERK activity, which ultimately inhibits the extrinsic and intrinsic apoptotic signaling pathways to block apoptosis[24].

By inhibition of ROS by liraglutide, the stimuli to microglia would be removed, microglia can promote neurogenesis by releasing the neurotrophins and anti-inflammatory cytokines, potentially leading to neuroregeneration and wound healing within the SN and striatum[25].

Conclusion:

Liraglutide treatment prevents histological changes, occurred with 6 –OHDA when given unilateral intrathecal injection.

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