Research Article

Potential Therapeutic Effect of Liraglutide in Male Rats Parkinson Disease Model

Ahmed H. Abdulkareem[a*], Mustafa G. Alabbassi[a]*, Mustafa M. AL-Obaidy[b*].
[a]Department of pharmacology/ College of Pharmacy/ the University of Mustansiriyah/ Baghdad/ Iraq
[b]Department of Anatomy/ College of medicine/ the University of Mustansiriyah/ Baghdad/ Iraq.
***Corresponding author's E-mail:** ahmedhazemph@gmail.com

Received: 25-02-2017; **Revised:** 25-04-2017; **Accepted:** 05-05-2017.

ABSTRACT

The objective of this study to investigate the potential therapeutic effect of liraglutide in male rats Parkinson's disease model. Glucagon like peptide (GLP-1) have been many effect on CNS. Thirty six adult male rats were divided into three equal groups. An Immuno histochemical change for glutathione peroxidase-4 and interleukin- 1betawere observed by light microscopy. The study has been started after get the approval at ethical committee at the college of Pharmacy/ Al-Must ansiriyah University. Immuno histochemical results showed that highly significant ($p<0.01$) changes occur between groups B and group C regarding glutathione peroxidase-4 immuno histochemical kit. Results of Interleukin- 1 beta (IL-1β) kit also showed a very highly significant ($P<0.001$) changes occur between 3 groups. Pursuant with the achieved of these results: It has been shown that liraglutide significantly lower inflammatory marker (IL-1β) in brain tissue. The ability of liraglutide to enhance endogenous anti-oxidant enzyme (glutathione peroxidase-4) and protect the dopaminergic cells.

Keywords: Parkinson model, 6- hydroxydopamine, liraglutide.

INTRODUCTION

In the brain, Glucagon like peptide -1 (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[1]. GLP-1R is a member of 7-transmembrane-spanning, hetero trimeric G protein-coupled receptors of the class B family[2]. There is another study demonstrate that GLP-1 receptors widely expressed in humans and rodents in all parts of the brain[3]. 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)[4]. Glucagon like peptide-1 promote cAMP-mediated pathways which lead to a central effect central as inhibition of apoptotic actions in β-cells[2], also the agents which elevate the cAMP appear to have neuroprotective effects in many neuronal cells[5]. 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[6].

MATERIAL AND METHODS

Chemicals

There are many chemicals and reagent in a very high level purifying and accuracy used in this research as listed in the tables below.

Table (2-1): Materials and solvents.

Chemical	Suppliers
6- Hydroxydopamine powder	Hyper chem – China
Diethyl Ether	QualiKems-India
DPX mounting medium	SyrBio-Switzerland
Ethanol (99%)	Scharlau-Spain
Eosin solution (water-based)	SyrBio-Switzerland
Formalin (37%-40%)	SIGMA CHEMICAL co.-USA
Hematoxylin solution	SyrBio-Switzerland
Ketamine Vial	Germany
Liraglutide prefilled injection	Novo nordisk-Denamark
Oxytetracycline	Norbrook Laboratories limited
Phosphate buffer solution (PBS)	DAKO, Denmark
Positively charged microscope slides	FisherbrandSuperfrost-USA
Paraffin wax (5 kilos)	Medite-USA
Tween 20	SCRC-China
Xylene	Scharlau-Spain
Xylazine Vial	Arendonk-Belgium

Table (2-2): The Secondaryimmuno-histochemical detection system.

Materials	Quantity	Part Number	Company
Peroxidase Block (H_2O_2)	5 ml	2D054051	Biovision
Protein Blocking solution	5ml	2D054052	Biovision
Primary Ab dilution buffer	8ml	2D054053	Biovision
HRP-anti-Mouse, Rat & Rabbit Polymer	5ml	2D054054	Biovision
Reagent BS (buffer & substrate)	5ml	2D054055	Biovision
Reagent C (conc. DAB chromogen)	1ml	2D054056	Biovision

Table (2-3): Interleukin-1 beta (IL-1β) antibody kit.

Materials	Quantity	Catalog no.	Company
KLH is connected with synthetic peptide to consist a series of the N-term region of human IL-1beta	1 mg	ABIN1582289	Abbiotec

Table (2-4): Glutathione peroxidase-4 antibody kit.

Materials	Quantity	Catalog no.	Company
Glutathione peroxidase 4 antibody	50 µg	ABIN1735233	Abbiotec

Devices:

Devices which used in this research are summarized in table (2-6) with their providers (company).

Table (2-5): Instruments and their suppliers.

Devices	Providers (company)
Leica DM4000 B LED refer to automatic microscope system intended LED Illumination are use Life Sciences.	Leica-microsystems
Leica EG1150 C refer to plate for modular tissue embedding.	Leica-biosystems
Centrifuge	Hettich Universal (Germany)
Electrical oven	Memmert(Germany)
Leica EG1150 H refer to heated paraffin embedding module.	Leica-biosystems
Microplate washer ELx50	Biotec ELx50 (Germany)
Microplate reader	Biotec ELx800 (Germany)
Multistainer Leica ST5020	Leica-biosystems
Mettler H54 A.R. Microbalance	Karl Kolb (Germany)
Orbital Shaker	GFL (Germany)
pH meter	Inolab (Germany)
Leica RM2245 identified as a semi-automated rotary microtome.	Leica-biosystems
Leica TP1020 is a benchtop for tissue processing.	Leica-biosystems
Thermostatic waterbath	Gemmy (Tiawan)
Vortex Mixer	Cleaver(Germany)

Animals and 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- Must ansiriyah University, which were found, 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 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. For keeping three animals / cage, they used plastic cages of (20x25x35 cm) dimension. In the same cage, there were differences of animals in tail marking by using water proof markers. Before starting study protocol, the animals were kept for 14 days under controlled conditional of temperature of (22 ± 1^0C) and light schedule should be 12 – 12 hour's light / dark cycles. It is necessary to prepare the animal house with an air vacuum adapt with the environment. As long as foods and water were freely found to these animals. This research completed in Baghdad at College of Pharmacy/ Al- Must ansiriyah University on 2016.

Model:

Group	Glutathione peroxidase 4
A	128±10.61
B	58±6.35
C	387±20.3 [b,] **
P-value (ANOVA)	**0.008**

**High significant difference of independent T-test compare with group A (P<0.01).

[b] mean significantly difference when compared with group B.

A control group (30 days IP saline)

B group (30 days IP saline but after unilateral intrathecal injection of 6-OHDA)

C group (30 days IP liraglutide but after unilateral intrathecal injection of 6-OHDA)

There are different methods to have Parkinson's model. In the present study, 6 – hydroxydopamine (6- OHDA) was selected as toxin given unilateral intrathecal injection in stereotaxic coordinate in SNpc to induce Parkinson[7].

Surgery

Upon studied research regarding rats which were fixed in the flat position, after the rats had been anaesthetized with I.P injection of xylazine (10mg/ kg) and ketamine (80 mg/ kg), were clearly shown reflexes loss of pad and corneal.

Then rat's hair was shaved and cleared with povidone iodine 10 %, and 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[(8)]. After making hole by drill, the 6-OHDA (8 µg/per rat in 2 µl distal water) was infused into the right substantianigra. It is important to keep syringe for an additional 2 min and withdraw it slowly[9].

Finally, non – absorbable suture is used to suture wound and then local oxytetracycline, gentian violet and povidone 10% were applied as prophylaxis from infection.

Treatment administration and Organ harvest

Each day of the experiment Liraglutide 25nmol/kg given I.P to group C[10]. On day thirty one of the experiment, the rats were sacrificed after they had been anesthetized by IP injection of xylazine (10 mg/kg) and ketamine (80 mg/kg). Then a decapitation were formed to euthanized them[11,12]. After cutting the skull as in U shape by using scissors, the skull bone is removed by forceps as parts then the brain is gently removed from the site, finally is washed by tape water and then is placed in formalin 10%.

Preparation and staining of tissue

Preparation of tissue

After fixation with 10% buffered formalin tissue processing will be started according to Bancroft and Stevens the tissues were processed as follows[13].

Statistical analysis

Data analysis has been done by using Statistical Packages for Social Sciences, version 16 (SPSS-16) for windows. The significance of difference of different means were tested using **ANOVA** test for difference among more than two independent means followed by Tukey test. Statistical significance was considered whenever the P value was less than 0.05 and highly significant if it was less than 0.01[14].

RESULTS

Glutathione peroxidase 4

The descriptive statistics, which represent the mean ± SD value for the score of glutathione peroxidase content (score * intensity) expression in 3 groups are displayed in table (3-1).

Table (3-1): Changes in brain glutathione peroxidase (Score*intensity). Data are expressed as mean ± SD.

Analysis of data statistically by independent T-test test revealed a significant difference (P< 0.05) between treated groups.

It has been shown that mean (58±6.35) of group B figure (3-5) shown a non-significant (p > 0.05) changes when compared by independent T- test with mean (128±10.61) of group A figure (3-4) regarding glutathione peroxidase-4 .

In addition there was a non-significant (p > 0.05) changes occur when compared mean (387±20.3) of group C figure (3-6) by independent T-test with mean (128±10.61) of group A regarding glutathione peroxidase-4.

Meanwhile a high significant (p < 0.01) changes occur regarding glutathione peroxidase-4 when compare mean (378±20.3) of group C figure (3-6) by independent T-test with mean (58±6.35) of group B figure (3-5). In comparison of all groups with group A there was highly statistical significant using ANOVA test where P < 0.01.

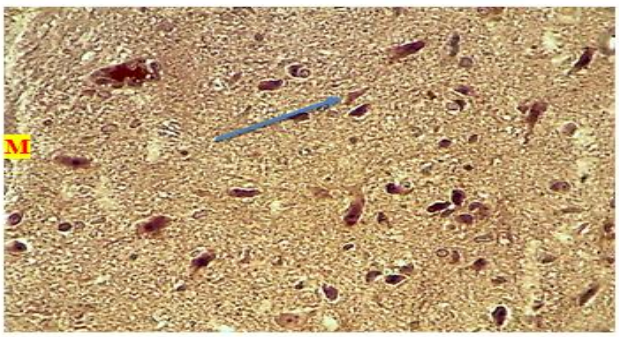

Figure (3-4): Microphotograph of immunohistochemical (IHC) staining of rat brain tissue of glutathione peroxidase 4 of control **group A** received IP saline for 30 days showed +ve brown cytoplasmic immunohistochemical stain of moderate intensity, (M: moderate intensity). (400x).

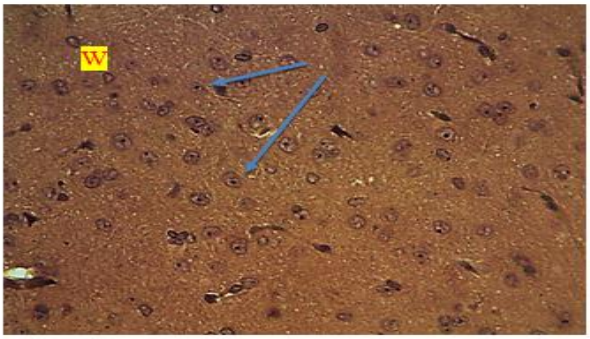

Figure (3-5): Microphotograph of immunohistochemical (IHC) staining of glutathione peroxidase 4 of rat brain tissue of**group B** which received 6- OHDA (8µg) unilateral intrathecal injection in the right sustantia nigra and IP saline for 30 days showing + ve brown cytoplasmic immunohistochemical stain of weak intensity, (w: weak intensity). (400x).

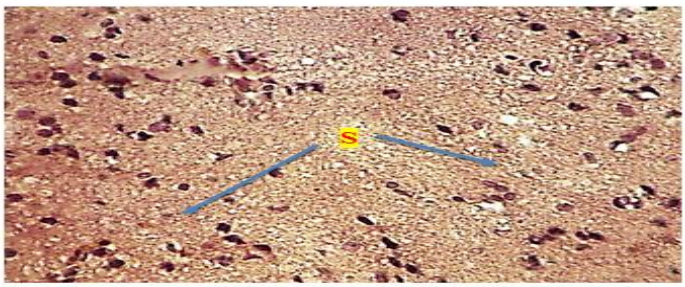

Figure (3-6): Microphotograph of immunohistochemical (IHC) staining of glutathione peroxidase of rat brain tissue **group C** received liraglutide (25 nmol/kg) intraperitoneally for 30 days after unilateral intrathecal injection of 6-hydroxydopamine (8µg) showing +ve brown cytoplasmic immunohistochemical stain of strong intensity.(S: Strong intensity). (400x).

Group	Interleukin 1 Beta
A	58±6.35 [b,]
B	485±27.38 [a, c,] **
C	60±6.32 [b,] **
P-value (ANOVA)	**0.0001**

**High significant difference of independent T- test when compared with group A (P less than 0.01).

[a] Mean significantly different when compared with group A (P value less than 0.05).

[b] Mean significantly different compared with group B (P value less than 0.05).

[c] Mean significantly different compared with group C (P value less than 0.05).

A control group (30 days IP saline)

B group (30 days IP saline but after unilateral intrathecal injection of 6-OHDA)

C group (30 days IP liraglutide but after unilateral intrathecal injection of 6-OHDA)

Interleukin 1 Beta:

The descriptive statistics, which represent the mean ± SD value for the score of interleukin 1 beta content (score * intensity) expression in 3 groups are displayed in table (3-2).

Table (3-2): Changes in brain rat interleukin 1 beta (Score*intensity). Data are expressed as mean ± SD

Analysis of data statistically by independent T-test test revealed a significant difference (P< 0.05) between treated groups. It has been shown that the mean value of interleukin 1 beta in group B showed a high significant elevation (p < 0.01) when compared by independent T- test with mean value of group A as in figure (3-7) and figure (3-8).

In addition there was a highly significant (p < 0.01) changes also occur when compared mean (485±27.38) of group B figure (3-8) by independent T-test with mean (60±6.32) of group C figure (3-9) regarding interleukin 1 beta.

Meanwhile a non-significant (p > 0.05) changes occur regarding interleukin 1 beta when compare mean (60±6.32) of group C figure (3-9) by independent T-test with mean (58±6.35) of group B figure (3-8). In comparison of all groups to group A there was a very highly statistical significant using ANOVA test where (P <0.001).

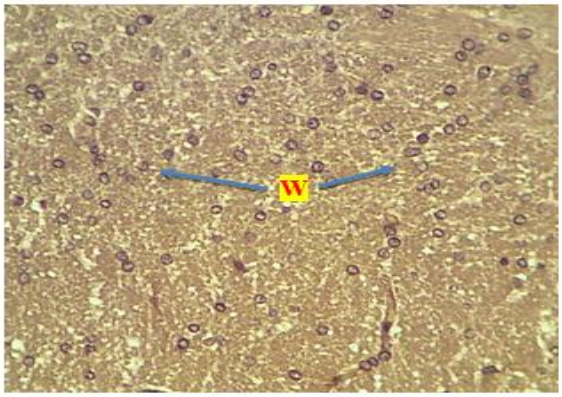

Figure (3-7): Microphotograph of immunohistochemical (IHC) staining of rat brain tissue of interleukin 1 beta of control **group A** received IP saline for 30 days of showed + ve brown cytoplasmic immunohistochemical stain of moderate intensity (W: Weak intensity). (400x).

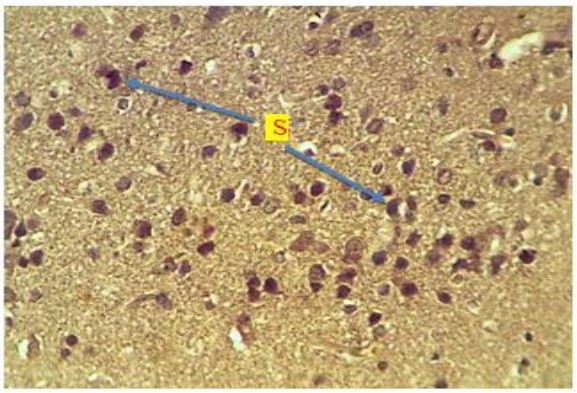

Figure (3-8): Microphotograph of immunohistochemical (IHC) staining of interleukin 1 beta of rat brain tissue of**group B** which received 6- OHDA (8µg) unilateral intrathecal injection in the right sustantia nigra and IP saline for 30 days showing + ve brown cytoplasmic immunohistochemical stain (S: strong intensity). (400x).

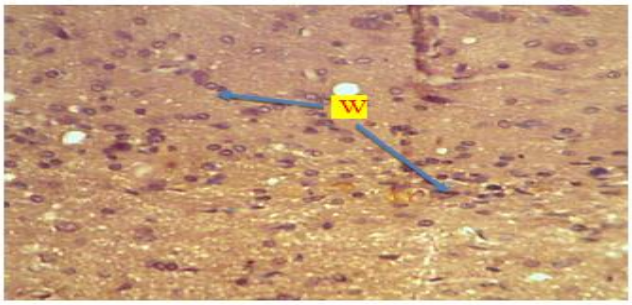

Figure (3-9): Microphotograph of immunohistochemical (IHC) staining of interleukin 1beta of rat brain tissue **group C** received liraglutide (25 nmol/kg) intraperitoneally for 30 days after unilateral intrathecal injection of 6-hydroxydopamine toxin showing +ve brown cytoplasmic immunohistochemical stain. (W: weak intensity) (400x).

DISCUSSION

Change of glutathione peroxidase 4

In this study, the result shows that group B receives IP saline after unilateral intrathecal injection 6-OHDA, clearly decrease GPX4 level in SNpc when it was compared with the control which is agreed with the previous study[15].

In addition, 6 – OHDA produce ROS extracellular and intracellular and this lead to decrease the level of glutathione peroxidase. These changes may be secondary to free radicals production after the infusion of 6-OHDA unilateral intrathecal in rat brain and lead to neurodegeneration as occurred in group B in present research and agreed with previous study mark the importance of the GPX4 (phospholipid hydro peroxidase) in all tissue but especially in the brain as two condition appear when deletion of GPX4 happen, embryonic lethal by genetic deletion of GPX4 and severe neurodegeneration in brain by conditional deletion of GPX4[16].

A result from group C which receive liraglutide IP after unilateral intrathecal injection of 6- OHDA showed that marked elevation of GPX4 as it is present in neuronal and in reactivated non-neuronal cell, and after 30 days of the experiment related to group C many of astrocyte cell still activated and the concentration of GPX4 high in these cell. While this matter dose not present in control group as GPX4 present only on neuronal cells so the level of GPX4 in group A less than group C that GPX4 present in neuronal cell and activated non-neuronal cells and these results to some extent identical with previous study about expression of GPX4 only on neurons in the developing brain, in contrast, following brain lesion, GPx4 is specifically up regulated in non-neuronal cells, as reactive astrocytes[17].

Change of Interleukin 1 β

The level of IL-1β showed elevated in Parkinson's model has been made by unilateral intrathecal injection of 6-OHDA to male rats in present study due to the 6-OHDA cause direct damage to neurons and this lead to reactive gliosis, the process of gliosis includes a series of cellular and molecular events that happen over several days, typically, the first response to lesion is the macrophages and local microglia migrations to the injury site and this lead to activation of microglia cell, activated microglia enhance the inflammatory condition science it has been secrete a different of inflammatory mediators as chemokines (monocyte chemo attractant protein, interferon inducible protein and macrophage inflammatory protein) and cytokines (interleukins IL-1β, TNF and IL-6).

In other hand treated group (group C) showed marked decrease in IL - 1β in brain tissue by the effect of liraglutide as the glucagon like peptide 1 and glucagon like peptide 1 receptor analogue bind to the glucagon like peptide 1 receptor, which blocks PKC or NF-B activation and subsequent expression of NLRP`3, IL-1β, TNF-α, IL-6, VCAM-1, IFN-γ, and MCP-1. In addition, GLP-1R signalling activates cAMP/Ca^{2+}, CAMKKβ, and pAMPK, which induces anti-inflammatory effects on monocyte adhesion. Can be concluded that glucagon like peptide 1 receptor analogue (liraglutide) have a modulator effect on inflammation within central nervous system[18,19].

REFERECES

1. Sharma, M. K., Jalewa, J. & Hölscher, C. Neuroprotective and anti-apoptotic effects of liraglutide on SH-SY5Y cells exposed to methylglyoxal stress. *J. Neurochem.*128, 2014,459–471.
2. Baggio, L. L. & Drucker, D. J. Biology of Incretins: GLP-1 and GIP. *Gastroenterology*132, 2007, 2131–2157.
3. Göke, R., Larsen, P. J., Mikkelsen, J. D. & Sheikh, S. P. Distribution of GLP-1 Binding Sites in the Rat Brain: Evidence that Exendin-4 is a Ligand of Brain GLP-1 Binding Sites. *Eur. J. Neurosci.*7,1995, 2294–2300.
4. Skoglund, G., Hussain, M. A. & Holz, G. G. Glucagon-like peptide 1 stimulates insulin gene promoter activity by protein kinase A-independent activation of the rat insulin I gene cAMP response element. *Diabetes* 49, 2000, 1156–1164.
5. Hanson, M. G., Shen, S., Wiemelt, A. P., McMorris, F. A. & Barres, B. A. Cyclic AMP Elevation Is Sufficient to Promote the Survival of Spinal Motor Neurons In Vitro. *J. Neurosci.* 18, 1998, 7361–7371.
6. Perry, T. *et al.* A Novel Neurotrophic Property of Glucagon-Like Peptide 1: A Promoter of Nerve Growth Factor-Mediated Differentiation in PC12 Cells. *J. Pharmacol. Exp. Ther.*300,2002, 958–966.
7. Blandini, F. & Armentero, M.-T. Animal models of Parkinson's disease. *FEBS J.* 279, 2012, 1156–1166.
8. Paxinos & Watson. The Rat Brain in Stereotaxic Coordinates, 6th Edition | George Paxinos, Charles Watson | ISBN 9780125476126, 2006.
9. Haddadi, R., Nayebi, A. M., Farajniya, S., Brooshghalan, S. E. & Sharifi, H. Silymarin improved 6-OHDA-induced motor impairment in hemi-parkisonian rats: behavioral and molecular study. *DARU J. Pharm. Sci.* 22, 2014, 38.
10. Liu, W. *et al.* Neuroprotective effects of lixisenatide and liraglutide in the MPTP mouse model of Parkinson's disease. *ResearchGate* 303, 2015.
11. Cressey, D. Best way to kill lab animals sought. *Nat. News*500, 2013, 130.
12. Leary, S. L. & American Veterinary Medical Association. *AVMA guidelines for the euthanasia of animals: 2013 edition*, 2013.
13. Bancroft, J. D. & Gamble, M. *Theory and Practice of Histological Techniques*. Elsevier Health Sciences, 2008.
14. Winner, L. Introduction to biostatistics. *Dep. Stat. Univ. Fla.* 2004.
15. Bellinger, F. P. *et al.* Glutathione Peroxidase 4 is associated with Neuromelanin in Substantia Nigra and Dystrophic Axons in Putamen of Parkinson's brain. *Mol. Neurodegener.*6,2011, 8.
16. Seiler, A. *et al.* Glutathione Peroxidase 4 Senses and Translates Oxidative Stress into 12/15-Lipoxygenase Dependent- and AIF-Mediated Cell Death. *Cell Metab.* 8, 2008, 237–248.
17. Savaskan, N. E., Ufer, C., Kühn, H. & Borchert, A. Molecular biology of glutathione peroxidase 4: from genomic structure to developmental expression and neural function. *Biol. Chem.* 388, 2007, 1007–1017.
18. Iwai, T., Ito, S., Tanimitsu, K., Udagawa, S. & Oka, J.-I. Glucagon-like peptide-1 inhibits LPS-induced IL-1β production in cultured rat astrocytes. *Neurosci. Res.* 55, 2006, 352–360.
19. Lee, Y.-S. & Jun, H.-S. Anti-Inflammatory Effects of GLP-1-Based Therapies beyond Glucose Control. *Mediators Inflamm.*2016.

Source of Support: Nil, **Conflict of Interest:** None.