Research Article

Mechanism of the Neuroprotective Effect of GLP-1 in a Rat Model of Parkinson's with Pre-existing Diabetes

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Abstrac:
Many studies have proposed the relations between neurodegenerative diseases and diabetes mellitus (DM), DM promotes to cognitive impairment with aging, but its effect in Parkinson's disease (PD) is not well studied. Glucagon-like peptide-1 (GLP-1), as a member of incretin family, has glycemic control roles. Moreover, it exerts many further effects on various tissues through the widespread expression of its receptor. **Objective:** Our aim is to investigate the effect of pre-existing diabetes on the severity of PD in male albino rats, and to find out if GLP-1 could improve symptoms of PD in the diabetic animals beside its hypoglycemic effect, and work out how it might do this. **Methods:** 75 adult male albino rats were equally divided into: Control, Parkinson's, Diabetic Parkinson's, Diabetic Parkinson's + low dose exenatide (GLP-1 receptor agonist), Diabetic Parkinson's + high dose exenatide group. Blood glucose and insulin, striatal dopamine, some striatal oxidative stress and inflammatory markers, and the catalepsy score were measured. **Results:** Pre-existing of diabetes before PD induction increase the severity of PD proved by the more significant increase in catalepsy score, and the more significant decrease in striatal dopamine level. GLP-1 effects extend beyond their hypoglycemic effects only since it has a direct neuronal anti-oxidative, and anti-inflammatory effect with increasing the striatal dopamine and improving the catalepsy score in a dose dependent manner. **Conclusions:** Diabetes increases the severity of impairment in PD, and GLP-1 improve it through its direct neuronal effect beside its indirect effect through producing hypoglycemia.
Key words: Diabetes mellitus, Parkinson's disease, Glucagon-like peptide-1, Anti-oxidative, Anti-inflammatory

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Intoduction

Parkinson's disease (PD) is one of the most common chronic neurodegenerative disorder come after Alzheimer disease, affecting more than 1% of the elderly people worldwide (Pringsheim et al., 2014). It is a progressive, disabling motor disorder, its symptoms (bradykinesia, rigidity, resting tremor, and postural instability) resulted mostly from the reduction in substantia nigra dopaminergic activity in the midbrain (Sethi 2008).

In spite of the availability of multiple effective symptomatic drugs, there is no treatment for PD and all the efforts to slow down the neuronal cell loss were unsuccessful. This is may be related to the fact that the homeostasis of substantia nigra pars compacta (SNc) is susceptible to different environmental, cellular, and genetic factors which individually or concurrently lead to death of the cell overtime (Lang and Espay 2018).

In the earlier 30 years, the study of the pathogenesis of PD has recognized a number of possible contributing factors, proposing that it has multifactorial origin. In addition to numeral genetic mutations, metabolic and nutritional factors seem to be complicated. Essential stages in this neurodegenerative pathway has been revealed emphasizing relations between the pathogenesis of PD and the mechanisms underlying insulin resistance development (Cereda et al., 2011). Whereas other studies stated that the pathogenic mechanisms of PD also include oxidative stress (Manoharan et al., 2016), inflammation

(Taylor et al., 2013), and apoptosis (Venderova and Park 2012).

Incretins are a group of Metabolic Hormones that motivate the blood glucose level reduction (Amori et al., 2007). There are two main candidate molecules which carry out incretin criteria; the Gastric inhibitory peptide (GIP) and the intestinal peptide Glucagon-like peptide-1 (GLP-1) (Drucker and Nauck 2006).

GLP-1 is an intestinal gut hormone secreted in a response to ingestion of food and potentiates the glucose-dependent insulin secretion from the pancreatic beta-cells. Moreover, GLP-1 suppresses glucagon secretion from alpha-cells, resulting in glucose-dependent reduction in the hepatic glucose production (Holst et al., 2011). Inddition to glycemic control improving, GLP-1 receptor agonists produce weight loss also (Gerich 2013). However, accumulating data from the preclinical and clinical studies show that GLP-1 receptor agonists effects go beyond the weight reduction and glycemic control alone (Seufert and Gallwitz 2014).

GLP-1 receptors present extensively in many tissues outside the pancreas, as the gastrointestinal system, kidneys, central nervous system and cardiovascular system, (Pyke et al., 2014). So, it is reasonable to consider that many physiological effects are mediated by GLP-1 receptor agonists, independent of their main actions of improving glycemic control and stimulating body weight loss.

In the central nervous system (CNS), the GLP-1 action has been concerned in food intake regulation (Barrera et al., 2011), hypothalamic-pituitary-adrenal (HPA) axis function (Ghosal et al., 2013), activation of sympathetic nervous system (SNS) (Yamamoto et al., 2002), and visceral illness (Kinzig et al., 2002). However, additional effects of the central stimulation of GLP-1 are unclear as yet, and until now there are many debates about its mechanism of action.

Some clinical trials reported that drugs used in diabetes treatment have shown positive effects on neurodegenerative processes and on clinical outcome, regarding memory and cognition, and could, hopefully, be developed into novel therapies against PD and related conditions (Green et al., 2019), and some of them reported the protective effects of the GLP-1 mimetic exendin-4 in patients with Parkinson's disease (Athauda et al., 2017; Athauda and Foltynie 2018; Athauda et al., 2019). However, the number of these clinical studies is low and further clinical trials are needed .

The aim of this work is to investigate the effect of preexisting diabetes induction on the severity of experimentally induced Parkinson's disease in male rats, and to discover if GLP-1 could reverse symptoms of Parkinson's disease in the diabetic animals beside controlling hyperglycemia, and work out how it might do this.

Materials and Methods

Ethics statement and animals

The protocol of this study accepted by Minia University Faculty of Medicine, Research Ethics Committee (FMREC), and it executed in conformity with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Manual and Manual 2010). All precautions taken to diminish the number and suffering of animals used in the experiment.

Seventy five (specific-pathogen-free) adult male albino Sprague–Dawley rats that weighing about 150–200 g were used. The rats received from the Faculty of Medicine, Minia University animal House. All rats housed at room temperature with normal light/dark cycles, water and food allowed ad-libitum. Rats randomly divided into five equal groups as follows:

I. Control group: vehicle-injected group that received daily normal saline by intraperitoneal injection for 28 successive days.
II. Parkinson's group: rats received 1-methyl-4-phenyl-1,2,3,6-tetrahydro-

pyridine (MPTP) that dissolved in normal saline by intraperitoneal injection in a dose of 30 mg/kg body weight at 24 h intervals for 28 successive days (Lee et al., 2011).

III. Diabetic Parkinson's group: induction of diabetes by injection of 50 mg/kg streptozotocin (STZ) single intra-peritoneal injection (Szkudelski 2001) four days before the beginning of MPTP-induced Parkinson's.

IV. Diabetic Parkinson's + low dose exenatide (GLP-1 receptor agonist) group: STZ diabetic induction four days before the beginning of MPTP Parkinson's induction + treating the rats with 1.0 µg/kg single subcutaneous exenatide daily dose on the 4th day of STZ treatment for 28 consecutive days (Elbassuoni 2014)

V. Diabetic Parkinson's + high dose exenatide group: STZ diabetic induction four days before the beginning of MPTP Parkinson's induction + treating the rats with 5.0 µg/kg single subcutaneous exenatide daily dose on the 4th day of STZ treatment for 28 consecutive days (Elbassuoni 2014) .

Induction of diabetes

Induction of diabetes done by STZ (50 mg/kg) injection. It was freshly dissolved in the sodium citrate buffer (pH 4.5) and intraperitoneally injected in a single dose (Szkudelski 2001). After four days of STZ injection, tail vein blood samples taken for blood glucose level assay. Rats with a fasting blood glucose level higher than 180 mg/DL (10 mmol/l) considered diabetic.

Behavioral tests

PD development detected after 28 days from induction with MPTP, by the occurrence of tremors and the observation of rigidity and bradykinesia in rats that further quantified by ‘‘Catalepsy test’’. The first part of this test was the grid test where the rat was hung on a vertical grid by its paws (the grid was 44 cm high and 25.5 cm wide with a space of one cm in between each wire), the time for each rat to move its paws or any other sort of the first movement recorded. The second part of the test was the bar test where the rat placed on a bar (9 cm above and parallel from the base) with both fore paws, the time of removal of the paw recorded (Alam and Schmidt 2004). These two test performed for all the rats that were included in the study. Many studies reported that these tests are sensitive methods for evaluating motor dysfunctions in the MPTP animal model of Parkinson's disease (Alam and Schmidt 2002; Abdin and Hamouda 2008; Kim et al., 2010)

Biochemical analyses of the blood and brain tissue homogenate

At the end of the work and after performing the behavioral tests, all rats sacrificed; blood samples were immediately collected in 10-ml Eppendorf tubes, left to clot, then delivered into centrifuge tubes and centrifuged at 3000 rpm for 20 min; serum samples separated in 2-ml Eppendorf tubes to be used immediately as fresh samples (preferred) or to be stored on − 20 °C until used. Serum samples used to determine serum glucose using colorimetric assay kit from MyBioSource, USA, insulin level using ELISA kit from MyBioSource, USA.

The brains removed quickly and washed using ice-cold saline. The striata of the hemisphere of each brain isolated, weighed, and homogenized using a homogenizer. Homogenization carried out in phosphate-buffered saline (pH = 7.4). The homogenate then centrifuged for 10 minutes. The supernatants kept at – 80°C until the analysis of: dopamine using Rat Dopamine (DA) ELISA Kit from CUSABIO, USA; malondialdehyde (MDA) using colorimetric assay kit from MyBioSource, USA; Superoxide Dismutase Activity using colorimetric assay kit (ab65354) from Abcam, USA; Catalase Activity using colorimetric assay kit (ab83464) from Abcam, USA; Reduced Glutathione (GSH) using colorimetric assay kit (ab235670) from Abcam, USA; tumor necrosis factor-α (TNF-α) by ELISA kit from MyBioSource, USA; Interleukin 1 beta (IL-1β) using ELISA Kit (ab100768) from Abcam, USA.

Streptozotocin, MPTP, exenatide, and other chemicals obtained from Sigma–Aldrich Chemical Co.

Statistical Analysis

Values expressed as mean ± SEM, analyzed statistically using SPSS program, version 17 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons test were applied for all analyses. *P* value less than 0.05 was considered statistically significant.

Results

Assessment of Motor Function

Parkinson's group exhibited a significant increase in catalepsy score of either grid test or bar test compared to control group. Diabetic Parkinson's group receiving no treatment showed more significant increase in catalepsy score of either grid test or bar test compared to Parkinson's group. Treatment of Diabetic Parkinson's group with exenatide showed a significant reduction in the catalepsy score of grid test and bar test in a dose-dependent way as compared to Diabetic Parkinson's group receiving no treatment.

Biochemical analysis

Serum glucose and insulin

Parkinson's group showed no significant change in serum glucose and insulin levels compared to control group. Diabetic Parkinson's group receiving no treatment showed a significant increase in serum glucose level and significant decrease in serum insulin level compared to Parkinson's group. Treatment of Diabetic Parkinson's group with exenatide significantly decrease serum glucose level, and significantly increase serum insulin level in a dose-dependent way when compared to Diabetic Parkinson's group receiving no treatment.

Striatal dopamine content

Parkinson's group showed a significant decrease in striatal dopamine levels compared to control group. Diabetic Parkinson's group receiving no treatment showed more decrease in striatal dopamine level compared to Parkinson's group. Treatment of Diabetic Parkinson's group with exenatide significantly improved the striatal dopamine level in a dose-dependent way as compared to Diabetic Parkinson's group receiving no treatment.

Striatal oxidative stress biomarkers

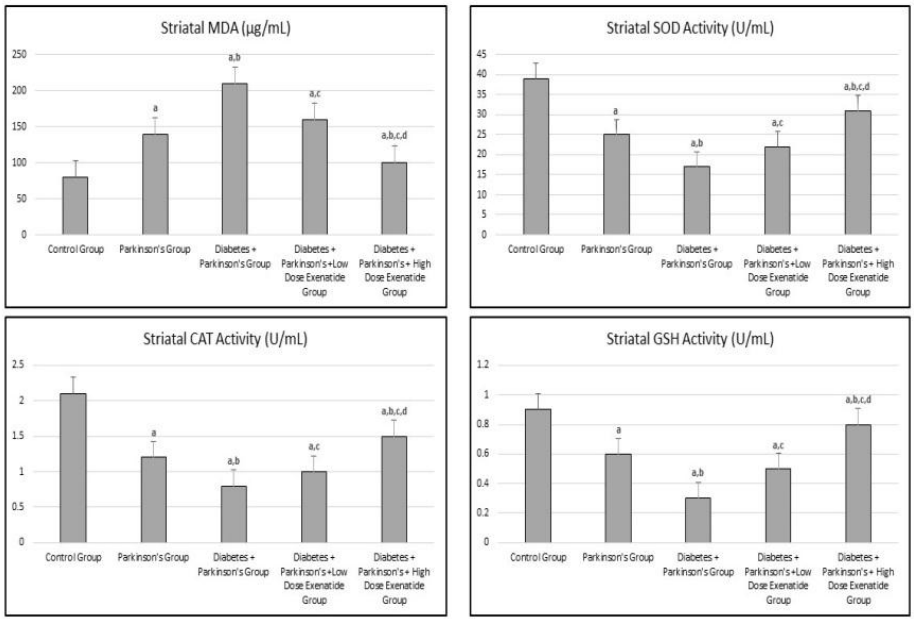
Measuring MDA level, as a stable product of lipid peroxidation, is a reliable tool to assess the extent of oxidative damage at the cellular level. As compared to the control group, there was a significant increase in striatal MDA level in the Parkinson's group, with more increase in its level in the Diabetic Parkinson's group. Treatment of Diabetic Parkinson's group with exenatide significantly decreased the striatal MDA level in a dose-dependent way as compared to Diabetic Parkinson's group receiving no treatment.

The Parkinson's group exhibited a significant decrease in the activities of the antioxidant enzymes SOD, CAT and GSH in the striata compared to the control group, with more decrease in their activities in the Diabetic Parkinson's group. Treatment of Diabetic Parkinson's group with exenatide significantly increased their activities in the striata in a dose-dependent way as compared to Diabetic Parkinson's group receiving no treatment.

Striatal inflammatory biomarkers

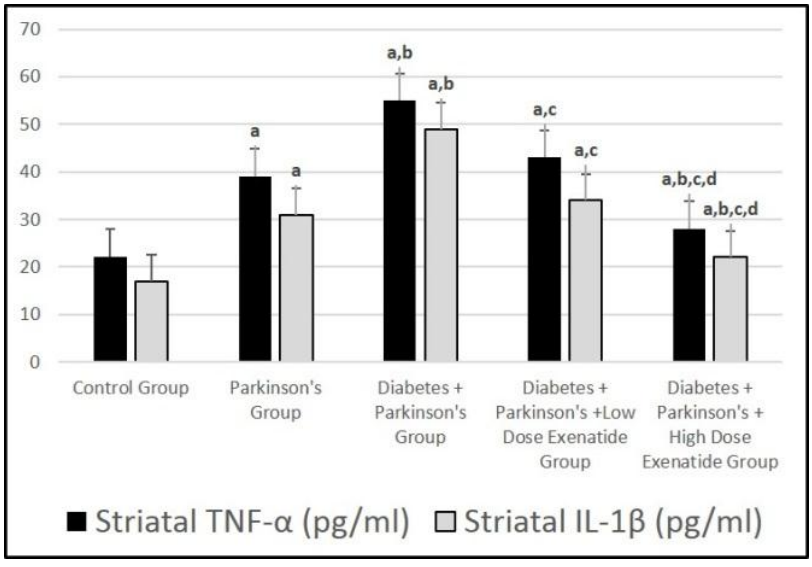
In the present study, striatal TNF-α and IL-1β levels were significantly increased in the Parkinson's group compared to the control group, with a more significant increase in the Diabetic Parkinson's group as compared to Parkinson's group. Treatment with exenatide significantly decreased TNF-α and IL-1β levels in a dose-dependent way as compared to Diabetic Parkinson's group receiving no treatment.

Figure 1: Striatal oxidative stress markers in the different studied groups



Values expressed as mean ± SEM. *n= 15, P* < 0.05 was considered statistically significant
[a] Significant from control group, [b] Significant from Parkinson's group, [c] Significant from Diabetic Parkinson's group, [d] Significant from Diabetic Parkinson's + Low Dose Exenatide group.

Figure 2: Striatal inflammatory markers in the different studied groups:



Values expressed as mean ± SEM. *n= 15, P* < 0.05 was considered statistically significant
[a] Significant from control group, [b] Significant from Parkinson's group, [c] Significant from Diabetic Parkinson's group, [d] Significant from Diabetic Parkinson's + Low Dose Exenatide group

Table 1: Striatal dopamine level in the different studied groups:

Parameter	Control Group	Parkinson's Group	Diabetic Parkinson's Group	Diabetic Parkinson's + Low Dose Exenatide Group	Diabetic Parkinson's + High Dose Exenatide Group
Dopamine (ng/ml)	7.3 ± 0.7	3.5 ± 0.5[a]	1.2 ± 0.2 [a,b]	2.8 ± 0.4 [a,c]	5.4± 0.5 [a,b,c,d]

Values expressed as mean ± SEM. *n= 15, P* < 0.05 was considered statistically significant
[a] Significant from control group, [b] Significant from Parkinson's group, [c] Significant from Diabetic Parkinson's group, [d] Significant from Diabetic Parkinson's + Low Dose Exenatide group

Table 2: Catalepsy score in the different studied groups:

Parameter	Control Group	Parkinson's Group	Diabetic Parkinson's Group	Diabetic Parkinson's + Low Dose Exenatide Group	Diabetic Parkinson's + High Dose Exenatide Group
Grid test (time in seconds)	6.9±0.8	29.5±4.5 [a]	49.9±5.6 [a,b]	30.8±4.8 [a,c]	16.2±2.7 [a,b,c,d]
Bar test (time in seconds)	7.4±0.9	22.6±3.7 [a]	46.3±6.4 [a,b]	31.2±3.5 [a,c]	17.7±1.8 [a,b,c,d]

Values expressed as mean ± SEM. *n= 15, P* < 0.05 was considered statistically significant
[a] Significant from control group, [b] Significant from Parkinson's group, [c] Significant from Diabetic Parkinson's group, [d] Significant from Diabetic Parkinson's + Low Dose Exenatide group

Table 3: Fasting serum glucose and fasting serum insulin in the different studied groups:

Parameter	Control Group	Parkinson's Group	Diabetic Parkinson's Group	Diabetic Parkinson's + Low Dose Exenatide Group	Diabetic Parkinson's +High Dose Exenatide Group
Fasting serum glucose (mmol/L)	5.1±0.4	5.6 ± 0.5	22.7 ± 2.3 [a,b]	5.9 ± 0.9 [c]	5.5± 0.7 [c]
Fasting serum insulin (µIU/ml)	33.7±1.9	34.9 ± 2.1	13.2 ± 1.3 [a,b]	35.5 ± 2.3[c]	31.9 ± 3.4 [c]

Values expressed as mean ± SEM. *n= 15, P* < 0.05 was considered statistically significant.
[a] Significant from control group, [b] Significant from Parkinson's group, [c] Significant from Diabetic Parkinson's group

Discussion

In the present study, the rat model of Parkinson's disease was induced by administration of MPTP that induced Parkinson's disease in all rats treated with it proved by the significant increase in the catalepsy score and the significant decrease in striatal dopamine level. It was established that MPTP enters the dopaminergic neurons through the dopamine transporter and induced their degeneration (Hirsch et al., 2003).

In the Parkinson's group, striatal antioxidant enzyme SOD, CAT and GSH activities were significantly decreased, with significant increase in striatal MDA, a marker of lipid peroxidation, suggesting that oxidative stress involved in the pathogenesis of MPTP-induced Parkinson's disease as reported by Shi et al., (Shi et al., 2016) ,and many post-mortem studies have shown that oxidative stress induces the antioxidant protective systems down regulation, including SOD, CAT, and GSH, and damages proteins, lipids, and DNA, eventually resulting in damage to the dopaminergic neurons in the substantia nigra (Maj et al., 2010).

Moreover, in the present study the levels of the proinflammatory cytokines, TNF-α and IL-1β, were significantly increased in the Parkinson's group. Ferger et al., (Ferger et al., 2004) reported that in the MPTP model of PD, the inflammatory reactions have been determined, and elevated level of the proinflammatory cytokines TNF-α was detected. This can be explained by the excessive microglial activation produced by MPTP, proposing that neuroinflammation and the activated microglia are crucial in PD pathogenesis (Tansey and Goldberg 2010). Microglia involved in the immune defense and surveillance, and it activates multiple proinflammatory cytokines, including IL-1β and TNF-α, which can directly induce apoptosis in the dopaminergic neurons (Niranjan 2014).

In order to study the effect of diabetes on the PD-induced biochemical and motor impairments; diabetes induced in the present study by streptozotocin (STZ), a Glucosamine–nitrosourea compound derived from Streptomyces achromogenes. STZ damages the pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia (Lenzen 2008).

We found that in the Parkinson's group with pre-existing diabetes, there is a significant elevation in the catalepsy score and a significant decrease in striatal dopamine level compared to the Parkinson's group. These results come in line with Aviles-Olmos et al., (Aviles-Olmos et al., 2012) who reported that hyperglycemia related to insulin resistance or hypoinsulinaemia, shown to decrease the basal striatal dopamine concentrations, this is because insulin receptors are represented densely in the substantia nigra and insulin increases the dopamine transporter mRNA in substantia nigra, it also regulates brain dopamine concentrations (Cereda et al., 2013).

In the present study, striatal antioxidant enzymes SOD, CAT and GSH activities were significantly decreased in the Parkinson's group with pre-existing diabetes, with significant increase in striatal MDA level compared to the Parkinson's group. These results come in line with earlier studies that reported that the activities of these antioxidant enzymes decreased in the diabetic brain (Alvarez-Nolting et al., 2012; Miranda et al., 2007). Chronic hyperglycemia leads to oxidative stress with the production of reactive oxygen species, production of reactive oxygen species can also be a mechanism that is underlying dopaminergic cell loss in the hyperglycemic animals (Stranahan and Mattson 2011).

The brain is mostly vulnerable to the oxidative injury due to its high rate of oxygen consumption, excessive production of reactive radicals, and high transition metals levels, such as iron, that catalyze reactive radical production. Furthermore, neuronal membranes are rich in the polyunsaturated fatty acids that are a source of lipid peroxidation (Ansari et al., 2008). Free radicals formed excessively in the diabetes by non-enzymatic glycation of

proteins, glucose oxidation, and the consequent oxidative degradation of the glycated proteins. Abnormally free radicals high levels, and the concurrent antioxidant defense mechanisms decline can lead to damage to the cellular enzymes and organelles, increased in lipid peroxidation, and development of insulin resistance. These oxidative stress consequences can promote the development of diabetic complications (Rains and Jain 2011).

Increased in systemic and cerebrovascular inflammation considered as one of key pathophysiological features in diabetes mellitus and its vascular complications (Goldberg 2009). Key mechanisms of the hyperglycemia-induced inflammation include the neuronal nuclear factor kappa-light-chain-enhancer of the activated B cells (NFκB)-dependent production of proinflammatory cytokines, inflammasome activation, and increased oxidative stress (Lee et al., 2013). These studies can explain the more increase in the levels of the proinflammatory cytokines, TNF-α and IL-1β that found in the present study in the Parkinson's group with preexisting diabetes compared to the Parkinson's group.

Exenatide is a synthetic agonist for GLP-1 receptor that has been approved for diabetic treatment with definite useful effects on glucose control, supposed to be mediated by β-cell proliferation, decreased gluconeo-genesis, increased insulin production, and weight loss that follows the chronic stimulation of GLP-1 receptor in the gastrointestinal tract (Aviles-Olmos et al., 2013). This explains the hypoglycemic effect of exenatide found in the present study in exenatide-treated group, and its ability to return the glucose and insulin of the diabetic rats to the normal level even in its low dose. Exenatide proved also to protect the β islet cells from apoptosis, prevent damage to the mitochondrial DNA encoded genes and stimulate the mitochondrial biogenesis (Fan et al., 2010).

,Expression of GLP-1 receptor is broadly detected in various cells and organs, including the heart, kidney, lung, endo-thelial cells, hypothalamus, astrocytes, microglia, and neurons besides pancreatic beta-cells (Arakawa et al., 2010; Fujita et al., 2014; Goke et al., 1995; Iwai et al., 2006; Romani-Perez et al., 2013; Thorens 1992), proposing that GLP-1 can have additional roles other than the glucose-lowering effects.

The results of the present study demon-strated that treatment of Diabetic Parkinson's group with exenatide signifi-cantly improved the striatal dopamine level, and decreased the raised catalepsy score in a dose dependent way as compared to Diabetic Parkinson's group receiving no treatment. These results come in line with earlier studies that reported that in a rat model of Parkinson's disease, GLP-1 analogue has revealed protection of the substantia nigra dopaminergic neurons and prevention of basal ganglia dopamine loss while preserving motor control (Tan et al., 2009), (Ji et al., 2016), (Chen et al., 2015). Moreover, it was reported that GLP-1 analogue reverses the biochemical and behavioral deficits in a rodent model of Parkinson's disease (Kim et al., 2017; Rampersaud et al., 2012).

As we mentioned above it was established that diabetic hyperglycemia has negative neuronal effect; so, GLP-1can produce in the neuroprotective effect through its glucose-lowering effect. However, we investigated also if GLP-1 has a direct neuronal effect by studying its effects on striatal inflammatory and oxidative stress status to conclude if it has its neuroprotective effect only indirectly through its hypoglycemic effect or also through its direct effect on the brain.

GLP-1 agonists can cross the blood–brain barrier (Hunter and Holscher 2012), so the effect of GLP-1 treatment on cellular pathways involved in inflammation and oxidation investigated. We found that treatment of Diabetic Parkinson's group with exenatide produced a block at the raised level of lipid peroxidation marker, MDA, and the decrease in the activity of antioxidant enzyme, SOD, GSH and CAT, seen in striatal tissue of Diabetic Parkinson's group receiving no treatment in

a dose dependent way. Similarly, Muscogiuri et al., (Muscogiuri et al., 2017), and Spielman et al., (Spielman et al., 2017) reported that incretins reduce brain oxidative stress through inhibiting the accumulation of intracellular reactive oxygen species (ROS) and the release of nitric oxide (NO), along with increasing the expression of superoxide dismutase 1 (SOD1) and the antioxidant glutathione peroxidase 1 (GPx1).

On studying the effect of GLP-1 on striatal inflammatory status, we found that GLP-1 has a definite anti-inflammatory effect, since treatment of Diabetic Parkinson's group with exenatide significantly decreased striatal TNF-α and IL-1β levels in a dose dependent way as compared to Diabetic Parkinson's group receiving no treatment. It was reported that GLP-1 receptor stimulation attenuate the synthesis of the pro-inflammatory cytokine interleukin-1β (IL-1β) in the activated astrocytes (Iwai et al., 2006). In astrocytes, GLP-1 prevented the lipopolysaccharide-induced IL-1β expression by increase of cAMP (Iwai et al., 2006).

The efficacy of GLP-1 in PD models discussed in earlier works from other points of view; Li et al., (Li et al., 2016) has shown that GLP-1 preserved neuronal cell viability, and prevent dopaminergic degenerative processes, and the apoptotic and neuronal death signaling pathways induced by rotenone or similar oxidants. This effect can be due to the activation of growth factor signaling via the GLP-1 receptor, that inhibits apoptotic signaling (Li et al., 2009). In addition, the GLP-1 receptor induces up regulation of the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) (Sharma et al., 2014). Bcl-2 acts to reserve mitochondrial integrity by preventing the loss of mitochondrial membrane potential and/or release of pro-apoptotic proteins such as cytochrome C into the cytosol (Harada and Grant 2003). Thus, this seems that GLP-1 act by improving the course of the pathology rather than just exerting a symptomatic and acute effect. An additional component of the effect mediated by GLP-1 could be a trophic effect on remaining dopaminergic neurons (Bertilsson et al., 2008).

Recently Erbil et al., (Erbil et al., 2019) study reported that GLP-1 is effective in partially or fully reversing the effects of neuropathological changes related with Alzheimer's disease, Parkinson's disease, neurovascular complications of diabetes, neurotoxic compounds, or vascular occlusion. Possible mechanisms that offer neuroprotection are enhancing the viability of the nerve cells and restoring neurite outgrowth by increased neurotrophic factors, decreasing apoptosis, decreasing the level of pro-inflammatory ingredients, reduced oxidative damage, decreased cerebral edema, and strengthening blood brain barrier.

Conclusion

The prominent outcome of this study was that GLP-1, in addition to its efficacy in diabetes management, exerts significant neuroprotection effects against neuronal damage in PD which worsened with the presence of diabetes. The mechanisms involved could be correlated to its anti-inflammatory and anti-oxidative stress effects through its ability to suppress the inflammatory cytokines expression, and increase the activity of the antioxidant defense enzymes. The preliminary results of the present study propose that GLP-1 can exert its neuronal protective effects via a direct effect on the brain independently of controlling the hyperglycemia and the consequent removal of neuronal glucot-oxicity effect. This proved by increasing the neuronal GLP-1 effect in a dose dependent manner although the blood glucose return to its normal level with the GLP-1 low dose. These visions afford the opportunity to plan therapeutic approaches directed at specific pathogenic mechanisms, including diabetic control, anti-inflammatory, antioxidant, and insulin-stimulatory drugs that can be effective for protection against neurode-generative central nervous system disorders by preventing or stopping the neuronal damage progression in the case of diabetes. However, it is clear that further basic mechanistic and clinical researches required before any potential therapeutic benefits

may be realized. Hoping this research will help people with PD, especially those with diabetes, relatively soon.

Limtation

As far as the results of the present study look promising, but longer and larger trials needed to fully test the effects of GLP-1 in diabetic people with Parkinson's since the animal model we've used involves using chemicals that damage dopamine-producing nerve cells specifically and very quickly. This is different to Parkinson's development in people that happen slowly, and affects other types of nerve cells, not only the dopamine-producing ones.

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