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Preventive effect of metformin in a rat model of parkinson's disease with diabetes mellitus

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Abstract

Metformin is a main first line medication in treatment of type 2 diabetes mellitus. Metformin is described as an anti-hyperglycaemic agent that does not cause clinical hypoglycaemia in patients with T2DM or alter glucose homeostasis in non-diabetic individuals. It also believes to attenuate the progress and severity of age-related diseases such as, neurodegenerative (e.g. Alzheimer's disease and Parkinson's disease). This study aims to elucidate the role of Metformin as a protective agent against further neurodegeneration in Parkinson's disease (PD) and its role in improving the motor manifestations of PD. This study was a prospective randomized controlled experiment conducted in the laboratory of the Medical Physiology department, faculty of Medicine, Minia University. It included a total of 40 rats. Statistically significant improvement was seen in metformin treated group as regards oxidative stress and inflammatory markers, Malondialdehyde (MDA), Total antioxidant capacity (TAC), Tumour necrosis factor-α (TNF-α) were measured in control group (C group), Parkinson's group (P group), Diabetes Parkinson's group(DP group) and Diabetes Parkinson's Metformin group(DPM group). Metformin was a safe, effective method in prevention of further neurodegeneration in adult diabetic albino rats with parkinsonism.

Keywords: Parkinson's disease (PD), Malondialdehyde (MDA), Total antioxidant capacity (TAC), Tumour necrosis factor-α (TNF-α).

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1. Introduction

Parkinsonism is a clinical syndrome, which is characterized by rigidity, bradykinesia, static tremors, and postural instability. James Parkinson was the first to describe it in the essay that was titled, "An Essay on the Shaking Palsy" in 1817 [1]. Mortality is not increased in the first decade after disease onset, but increases thereafter, eventually doubling in comparison with the general population. Beyond the perception of PD as a disorder of movement, it has been proved that it also includes non-motor features, such as cognitive impairment, autonomic dysfunction, sleep disorders, depression and hyposmia [2]. Pathophysiology of PD includes neuronal loss in specific areas of the SN and widespread accumulation of an intracellular protein called α-synuclein. α-Synuclein is a member of the synuclein family of proteins, it is abundantly expressed in the nervous system at presynaptic terminals and it acts mainly as a modulator of synaptic transmission. Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both. According to the International Diabetes Federation, Adults between the ages of 20 to 79 years nearly 415 million of them had diabetes mellitus in 2015. DM is a global public health burden as this number is expected to rise to another 200 million by 2040 [3]. DM is known to be an inflammatory condition, and has also been linked to neurodegenerative diseases through abnormal insulin pathways [4]. The results of several epidemiological studies searching for evidence of a relationship between PD and DM are controversial. However, strong evidence suggests that DM might be a risk factor for PD, and that it might be limited to those patients with long diabetic disease duration (over 10 years) [5]. DM and neurodegeneration share several pathological pathways, such as oxidative stress, mitochondrial dysfunction and neuro-inflammation. Furthermore, significant evidence supports the role of insulin resistance in the interplay between DM and PD. The existence of common molecular mechanisms encourages the use of drug repurposing treatments to modify disease progression in parkinsonian patients [6]. Metformin is an anti-diabetic drug, reduces inflammation and oxidative stress in the brain as well as in the periphery by reducing the level of leukocytes and circulating pro-inflammatory cytokines

interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [7].

2. Material and methods

2.1 Animals

A total of 40 adult male albino rats of local strain, of average body weight 180 g, ranging from 150 to 200 g, at the beginning of this study were used. They were housed in eight wire mesh cages (5 rats/cage) at room temperature with natural light\dark cycles for one week of acclimatization to lab conditions. The protocol of this study was documented according to the rules of the Institutional Animal Care and Use Committee (IACUC), Faculty of Medicine, Minia University. The whole practical work was conducted in the laboratory of the Medical Physiology department, faculty of Medicine, Minia University.

2.2 Chemicals, drugs, and kits

2.2.1 Drugs

1. Streptozotocin (STZ): Sigma-Aldrich, MO, USA.
2. Rotenone (ROT): Sigma-Aldrich, MO, USA.

2.2.2 Chemicals

1. Metformin (500mg/kg daily oral).
2. Phosphate buffered saline (PBS): with pH =7.4
3. 10% buffered formalin.

2.2.3 Kits

2.2.3.1 Kit for determination of striatal malondialdehyde (MDA) (ELISA)

Elabscience, China– CAT. No. E-BC-K025-S.

2.2.3.2 Kit for determination of striatal total antioxidant (TAC) (colorimetry)

Bio-diagnostic, EGYPT – CAT. No. TA 25 13.

2.2.3.3 Kit for determination of striatal tumor necrosis factor-α concentration (TNF-α) (ELISA)

Sigma-Aldrich, USA. – CAT. No. RAB0480.

2.3 Experimental design

2.3.1 Experimental groups

The rats were randomly divided into six main groups:

1-Control groups (CG): subdivided into two subgroups

a- Control group (5 rats/group): in which rats didn't receive medications.

b- Control group treated with vehicle (5 rats/group): In which rats received the vehicle, 0.01 M citrate buffer at pH 4 once at the time of induction of T2DM and dimethyl sulfoxide (DMSO), injected intraperitoneally at a daily dose level 1ml/kg for 28 days at the time of parkinsonism induction.

2-Parkinson's group (PG).

3-Diabetes Parkinson's group (DPG).

4- Diabetes Parkinson's group + Metformin (DPMG).

2.3.2 Induction of T2DM

T2DM was induced by giving high-fat diet (HFD) containing 17% carbohydrate, 25% protein, and 58% fat, as a percentage of total kcal for 4 weeks, followed by intraperitoneal injection of streptozotocin at a single dose of 35 mg/kg dissolved in freshly prepared 0.01 M citrate buffer at pH 4 (Sohrabipour, et al. 2018). Three days after the STZ injection, the blood glucose was measured by a blood sample obtained from the rat tail. The rat whose blood glucose was ≥ 200 mg/dl was considered diabetic [4].

2.3.3 Induction of Parkinsonism:

Parkinsonism was induced by intraperitoneal injection of rotenone (inhibitor for complex I of the mitochondrial electron transport chain), dissolved in DMSO, at a daily dose of 0.5 mg/kg for 28 days. In the diabetes Parkinson's groups, the induction of parkinsonism was started the day after the induction of T2DM.

2.3.4 Intermittent Fasting Regimen

2.3.5 Metformin dosage

After 3 days of streptozotocin injection, each rat in DPMG received Metformin at an oral daily dose of 500mg/kg/day for 28 days.

2.4 Parameters measured

Brain tissue parameters: malondialdehyde (MDA), total antioxidant capacity (TAC) and tumor necrosis factor-alpha (TNF-α).

3. Results

3.1 Effect of Parkinsonism and Parkinsonism on top of DM with or without treatment on striatal levels of MDA and TAC

Our results show a significant increase in the brain tissue level of malondialdehyde (MDA) associated with a significant decrease in total antioxidant capacity (TAC) in P group compared to the C group. The DP group shows a significant increase in the brain tissue level of MDA associated with a significant decrease in TAC in DP group in comparison with C group and P group. The DPM group shows a significant decrease in the brain tissue level of MDA associated with a significant increase in TAC as compared to the DP group.

3.2 Effect of Parkinsonism and Parkinsonism on top of DM with or without treatment on striatal levels of TNF-α

As regards TNF-α, our results show a significant increase in the brain tissue level TNF-α levels in the P group than in the C group. The DP group shows a significant increase in the brain tissue level TNF-α in comparison with C group and P group. The DPM group shows a significant decrease in the brain tissue level of TNF-α as compared to the DP group.

4. Discussion

Diabetes mellitus (DM) is known to be one of the most common chronic metabolic diseases worldwide. Recently, DM represents one of the most significant health burdens worldwide. There are many similarities between PD and DM. In the present study, induction of PD was performed by using rotenone which is a naturally occurring insecticide extracted from Leguminosa plants. Rotenone is a potent dopaminergic neurotoxic agent. Being highly lipophilic, it easily crosses the blood–brain barrier (BBB), and, unlike many other toxic agents, bypasses the dopamine transporter for cellular entry. Induction of DM type 2 (T2DM) was performed in this study by feeding rats high fat diet (HFD) for 4 weeks followed by injection of streptozotocin (STZ) which is an antibiotic drug formed by Streptomyces achromogenes bacteria. It has damaging effects on the β-cells

in the islets of Langerhans via stimulation of oxidative stress
and suppression of antioxidant defense [8].

Table 1: Effect of induction of parkinsonism and parkinsonism on top of diabetes mellitus on oxidative stress markers:

Groups Parameters	C group	P group	DP group	DPM group
MDA (pmol/mg)	6.45± 0.14	8.35 ± 0.23 [a]	12.07±0.17 [a,b.]	7.87±0.27 [a,b,c]
TAC (mmol/mg)	7.14±0.10	4.12±0.05 [a]	3.78±0.04 [a,b]	8.50±0.06 [a,b,c]

Data are expressed as mean ± SE of 10 rats in each group, means in the horizontal row with different subscripts a,b,c are significantly different ($p \leq 0.05$): (a) versus control group (C), (b) versus parkinsonism group (P), (C) versus diabetic Parkinson group (DP), malondialdehyde (MDA) and total antioxidant capacity (TAC).

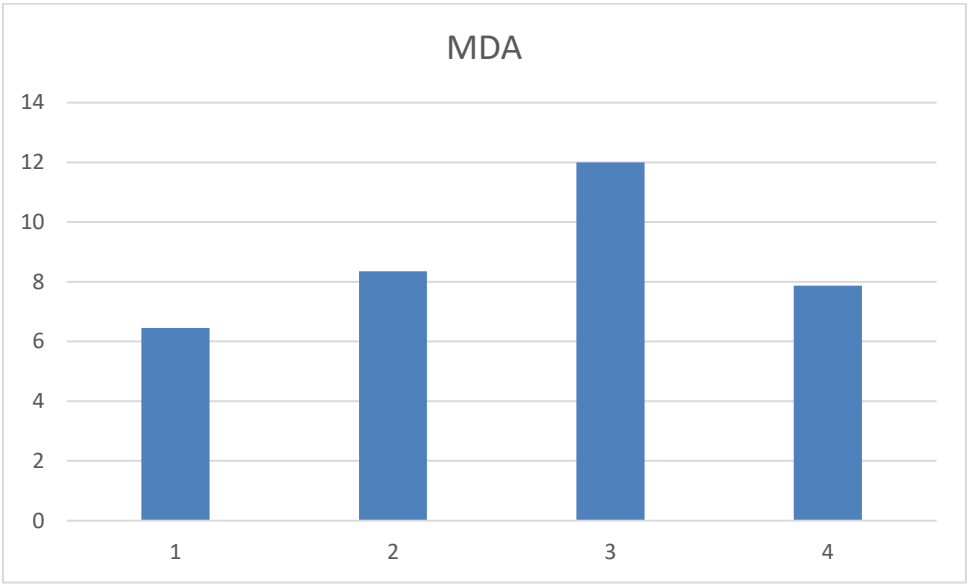

Figure 1: Effect of induction of parkinsonism and parkinsonism on top of diabetes mellitus on MDA

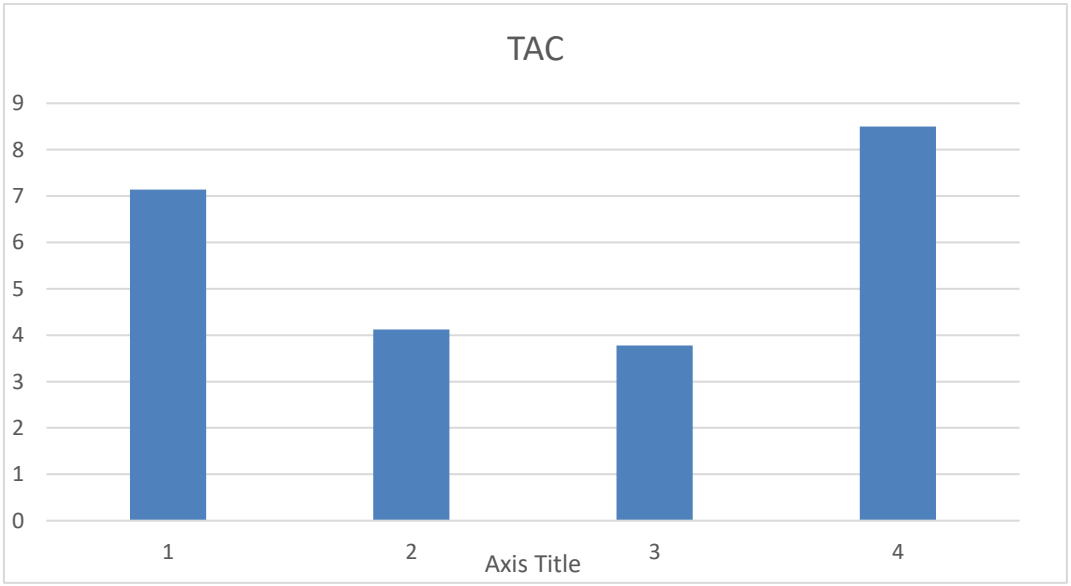

Figure 2: Effect of induction of parkinsonism and parkinsonism on top of diabetes mellitus on TAC.

Table 2: Effect of induction of parkinsonism and parkinsonism on top of diabetes mellitus on inflammatory markers:

Groups Parameters	C group	P group	DP group	DPM group
TNF-α (ng/mg)	147±2.98	475.8±9.46 [a]	566.67±2.96 a,b	309.8±6.57 a,b,c

Data are expressed as mean ± SE of rats in each group, means in the horizontal row with different subscripts a,b,c,d,e are significantly different ($p \leq 0.05$): (a) versus control group (C), (b) versus parkinsonism group (P), (C) versus diabetic Parkinson group (DP), tumor necrosis factor-α (TNF-α).

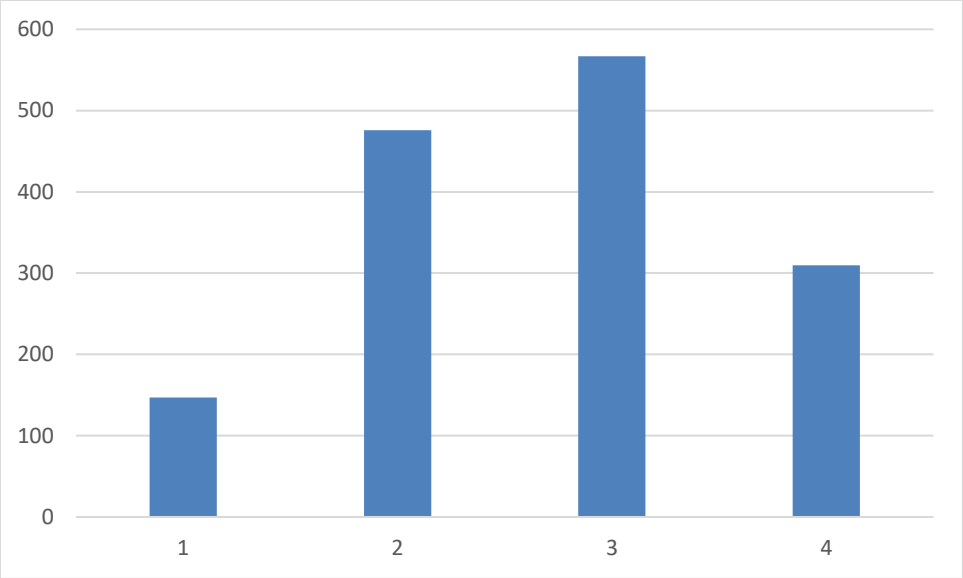

Figure 3: Effect of induction of parkinsonism and parkinsonism on top of diabetes mellitus on TNF-α.

As regards brain tissue oxidative stress and inflammatory markers, Rotenone inhibits electron transfer from iron-sulfur centers in complex-I to ubiquinone resulting in the inhibition of the oxidative phosphorylation process, with an increase of ROS [9]. Rotenone also decreases the level of glutathione (GSH) which has an antioxidant function that arises from its ability to scavenge reactive oxygen species or to serve as an essential cofactor for GSH S-transferases and peroxidases [10]. The increased level of TNF-α in our study is agreed by [11] in their study on the implication of rotenone in Parkinsonism disease in the rat model. They explained it by the ability of rotenone to activate microglial cells, with subsequent release of cytotoxic inflammatory cytokines, e.g., TNF-α. The DP group shows a significant increase in the brain tissue level of MDA and TNF-α associated with a significant decrease in TAC in DP group in comparison with C group and P group. Showing significant worsening in the level of striatal oxidative and inflammatory markers in comparison with P group. These results can be attributed to the effects of both rotenone used to induce PD, and STZ used to induce DM. STZ decreases the antioxidant enzymes such as CAT, glutathione peroxidase (GPx), and SOD [12].

STZ-induced hyperglycemia increases the ROS release through stimulation of the production of AGEs, enhanced polyol pathway, and activated protein kinase C. The generated ROS leads to excess lipid peroxidation and increases the level of MDA [13]. TNF-α is an adipokin and a cytokin, its measurement is a useful tool to assess inflammatory responses in the brain. The increased level of TNF-α in the DP group can be explained by the pro-inflammatory action of rotenone, in addition to the effect of HFD. The DPM group shows a significant decrease in the

brain tissue level of MDA and TNF-α associated with a significant increase in TAC as compared to the DP group. With significant improvement in both oxidative and inflammatory markers in comparison with P group which ensures its direct protective effect on PD. This protective effect of metformin against oxidative stress is in line with [14] who reported a significant increase in TAC as a result of treatment with metformin. Metformin decreases MDA by decreasing ROS production and subsequently decreases lipid peroxidation. The decreased level of TNF-α in the DPM group is in line with [15] whose study explained the effect of metformin on the level of TNF-α in rats. The percentage of macrophages in the increased adipose tissue by HFD is increased by 40%. Macrophage infiltration is promoted by chemokines from adipocytes. Infiltrated macrophages attract other macrophages and the accumulated macrophages secrete pro-inflammatory cytokines, *e.g.,* TNF-α. Macrophages express many types of scavenger receptors which bind and internalize modified low-density lipoprotein (LDL). Scavenger receptors enhance NF-κB activity through the uptake of lipopolysaccharide (LPS) and oxidized LDL, leading to pro-inflammatory cytokine production. Metformin downregulates the NF-κB translocation in macrophages and attenuates the expression of scavenger receptors in macrophages. Furthermore, the metformin-induced activation of AMPK not only facilitates its antioxidant action but also enhances its anti-inflammatory action as AMPK is crucial for the suppression of lipopolysaccharide-induced expression of proinflammatory molecules and mediators including TNF-α [16].

5. Conclusion

DM worsening the complication of PD by increasing the oxidative stress and inflammation in substantia nigra cells. Metformin was a safe, effective method in prevention of further neurodegeneration in adult diabetic albino rats with parkinsonism through its ability to improve DM and through improving of neuroinflammation and oxidative stress in brain cells.

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