

The Effect of Empagliflozin on the Brain Toll Like Receptor-4 Gene Expression in the Experimental Models of Parkinson's Disease

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in Parkinson's
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ABSTRACT

Objective: To investigate the effect of empagliflozin on brain toll like receptor 4 gene expression in animal models of Parkinson and to assess the neuroprotective effect of this intervention

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Pharmacology, College of Medicine, University of Babylon, Hilla, Iraq from 1st December 2025 to 30th April 2026.

Methods: Thirty healthy male albino rats were divided randomly in 5 experimental groups. The time spent on rotarod apparatus was used to assess anti-parkinsonism effect of levodopa/carbidopa and empagliflozin and the real time polymerase chain reaction was used to measure toll like receptor 4 gene expression.

Results: Rotenone administration leads to upregulation of toll like receptor 4 gene expression and significant decrease in body weight, also time spent on rotarod apparatus was considerably lower than the control group ($p \leq 0.05$). Conversely, therapeutic intervention with levodopa/carbidopa and empagliflozin in both doses showed a significant increase in locomotor activity and the body weight when compared with group 2. Groups 3, 4 and group 5 demonstrated significant down regulation of toll like receptor 4 gene expression when compared with rotenone group 2.

Conclusion: Empagliflozin exhibits promising anti-Parkinsonian like effects and it appears to attenuate the neurodegeneration effect induced by rotenone potentially through down-regulation of toll like receptor 4 in the mid brain tissue.

Key Words: Empagliflozin, Rotenone, Parkinson's disease, Neuroinflammation, Toll-like receptor 4.

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INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative condition marked by severe motor impairment due to dopamine depletion in the striatum and chronic degradation of dopaminergic neurons in the substantia nigra pars compacta. Despite the fact that the exact pathophysiology of PD is still unknown, mounting data indicates that neuroinflammation is essential to the development and course of the illness¹. One of the critical mediators of neuroinflammation is toll like receptor 4 (TLR4), a pattern recognition

receptor that is highly expressed in microglia, the primary immune cells of the central nervous system. Activation of TLR4 triggers intracellular signaling cascades leading to activation of transcription factors including nuclear factor-kappa B and the production of pro-inflammatory cytokines. In Parkinson's disease, these inflammatory mediators lead to oxidative stress, neuronal injury, and the gradual loss of dopaminergic neurons¹.

Several experimental studies have demonstrated that TLR4 expression is significantly increased in both human PD patients and animal models of the disease². Conversely, genetic deletion or inhibition of TLR4 has been shown to reduce neuroinflammation, improve dopaminergic neuron survival, and attenuate motor deficits in toxin-induced animal models of PD^{3,4}. TLR4 signaling is an important therapeutic target in the management of neurodegenerative conditions such as Parkinson's disease.

A common medication for type 2 diabetes is empagliflozin, a selective inhibitor of sodium-glucose cotransporter-2 (SGLT2). Beyond its ability to decrease glucose, empagliflozin has shown neuroprotective qualities^{5,6}. Recent studies suggest that SGLT2

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inhibitors may modulate inflammatory signaling pathways and reduce oxidative stress in various tissues, including the CNS. These pleiotropic effects raise the possibility that empagliflozin may influence neuroinflammatory mechanisms involved in neurodegenerative diseases.

Given the pivotal role of neuroinflammation in Parkinson's disease and the emerging anti-inflammatory properties of empagliflozin and investigating its potential effect on TLR4 signaling represent an important area of research.

METHODS

This experimental study was conducted at Department of Pharmacology, College of Medicine, University of Babylon, Hilla, Iraq from 1st December 2025 to 30th April 2026 vide letter No. 4-43 dated 6th November 2025 and 30 mature male albino rats were used. They weighed between 200 and 300 grams. The College of Medicine/University of Babylon's animal house kept the rats at 25°C with 10 hours of darkness and 14 hours of light, along with food and drink. Following two weeks of adaptation, the animals were divided into five groups at random in accordance with the experimental protocol. The weight of each rat was taken on day 0, 10 and 21 day and the rotarod test was performed at the day 22 to assess motor coordination and balance and to evaluate the effectiveness of treatments on animal models. The healthy, rotenone-free rats in group 1 are the negative control. For 21 days, every other day, rats in groups 2 (positive control), 3, 4, and 5 received rotenone. Group 2 rats were given 2.5 mg/kg of rotenone IP every other day without treatment⁷. Group 3 rats received 10 mg/kg/day of levodopa/carbidopa PO⁸. Each rat in groups 4 and 5 received 10 and 20 mg/kg/day of empagliflozin, respectively. The rotarod test was performed at the day 22 to assess motor coordination and balance and to evaluate the effectiveness of treatments on animal models.

The rotarod test is frequently employed to assess motor coordination and balance in animal models of PD. Each rat was placed on the rotarod apparatus for 3 minutes at speed 20rpm. The number of Rotation and the time

spent on the rotarod apparatus were recorded for each rat⁹.

On the twenty-second day, twenty-four hours after the last doses were given, the animals were beheaded. The brains were removed following the posterior foramen magnum incision in the skull. The olfactory bulbs and cerebellum were removed. Anatomical instruments were used to separate the midbrain tissue from the remainder of the brain. The separated midbrain tissue was sent to the lab for RNA extraction after being immediately stored in Trizol reagent Eppendorf.

Using the NCBI GeneBank database, primers for the target gene (TRL-4) and housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were designed by the online Primer3 design programme (Table 1).

Total RNA was isolated from midbrain using the AccuZol™ Total RNA Extraction Kit (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. NanoDrop Spectrophotometer was used to evaluate the quantity and purity of the isolated RNA. Treatment with a DNase I enzyme kit (Promega, Madison, USA) was performed to eliminate residual genomic DNA. Lastly, the cDNA for TLR-4 and GAPDH genes were created with the M-MLV Reverse Transcriptase kit, according to the manufacturer's protocol.

In a reverse transcriptase quantitative polymerase chain reaction (PCR), relative RNA expression was assessed using the SYBR green reporter dye. The TLR-4 gene and the housekeeping gene GAPDH were quantitatively expressed in template cDNAs from each sample using Bio-Rad iQ5 detection equipment (Bio-Rad, Richmond, Virginia)¹⁰.

GAPDH is a commonly used housekeeping gene, especially in gene expression studies. It encodes an enzyme of major regulatory importance that catalyzes oxidative phosphorylation of glyceraldehyde-3-phosphate in the glycolytic pathway. Importantly, its expression is relatively constant across multiple experimental conditions¹¹. The data was analyzed through SPSS-25. The one-way ANOVA followed by post hoc test was used.

Table No. 1: Primer of Toll like receptor 4 (TLR-4) and housekeeping gene (GADPH)

PRIMER	SEQUENCE (5'-3')	NCBI-Reference code
TLR4	TCCGCTGGTTGCAGAAAATG	NM-019178.2
qPCR primer	AGGCAGGAAAGGAACAATGC	
GAPDH	CAAATGGGGTGATGCTGGTG	NM-017008.4
qPCR primer	ATCACAAACATGGGGGCATC	

RESULTS

In comparison to the beginning of the trial on day 0, the control group 1's mean body weight increased significantly ($p=0.009$) on days 10 and 21. In contrast, the mean body weight of the rats in group 2 decreased

significantly ($p=0.025$) on days 10 and 21 in comparison to day 0. The mean body weight of the (L/C) group on day 21 was not significantly different from day 0 ($p=0.527$). The means of weight on days 10 and 21 did not differ significantly ($p>0.05$) between the groups of rats treated with EMPA concentrations of 10, 20 mg/kg and day 0. (Table 2, Fig. 1)

There was a significant decrease ($p=0.001$) in number of rotation in group 2 that were exposed to rotenone as compared to healthy group 1. Additionally, there were a significant increase ($p\leq 0.05$) in groups 3, 4 and group 5 as compared with group 2. Group 5 showed highly significant improvement ($p<0.05$) among all treated groups (Fig. 2)

Comparison of mean difference of time spent on rotarod among group showed that the group 2 had a significant ($p=0.001$) decrease on time spent on rotarod apparatus as compared to healthy group 1. Furthermore, there were a significant ($p\leq 0.05$) increase in groups 3, 4 and group 5 as compared to group 2 (Fig. 3).

Compared to the control group (Group 1), Group 2 treated 21 days with rotenone presented a statistically significant increase ($P < 0.05$) in gene fold change for TLR-4. By contrast, treatment with 10 mg/kg L/C

significantly decreased ($P < 0.05$) TLR-4 gene expression (group 3) figure 4 compared with that of animals exposed to rotenone alone for the same period of time. Likewise, 10 mg/kg empagliflozin-treated rats (Group 4) had a significant reduction ($P < 0.05$) in TLR-4 gene fold change relative to Group 2. The most evident decrease ($P < 0.05$) was found in Group 5 (20 mg/kg empagliflozin $\times$ 21 days). Additionally, the fold change in TLR-4 gene in Group 5 was significantly lower ($P < 0.05$) than Groups 3 and 4 (Fig. 4). For Ct values, lower Ct values were shown in Group 2, indicating an increase in TLR-4 gene expression. On the contrary, Groups 3, 4 and 5 had higher Ct values when compared to Group 2, indicating less expression of TLR-4. Stable GAPDH expression across experimental conditions was verified, as Ct values for each intracellular group were similar (Fig. 5).

Table No. 2: Mean of weight on day 0, 10 and 21 $\pm$ SEM

Weight	Group 1	Group 2	Group 3	Group 4	Group 5
Day 0	263.50 $\pm$ 7.80	244.00 $\pm$ 7.04	255.83 $\pm$ 11.39	257.83 $\pm$ 5.98	251.50 $\pm$ 7.77
Day 10	280.33 $\pm$ 8.48*	232.67 $\pm$ 6.63**	238.83 $\pm$ 10.54	249.67 $\pm$ 5.91	231.17 $\pm$ 7.08
Day 21	304.00 $\pm$ 7.61*	215.33 $\pm$ 6.09**	253.00 $\pm$ 11.44	268.50 $\pm$ 6.88	249.50 $\pm$ 7.61

*Progressive increase from day 0 $\rightarrow$ day 10 $\rightarrow$ day 21 **Significant decrease from day 0 $\rightarrow$ day 21

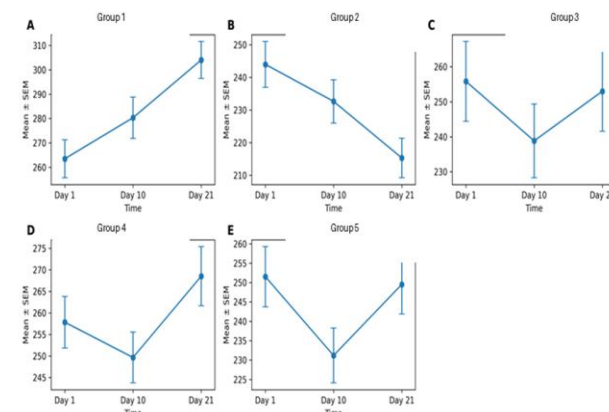


Figure No. 1: Mean of weight on day 0, 10, and 21 $\pm$ SEM

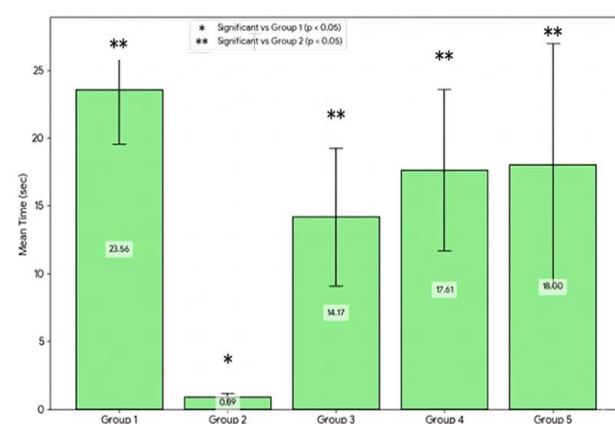


Figure No. 3: Mean of rotation (second) $\pm$ SEM in all rat groups

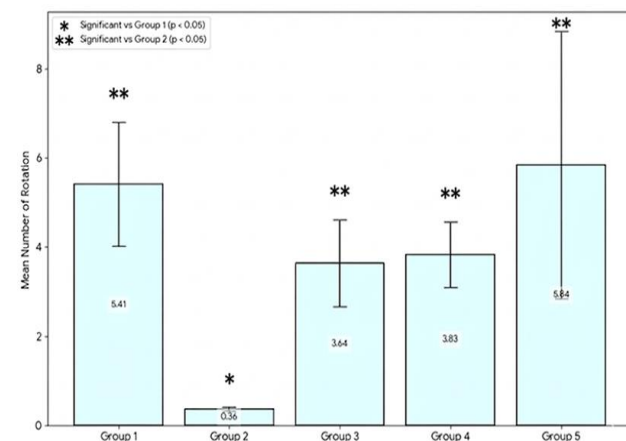


Figure No. 2: Mean of no. of rotations (revolution/minute) $\pm$ SEM

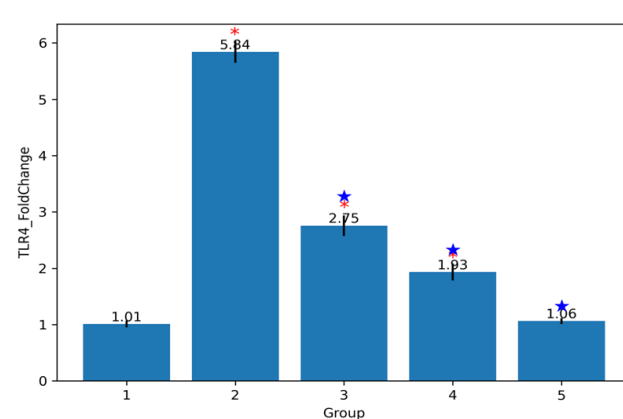


Figure No. 4: Mean of TLR4 fold change $\pm$ SEM

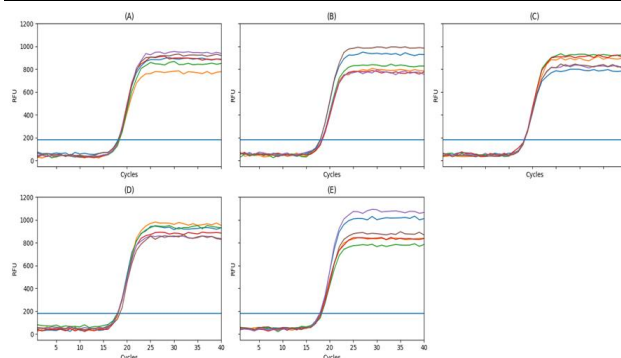


Figure No. 5: The Real Time plots of GAPDH gene expression

DISCUSSION

The changes in body weight are commonly reported in experimental models of PD and may reflect systemic metabolic disturbances and the disease progression. In the present study, rodents in the Parkinsonian group (group2) showed a significant reduction in body weight compared with the control group (group 1). This observation is consistent with previous studies demonstrating that neurodegeneration and metabolic dysregulation contribute to weight loss in Parkinsonian models¹². On the other hand, Treatment with empagliflozin partially ameliorated this reduction in body weight of rotenone exposed rats suggesting that empagliflozin may improve metabolic balance and overall physiological status in affected animals and this finding is consistent with Motawi et al¹³. Empagliflozin has been shown to exert pleiotropic effects beyond glycemic control, including anti-inflammatory and neuroprotective actions within the CNS¹⁴.

Motor impairment is a cardinal feature of PD and reflects the progressive degeneration of dopaminergic neurons in the substantia nigra. In the present study, the induction group demonstrated impaired motor coordination as indicated by decreased latency to fall from the Rotarod apparatus and decrease the number of Rotation. These findings are consistent with previous studies of Parkinson's disease models showing significant deficits in motor performance¹⁵. Additionally, treatment with empagliflozin significantly improved motor performance compared with untreated Parkinsonian rats and this finding is not contradict with the improvement in motor function that have been reported by Mohammed et al¹⁶ investigating the effect of SGLT2 inhibitors in neurodegenerative disorders suggesting that these agents may attenuate neuronal dysfunction and protect dopaminergic pathways. Neuroinflammation is increasingly recognized as a crucial contributor to the progression of PD. TLR4 plays a key role in activating innate immune responses and promoting the release of pro-inflammatory cytokines. In the present study, the induction group exhibited significant upregulation of TLR4 gene

expression compared with the control group and this finding supporting the involvement of inflammatory signaling pathways in PD pathology and this is consistent with Zaho et al¹⁷. Activation of the TLR4 signaling cascade can trigger the downstream inflammatory pathways which ultimately contribute to microglial activation and neuronal damage.

Additionally, when compared to the untreated Parkinsonian group, empagliflozin treatment significantly decreased TLR4 expression levels, especially at high doses. Research on rats revealed that empagliflozin, at a dose of 10 mg/kg/day, decreases TLR-4 gene expression in kidney tissues, demonstrating its capacity to inhibit TLR-4 gene expression¹⁸. This finding suggests that empagliflozin may exert anti-inflammatory effects by modulating TLR4 signaling pathways and was consistent with previous studies which demonstrated that suppression of TLR4 related inflammatory pathways can alleviate neuroinflammation and improve neuronal survival in experimental models of PD^{19,20}. However, to our knowledge, this is the first studies to provide direct evidence of empagliflozin' s ability to downregulate TLR4 gene expression in neurodegenerative model of PD. This novel finding highlights the translation potential of Empagliflozin in modulation crucial inflammatory pathways relevant to pathogenesis of PD. Limitations: First off, even though the rotenone-induced Parkinson's disease model in rats is well-established, it might not accurately reflect the complexity of the human condition, which would restrict how broadly the findings can be applied. Second, the study's brief duration may have limited its ability to show the long-term impacts of empagliflozin medication or the chronic course of Parkinson's disease. Thirdly, since changes in mRNA expression do not always accurately reflect protein level or activity, future research using immunohistochemistry to assess the amount of TLR4 protein in the brain tissue would offer more convincing evidence. The present study indicated that empagliflozin may exert neuroprotective effects in Parkinson's disease through multiple mechanisms including improvement of systemic metabolic status, enhancement of motor performance, and suppression of TLR4-mediated neuroinflammatory responses.

CONCLUSION

Empagliflozin ameliorated Parkinsonism like behavioral deficit induced by rotenone and also showed anti-inflammatory effect in the Parkinsonism induced model, it down-regulated TLR4 gene expression and its neuroprotective potential in the experimental model.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Riham Fadhil Jawad Kadhim, Selman Mohammed Selman
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Drafting or Revising Critically:	Riham Fadhil Jawad Kadhim, Selman Mohammed Selman
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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