Original Article

Study the Effects of Empagliflozin on Model of Chronic Depression and Interleukin-6 in the Brain of Male Rats

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Abstract

Background: Empagliflozin (EMP) is an oral anti-diabetic drug with pleiotropic effects such as anti-inflammatory properties. **Objectives:** To evaluate the antidepressant effect of EMP and describe the link between stress and interleukin-6 (IL-6) level in the brains of male rats. **Materials and Methods:** In this experiment, 50 rats were separated into five groups G1–G5. The sucrose preference test (SPT) was used to examine the antidepressant effects of fluoxetine and EMP. Rat interleukin enzyme-linked immunosorbent assay kit was used to measure the IL-6 level in rat brain tissue. SPT was performed on each rat on days 0, 10, and 25. Chronic unpredictable stress (CUS) was performed on each rat for 24 days. **Results:** By the end of day 10, all rats subjected to the CUS program had a substantial ($P < 0.05$) reduction in sucrose intake index compared to day 0. EMP significantly increases sucrose intake compared to the stressed group. In comparison to the CUS group, fluoxetine significantly increases sucrose intake ($P < 0.05$). In terms of IL-6, the mean IL-6 level in G2 was considerably greater than in G1. When compared to group 2, the mean IL-6 level was considerably lower in G3 and G5. **Conclusions:** EMP has antidepressant-like effects and can counteract the impact of stress-increased IL-6 levels in the brains of depressed rats.

Keywords: Chronic unpredictable stress, depression, empagliflozin, interleukin-6, sucrose preference test

Introduction

Depression is mental disorder widely spread throughout the world with a prevalence rate of 2.6%–5.9% worldwide.[1] Between 1990 and 2017, the estimated global prevalence of depression grew by 49.86%.[2] By 2030, the major depression disorder (MDD) is expected to be the leading contributor to the burden of all medical disorders globally.[3] Periods of low mood, disturbed cognition, a significant functional load, poor occupational functioning, and psychosocial impairment are all characteristics of depression.[4] Depression is treated with medication and non-medication therapies, including psychotherapy, electroconvulsive therapy, and transcranial management. Fluoxetine is a selective serotonin reuptake inhibitor of the second generation selective serotonin reuptake inhibitor and it shall exert its effects by blocking the reuptake of serotonin into presynaptic serotonin neurons by blocking the reuptake transporter protein located in the presynaptic terminal.[5] There is no convincing evidence that present medications may change the course of the disease in depressed patients, despite the fact that 30%–60% of people with major depression are nonresponsive to available treatments and the disease frequently remits at a rate of less than 50%.[6] The need to revitalize psychiatric therapies with new alternatives that engage non-monoaminergic biological pathways is, therefore, reflected in the therapeutic shortfall in treatment results.

A significant amount of data point to the essential role that inflammation may play in the development of MDD.[7,8] However, the precise processes causing depression brought on by inflammation remain poorly understood. The "monoamine-depletion hypothesis" has historically been

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the predominant pathophysiology put forward[9]; however, this explanation cannot adequately explain the etiology of MDD on its own. "Inflammatory hypothesis" has been suggested recently.[10] Additionally, in cases of severe depression, associations of interleukin-6 (IL-6) level and hyperactivity of the hypothalamic-pituitary-adrenal axis have been proposed.[11]

Empagliflozin (EMP) is a strong and specific inhibitor of sodium-glucose cotransporter 2 (SGLT2). Since August 2014, the food and drug administration has approved EMP for the treatment of type II diabetes mellitus and it has pleiotropic effects such as anti-inflammatory properties.[7,12] This study aimed to determine the association between stress and pro-inflammatory cytokines and study the antidepressant effects of EMP.

Materials and Methods

Animals

Fifty mature male albino rats were used in this study. Their weights varied from 245 to 300 g. The rats were kept in the College of Medicine, University of Babylon's animal facility at a constant temperature of 25°C with 14 h of light and 10 h of darkness, along with food and drink. Following a 2-week period of acclimatization, the rats were randomly divided into groups.

General experimental procedure

1. Sucrose preference test (SPT) was performed on each rat on days 0, 10, and 25.
2. The rats in G1 did not get therapy and were not exposed to stress.
3. Chronic unpredictable stress (CUS) was conducted on each rat in groups 2, 3, 4, and 5 for 24 days.
4. Without treatment, each rat in G2 received 0.5 mL of distilled water orally.
5. For 2 weeks, each rat in group 3 was given 10 mg/kg/day P.O.[7]
6. For 2 weeks, each rat in groups 4 and 5 were given 10 and 20 mg/kg/day, respectively.[7]

Chronic unpredictable stress

The chronic unpredictable stress was induced on each rat in groups 2, 3, 4, and 5 for 24 days. The stressors that given to them were (15 min forced swimming, 12 h crowded cages, 12 h cage tilting (45°), 1 h restraint, reversal of the light/dark cycle, 12 h wet bedding, 24 h food deprivation, 24 h food and water deprivation, tail pinch, and 12 h water deprivation). Each day two stressors were applied in a manner to prevent adaptation of rats to these stressors.[7]

Sucrose preference test

SPT was used to assessed anhedonia in rats as one of the fundamental symptoms of depression. Rats were initially trained to drink 2% sucrose solution for 3 consecutive days in two bottles for a cage without water access. Then one of these bottles was replaced with a bottle of tap water for the following 24 h. The rats then spent the next 24 h without food or drink. After this period of deprivation, rats were subjected to SPT for one hour as follows: each rat was removed from its cage and placed in another one that was supplied with two bottles, one of which contained 2% sucrose solution and the other one, tap water. Calculation of sucrose preference was performed according to the following formula: sucrose preference (%) = sucrose intake/(sucrose intake + water intake) × 100%.[13]

Preparation the samples

The rats were decapitated on the 25th day, 24 h after their final treatment. After dissecting the skull from the posterior foramen magnum, the brains were extracted. The cerebellum and olfactory bulbs were removed, and the brain was carefully removed from the skull. The total brain tissue was rinsed thoroughly in phosphate buffer saline (PBS) to remove excess blood and weighed before homogenization. It was homogenized in PBS (1 g of total brain tissue was homogenized in 10 mL of PBS with a glass homogenizer on ice.

Procedure

According to the protocol of Rat Interleukin enzyme-linked immunosorbent assay Kit (BT LAB, Shanghai Korain Biotech, China).

1. Prepared all reagents, standard solutions, and samples as instructed.
2. A 50 µL of the standard was added into a standard well.
3. A 40 µL of the sample was added to sample wells and then added 10 µL rat IL-6 antibody to sample wells, followed by addition of 50 µL streptavidin-horseradish peroxidase to sample wells and standard wells. Wells were mixed and covered the plate with a sealer and incubated for 60 min at 37°C.
4. The sealer was removed and the plate was washed 5 times with wash buffer, and the wells were soaked with 300 µL wash buffer for 1 min for each wash. Paper towels were placed on the plate to dry it.
5. A 50 µL (substrate solution A) was added to each well and then added 50 µL (substrate solution B) to each well. The plate was covered with a new sealer and incubated for 10 min at 37°C in the dark.
6. A 50 µL stop solution was added to each well, the blue color changed into yellow immediately.
7. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 min after adding the stop solution.
8. A standard curve was constructed by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best-fit curve was drawn through the points on the graph. In order to determine the concentration of each sample, these calculations were performed with

computer-based curve-fitting software, and the best-fit line was determined by regression analysis.

Statistics analysis

The statistical package for the social sciences (SPSS) program version 25.0 (SPSS, IBM Company, Chicago, IllinoisIL) for Windows® 10 was utilized. The data were given as a mean ± standard error of mean. Kruskal–Wallis test was used in the statistical analysis. A P <0.05 was regarded as statistically significant.

Ethical consideration

The study was conducted in conformity with the moral guidelines that the Helsinki Declaration served as the foundation for. On July 6, 2022, a local ethics committee examined and approved the research protocol using the reference number 4-1 to receive this permission.

Results

Sucrose preference test

The mean of sucrose intake on days 10 and 25 not significantly differed as compared to day 0 in the control group, untreated and unexposed to CUS G1. In G2, the mean of sucrose intake on days 10 and 25 significantly decreased ($P < 0.05$) in contrast to day 0. Furthermore, the mean of sucrose intake on day 10 significantly decreased ($P < 0.05$) in contrast to day 0 in G3, G4, and G5. On day 25 there was a substantial improvement in sucrose preference index from day 10 in G3, G4, and G5 [Table 1].

G1 (untreated and unexposed to CUS), G2 (untreated and exposed to CUS), G3 (treated with 10 mg/kg fluoxetine + CUS for 14 days), G4 (treated with 10 mg/kg EMP + CUS, G5 (treated with 20 mg/kg EMP+ CUS).

Comparison between the mean of sucrose preference index among rat groups on day 25

On the day (25) the consumption of sucrose was significantly ($P < 0.05$) lowered in (G2) compared to (G1), and a significant ($P < 0.05$) increase in mean of sucrose intake in (G3), (G4), and (G5) was observed compared to (G2) [Figure 1].

G1 (control group, untreated and unexposed to CUS), G2 (untreated and exposed to CUS), G3 (treated with 10 mg/kg fluoxetine + CUS for 14 days), G4 (treated with 10 mg/kg EMP + CUS, G5 (treated with 20 mg/kg EMP + CUS) ($P < 0.05$).

IL-6 concentration on day 25

A significant ($P < 0.05$) increase in IL-6 means concentration in G2 and G4 was observed compared to G1. In G3 and G5 there was a significant ($P < 0.05$) decrease in IL-6 mean concentration compared to G2 and G4 [Figure 2].

G1 (control group, untreated and unexposed to CUS), G2 (untreated and exposed to CUS), G3 (treated with 10 mg/kg fluoxetine + CUS for 14 days), G4 (treated with 10 mg/kg EMP + CUS, G5 (treated with 20 mg/kg EMP + CUS) ($P < 0.05$).

Discussion

Cytokines are connected to the development of MDD.[14] In fact, there is mounting evidence that the pathophysiology of depression involves pro-inflammatory cytokines.[15] Changes in IL-6 serum levels have been identified as one of the most repeatable variations in MDD among pro-inflammatory cytokines.[16] To our knowledge, this is the first study that analyzed the antidepressant effect of EMP. The rats that were subjected to unexpected chronic stress showed less consumption of sucrose solution on the tenth day of applying the protocol, and this was expected, as many previous studies showed that exposure of rats to different stress factors lead to a decrease in the rats' preference for sweetener solutions.[17] The decrease in sucrose consumption is one of the signs of depression development in animal rats.[18] After giving fluoxetine to rats in the third group, starting from the tenth day until the 24th day, with the continuation of the stress protocol, these rats showed a clear improvement in sucrose consumption. These results are consistent with the previous studies that administrations of fluoxetine improve sucrose intake in male rats exposed to chronic unpredictable stress.[19,20] In the fourth and fifth groups that were given EMP 10 mg and

Table 1: Comparison of the sucrose preference index in all rats groups

	Sucrose preference index				
	G1	G2	G3	G4	G5
Day 0	70.07 ± 1.81	68.35 ± 2.12	74.5 ± 1.32	70.17 ± 1.36	73.5 ± 1.19
Day 10	72.2 ± 2.24	61 ± 1.22*	64.9 ± 1.5*	62 ± 1.04*	65.3 ± 1.34*
Day 25	69.3 ± 1.22	58.4 ± 1.40*	72.1 ± 1.74**	68.3 ± 1.57**	70.1 ± 1.99**

*Significant decreased compared to day 0 ($P < 0.05$)

** Significantly increased compared to day 10 ($P < 0.05$)

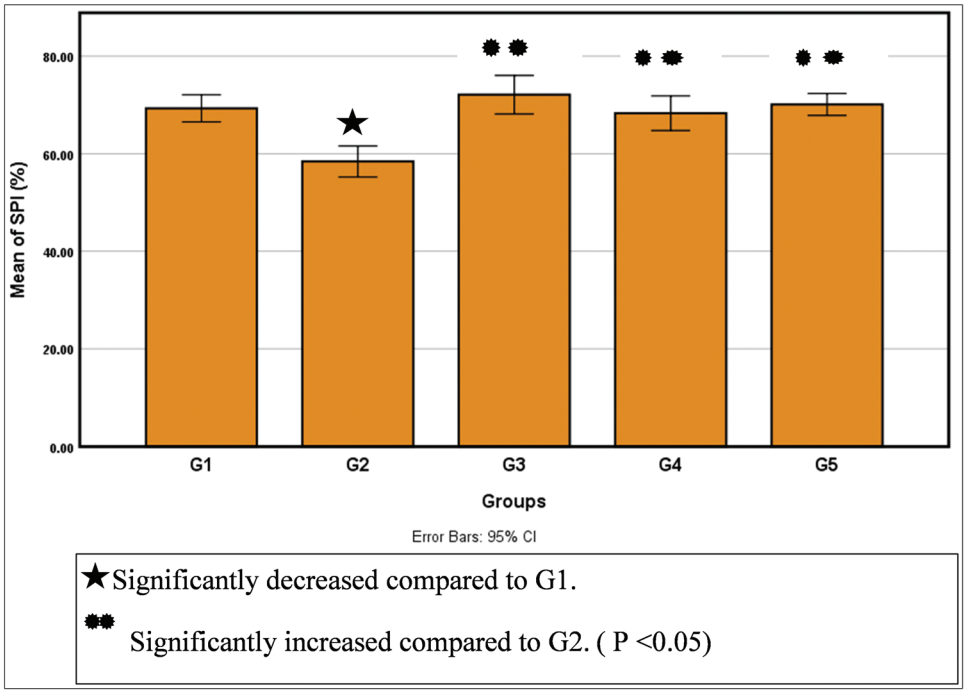

Figure 1: Comparison of the mean ± standard error of mean of sucrose preference index among rat groups on day 25

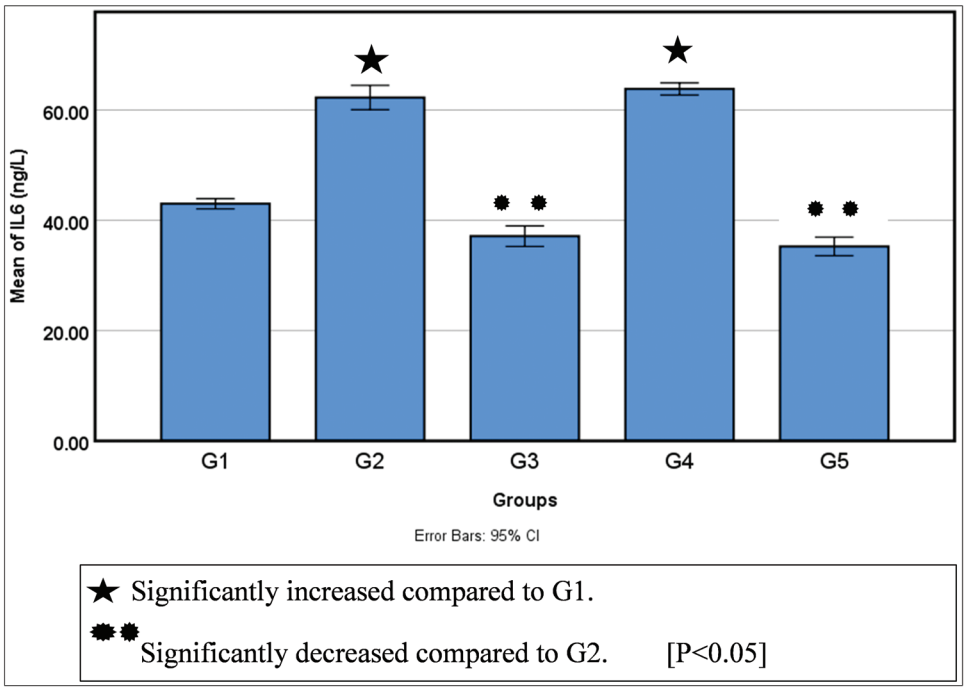

Figure 2: The mean of IL-6 concentration ± standard error of mean in all rats groups on day 25

20 mg respectively, a significant improvement in sucrose consumption on day 25 was shown. With regard to the concentration of IL-6 on day 25, there was a significant increase in the concentration of IL-6 in the group of rats that were exposed to chronic stress only. This is consistent with a study that found an increase in the level of IL-6 in the brain of rats exposed to chronic, unpredictable stress.[21] In the group that was given fluoxetine, the concentration of IL-6 decreased, and this is consistent with study that found that fluoxetine decreased the concentration of IL-6 in the brains of male rats exposed to CUS.[22] Fluoxetine reduces the concentration of this cytokine through several mechanisms, including inflammasomes, peroxisome proliferator-activated receptor gamma, and nuclear factor kappa-light-chain-enhancer of activated B cells.[23] In the group of rats treated with EMP 10 mg, there was no

decrease in the concentration of IL-6 on day 25, whereas the rats treated with EMP 20 mg showed a decrease in the concentration of IL-6 on day 25. This is compatible with studies that showed anti-inflammatory effects of EMP.[7,24] In earlier, it was found that CUS increased the expression of toll-like receptor 4 in the brain of male rats, and activation these receptors leads to increased production of pro-inflammatory cytokines like IL-6,[25-27] and treatment with 20 mg/kg/day led to reduced gene expression of these receptors and improvement in depressive-like behavior.[7] This may explain the mechanism through which EMP reduces the concentration of IL-6.

Conclusion

we can conclude that EMP has an antidepressant effect, as it reduced the anhedonia-like effect induced by CUS and decrease IL-6 levels in the brain of male rats.

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Conflicts of interest

There are no conflicts of interest.

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