



AMELIORATIVE EFFECTS OF PROGESTERONE ON PREFRONTAL CORTEX PLAQUES AND NEUROFIBRILLARY TANGLES ASSOCIATED WITH STREPTOZOTOCIN-INDUCED DIABETIC RATS BRAIN

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ABSTRACT

Progesterone, secreted and synthesized by nerve tissues has been implicated in brain functions, such as its neuro-protective and regenerative potentials on the damaged neurons. This study seeks to evaluate the therapeutic potentials of progesterone on the microanatomy of the prefrontal cortex (PFC) in streptozotocin-induced diabetic rats' brain. Forty eight (48) male Wistar rats with an average weight of $225g \pm 5$ was used for a duration of 16 weeks. The rats were divided into 6 groups comprising of 8 rats each. To induce diabetes; 30 mg/kg body weight of Streptozotocin was injected intraperitoneally along with an oil vehicle of 0.1ml of citrate buffer solution at pH of 4.5. Groups A served as the control, received 1ml of distilled water, group B received 4 mg/kg/body weight/day of progesterone injection intraperitoneally, group C received 8 mg/kg/body weight/day of progesterone injection intraperitoneally, group D received double doses of 30 mg/kg body weight/day of Streptozotocin intraperitoneally, group E received double doses of 30 mg/kg body weight/day of Streptozotocin and 4 mg/kg/body weight/day of progesterone injection intraperitoneally and group F received double doses of 30 mg/kg body weight/day of Streptozotocin and 4 mg/kg/body weight/day of progesterone injection intraperitoneally. Blood glucose levels and histological studies of the Prefrontal cortex of the rats brain was analyzed. Increase in consumption of food, water, and glucose levels was observed in the diabetic animals when compared with control. Body weight decreased in the diabetic animals when compared with control while histological analysis in group D showed the presence of plaques and multiple neurofibrillary tangles in the polymorphic, pyramidal and molecular layers of the cortex while a dose dependent significant decrease ($P < 0.05$) in neurofibrillary tangles and plaques was observed in groups E and F after exposure to progesterone injection compared with control. Conclusively, this further highlights the ameliorative effects of progesterone injection on the prefrontal cortex of streptozotocin induced diabetic rat brain.

Key words: progesterone, Streptozotocin, Blood glucose levels, prefrontal cortex, diabetes

Introduction

For decades, Streptozotocin (2-deoxy-2-(3-(methyl-3-nitrosoureido)-Dglucopyranose)) (STZ); a betacytotoxic substance has been implicated to cause Type 1 diabetes mellitus in mice and rats experiments^{[[17],[13],[21]]}. Streptozotocin (STZ) injection impairs cognitive function through glucose metabolism dysfunction as well as suppressing the activation of essential enzymes in the brain to produce insulin resistance by selectively reducing the process of autophosphorylation on insulin receptor^[6]. Decreased glucose and energy metabolism has been reported in STZ-induction, affects the dendritic morphology in the limbic structures such as occipital cortex, prefrontal cortex, and the hippocampus; which are all implicated in cognitive disorders^{[[19], [17]]}.

^[1] reported that untreated STZ-induced diabetes mellitus was associated with prefrontal Nissl body deficit and oxidative stress in exposed Wistar rats. This was further corroborated by^[14] who reported that the diabetes induction in Goto-Kakizaki rats by STZ administration, resulted in altered expression levels of genes related to neurotransmission, lipid metabolism, neuronal development, insulin secretion, oxidative damage and DNA repair from the hippocampus and the prefrontal cortex in comparison to non-diabetic control animals. STZ treatment in the brain of rats have been associated with an increase tau hyperphosphorylation and neuro-inflammation, which leads to the disturbance of brain insulin signaling, reduced synaptic plasticity and amyloid β peptides^[22].

Progesterone (PG); secreted and synthesized by nerve tissues has been implicated in brain functions, such as its neuro-protective and regenerative potentials on the damaged], neurons ^{[[23} ^[9]] reported that progesterone, with respect to neuro-protection, promotes nerve growth and inhibits inflammation and suppresses neural cell apoptosis. Progesterone (PG) not only functions in reproduction but also plays a beneficial role in injured brains. Its neuro-protective properties includes: voltage-gated calcium channels inhibition and reduced excitotoxicity ^[10]. Progesterone and its 5-alpha-reduced derivatives dihydroprogesterone (DHP) and tetrahydroprogesterone (or

allopregnanolone) promotes Schwann cell proliferation and activation of the myelination in brain cells ^[11].

Moreover, there are paucity of knowledge on the therapeutic potentials of progesterone on memory and cognitive impairment, corticohippocampal-dependent learning and motor imbalance resulting from the combination of oxidative damage, cell death and bioenergetics energy of dysfunctional neurons. Therefore, this study seeks to evaluate the therapeutic potentials of progesterone on the microanatomy of the prefrontal cortex (PFC) in streptozotocin-induced diabetic rats brain.

Materials and Methods

The experimental protocol was by the University ethical review committee, University of Ilorin, Ilorin, Nigeria. The research was approved to be in compliance with the institutional animal care and Use committee (IACUC).

Streptozotocin (Cat.No: 512378) is a product of Sigma-Aldrich (USA) sourced from LabTrade Limited®, Ilorin, Nigeria while Progesterone Injection was sourced from Aromokaye Pharmacy Limited, Ilorin Nigeria.

Forty eight (48) male Wistar rats with an average weight of 225g were used for duration of 16 weeks. The rats were divided into 6 groups comprising of 8 rats each. Groups A served as the control, received 1ml of distilled water, group B received 4mg/kg/body weight/day of progesterone injection intraperitoneally, group C received

8mg/kg/body weight/day of progesterone injection intraperitoneally, group D received double doses of 30mg/kg body weight/day of Streptozotocin intraperitoneally, group E received double doses of 30mg/kg body weight/day of Streptozotocin and 4mg/kg/body weight/day of progesterone injection intraperitoneally and group F received double doses of 30mg/kg body weight/day of Streptozotocin and 4mg/kg/body weight/day of progesterone injection intraperitoneally.

Preparation of Treatment Solutions

To induce diabetes; a double dose of 30 mg/kg body weight of Streptozotocin was injected intraperitoneally along with an oil vehicle of 0.1ml of citrate buffer solution at pH of 4.5. The rats body weight was monitored daily after streptozotocin injection to groups D-F

until a diabetic state was confirmed by the glucose dehydrogenase method.

Rats were inspected daily for signs of pain or distress, including changes in respiration, appetite, urine output, excessive thirst, dehydration, activity, weight loss exceeding 10% of the initial value, unkempt appearance, abnormal posture, and twitching or trembling. The measurement of body weight and observation of body condition (for example, thin, normal, overweight) acted as a marker of appetite. The measurement of skin turgor and observation of cage bedding for urine output acted as a marker of thirst.

On completion of treatments, rats for histological analysis were euthanized using 20 mg/kg of ketamine (intraperitoneal). Transcardial perfusion was done by exposing the left ventricle and injecting 50 ml 0.1 M PBS (pH 7.4) followed by 300 ml 4% paraformaldehyde (PFA) while the rat was suspended in an inverted position (gravity). Excised brains were then rinsed in 0.25 M sucrose 3 times for 5 minutes each and then post-fixed in 4% PFA for 24 hours before being stored in 30% sucrose at 4°C until further

processing. Rats for enzymatic assays were sacrificed by cervical dislocation, to avoid the interference of ketamine with biochemical redox; brains were then excised, rinsed in 0.25 M sucrose 3 times for 5 minutes each and placed in 30% sucrose in which they were stored at 4°C.

Coronal sections of PFC were obtained stereotaxically (+4 mm and -3 mm from the bregma respectively) from each brain. Subsequently, sections were processed routinely to obtain paraffin wax embedded blocks for histology and Bielschowsky's Silver staining to demonstrate Nissl substances in neurons.

Histochemical demonstration of Nissl substances was done with slight modification to the method published by [7].

Determination of superoxide dismutase (SOD)^[12], and catalase (CAT)^[20] activities was carried out on whole brains of treated rats using spectrophotometric technique. Each of the assay kits were procured from Randox Laboratories Limited, San Diego, CA, USA. Whole brain (in sucrose at 4°C) from rats across groups were weighed and

pulverized in 0.25M sucrose (Sigma) with the aid of an automated homogenizer at 4°C. Lysates from the brain were centrifuged for 10 min at 12,000 rpm to obtain the supernatant containing organelle fragments and synaptosomes. The supernatants were aspirated into plain labeled glass cuvette placed in ice. SOD, GPx, CAT and MDA activities were assayed according to the manufacturer's instruction in the assay kit pack.

For light microscopic studies, the PFC sections on glass slides were captured using Olympus binocular research microscope (Olympus, New Jersey,

USA) which was connected to a5.0 MP Amscope Camera (Amscope Inc, USA).

All data were analyzed using GraphPad Prism software (V6: GraphPad Inc., USA). SOD, GPx, CAT and MDA outcomes were plotted using one way ANOVA with Tukey's multiple comparisons test. Significance was set at $p < 0.05^*$. The outcomes were represented in bar charts with error bars to show the mean and standard error of mean respectively.

Results

A significant increase ($P < 0.05$) in body weight changes was observed in groups C and F when compared to control group, while a significant decrease

($P < 0.05$) in body weight changes was observed in group D compared to the control group (Table 1).

Table 1: Showing weight changes in the treatment and control groups

Groups	Initial Weight (g)	Final Weight (g)	Weight Difference (g)
A	166±7.60	179±8.10	13.13±1.88
B	198±2.80	216±3.37	17.50±2.31
C	212±2.98	231±3.40	20.00±1.34*
D	223±2.63	201±3.05	22.03±1.76*
E	230±2.23	241±2.01	11.04±1.13
F	225±1.63	245±2.63	20.17±0.07*

Values are expressed as mean ± SEM showing the level of significance in *P< 0.05 compared to the control group.

Blood glucose levels in group D significantly increased (P<0.05) after Streptozotocin (STZ) administration to the animals in that group when compared to control while a significant reduction in blood glucose levels in groups E and F was observed after progesterone administration when compared to the control (Figure 1).

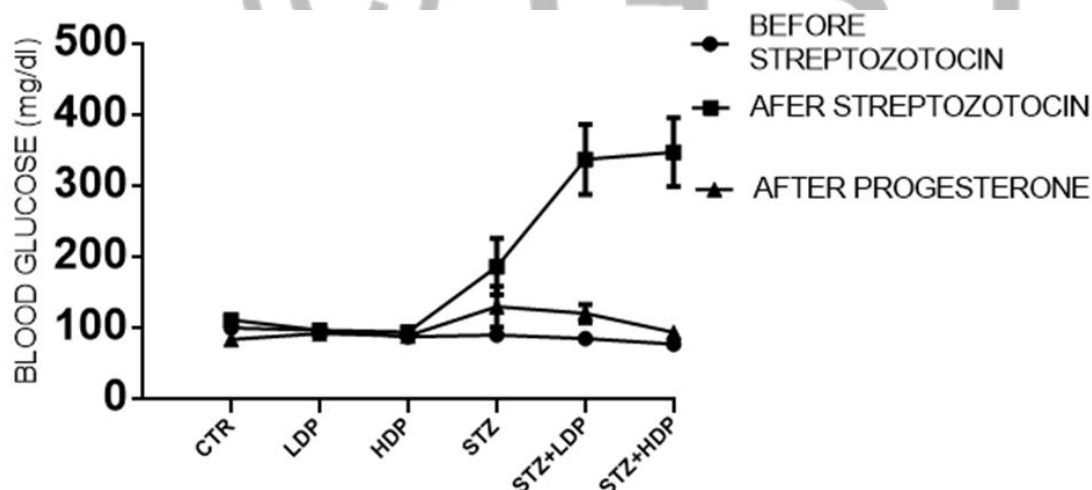


Figure 1: Showing a Line Graph of rats blood glucose levels across all groups. Level of significance in *P<0.05 compared to the control group.

The results of brain SOD, was assessed its involvement in the degenerative processes associated with STZ toxicity in the brain of treated rats to investigate

and to understand the mechanisms involved. Results showed an insignificant reduction ($P>0.05$) in group D when compared to the control while brain SOD profiles of rats in groups E and F were relatively at par with levels with the control and group C with no significant differences (figure 2a).

The results of brain catalase activities, was assessed in the brain of treated rats

to investigate its involvement in the degenerative processes associated with STZ toxicity and to understand the mechanisms involved. Results showed a significant increase ($P<0.05$) in group F alone that received 8 mg/kg body weight of Progesterone after STZ administration when compared with the control (figure 2b).

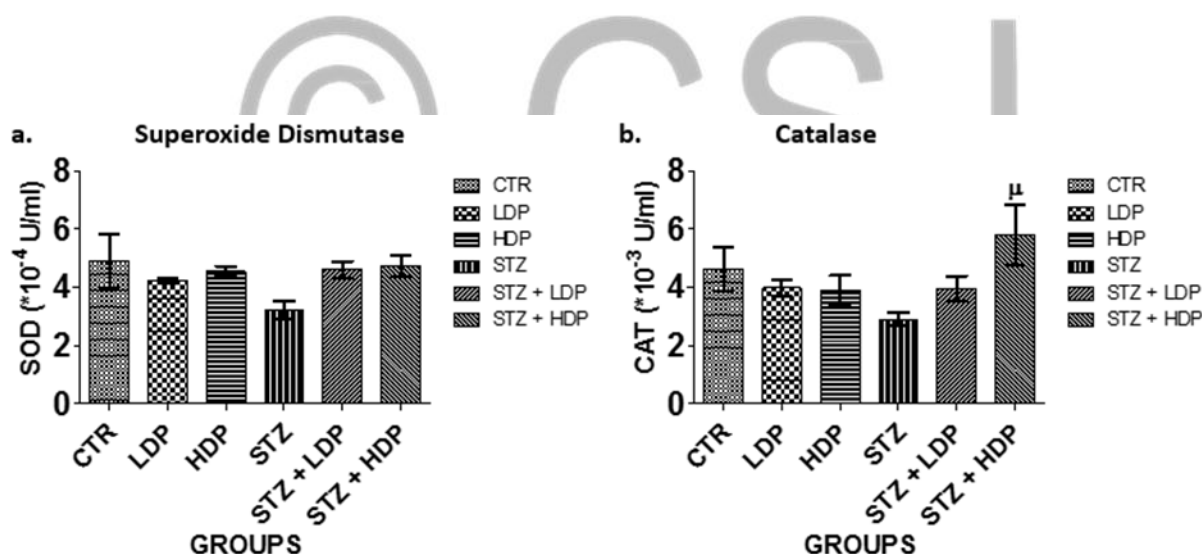


Figure 2 (A and B): Showing Brain SOD and Catalase levels across all treatment groups and control. μ - ($P<0.05$) significant levels.

The ventrolateral prefrontal cortex (VLPFC) regions of the granular layer of the prefrontal cortex showed no visible neurofibrillary tangles or plaque formation in the layers of the prefrontal

cortex (figure 3A-C), There was presence of amyloid plaques (black arrows) and neurofibrillary tangles (red arrow) scattered throughout the prefrontal cortex (figure 3D), group E

showed the presence of few but diffused presence of amyloid plaques (black arrows), neurofibrillary tangles (red arrow) and normal appearance of Nissl granules (white arrows) scattered throughout the prefrontal cortex while group F showed the presence of much lesser presence of diffused presence of

amyloid plaques (black arrows), neurofibrillary tangles (red arrow) and normal appearance of Nissl granules (white arrows) scattered throughout the prefrontal cortex compared to the control and group E (figure 3F) after 8 mg/kg body weight of Progesterone on STZ toxicity.

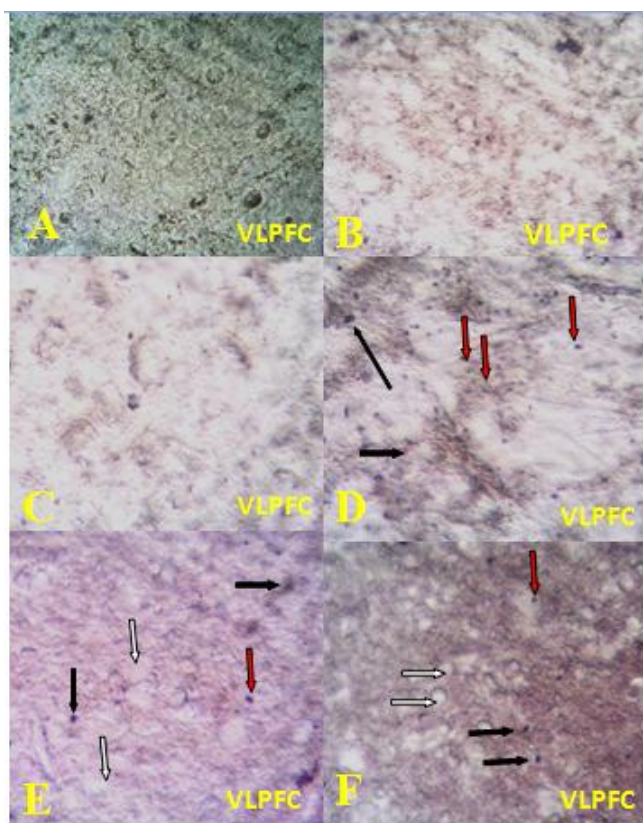


Figure 3 (A-F): Showing photomicrographs of the ventrolateral prefrontal cortex (VLPFC) regions of the granular layer of the prefrontal cortex in adult male Wistar rats. The black arrows point indicates amyloid plaques, Red arrow indicates neurofibrillary tangles and white arrows indicate the normal neurons (Bielshowsky stain x 400).

The dorsolateral prefrontal cortex (DLPFC) regions of the granular layer of the prefrontal cortex showed no visible neurofibrillary tangles or plaque formation in the layers of the prefrontal

cortex (figure 4A-C), There was presence of amyloid plaques (black arrows) and neurofibrillary tangles (red arrow) scattered throughout the prefrontal cortex (figure 4D), group E

showed the presence of few but diffused presence of amyloid plaques (black arrows), neurofibrillary tangles (red arrow) and normal appearance of Nissl granules (white arrows) scattered throughout the prefrontal cortex while group F showed the presence of much lesser presence of diffused presence of

amyloid plaques (black arrows), neurofibrillary tangles (red arrow) and normal appearance of Nissl granules (white arrows) scattered throughout the prefrontal cortex compared to the control and group E (figure 4F) after 8 mg/kg body weight of Progesterone on STZ toxicity.

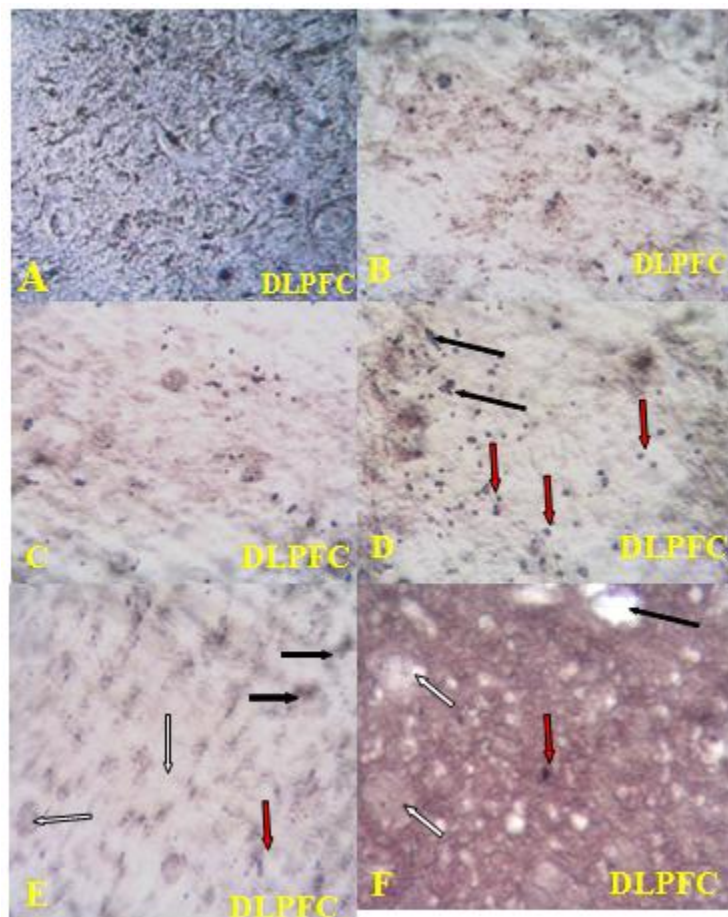


Figure 4 (A-F): Showing photomicrographs of the dorsolateral prefrontal cortex (DLPFC) regions' of the granular layer of the prefrontal cortex in adult male Wistar rats. The black arrows point indicates amyloid plaques, Red arrow indicates neurofibrillary tangles and white arrows indicate the normal neurons (Bielshowsky stain x 400).

Discussion

This study showed that Progesterone administration reversed the degenerative processes brought about by STZ toxicity within the PFC of rats by restoration of biochemical, physiological, cellular parameters to normal levels.

The decrease in weight observed in group D was due to the reduction in water and feed intake-as this mainly controls body weight in mammals according to [5]. This corroborated reports by [[2],[8],[10]] who that intracerebral microinjection of streptozotocin can specifically destroy the granular neurons of various brain areas (e.g. orbitofrontal prefrontal cortex, globus pallidus and ventromedial hypothalamus) leading to alterations in taste perception, severe deficits of feeding and metabolism. The significant increase in percentage body weight of rats in group F compared to the group D was in line with observed gradual regain of appetite and increased food consumption, suggesting a prophylactic role for PG against STZ-induced metabolic imbalance.

Blood glucose levels in group D significantly increased after Streptozotocin (STZ) administration

while the significant reduction in blood glucose levels in groups E and F was observed after progesterone administration (figure 1). This showed that Progesterone stabilized blood glucose in rats pre-treated with streptozotocin.

Induction of diabetes in Goto-Kakizaki rats by streptozotocin administration, results in altered expression levels of genes related to neurotransmission, lipid metabolism, neuronal development, insulin secretion, oxidative damage and DNA repair from the hippocampus and the prefrontal cortex in comparison to non-diabetic control animals [14]. This was in consonance with our reports from this study showed that STZ toxicity caused severe reductions in SOD and Catalase activities (figures 2A and B). However, Progesterone improves brain SOD profiles following STZ-induced oxidative stress. Following the results in groups E and F which were at par with

the control, it suggests that progesterone was able to regulate the activities of superoxide dismutase in the PFC. This corroborated reports by ^[15], ^[9], ^[3] who reported that PG had protective roles on the PFC of adult male Wistar rats.

Reports by ^[16] showed a significant reduction in catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx) in the liver and kidney of STZ-induced diabetic rats; thereby corroborating the results got from this study. However, the extenuative properties of PG were highlighted on brain catalase activities following STZ-induced oxidative stress. This result showed a significant increase in group F alone that received 8 mg/kg body weight of Progesterone after STZ administration when compared with the control (figure 2b); further highlighting a dose dependent ameliorative effects.

This was in consonance with reports by ^[10] who reported that Progesterone had neuroprotective properties which reduced excitotoxicity and inhibited voltage-gated calcium channels which are progenitors of oxidative stress.

The cytoarchitectural disposition of neural cells confirmed that STZ initiated cell death within PFC sections of rat brains. Neurons in this area showed pyknosis, cytoplasmic condensation, and apoptotic signs, hallmarked by nuclear materials degradation and fragmentation of subcellular components in the brain. Such degenerative modifications to the neuronal structure has been attributed to oxidative impairment to DNA, which can be induced by a potent neurotoxins like STZ, particularly by raising the levels of O^{2+} (which correlated observed SOD profiles and Catalase activities) in this study.

This study also showed that progesterone reduced the plaques and neurofibrillary tangles in the PFC corroborating reports by [4] who suggested that progesterone significantly reduced tau hyperphosphorylation when administered alone in female triple transgenic–Alzheimer’s Disease mice. The formation of plaques and neurofibrillary tangles which are regarded as abnormal neurons is as result of structural protein misfolding [18]. Plaques are formed from aggregation of beta amyloid proteins while the neurofibrillary tangles are as a result of in ability of the Tau protein to hold the microtubules in order to function properly.

The underlying mechanism through which PG may have exerted its therapeutic potentials in this study was by inhibiting neuronal death in the prefrontal cortex. This inhibition may involve its

roles in preserving neuronal antioxidant defense system and also by preventing the dysregulation of neuron bioenergetics. It’s good to note that PG may have halted the biochemical cascade that activate proteases which destroy molecules expedient for cell survival, and others that mediate a program of cell suicide in neuronal apoptosis and pyknosis.

In conclusion, this study duly identified the interconnections between the therapeutic potentials of PG in inhibiting biochemical alterations and its putative neuroprotective targets within the cell death machinery on a dose dependent manner. PG improved the expression of important neurochemicals generated as a result of STZ toxicity and the associated cellular damage within the PFC of rats. Therefore, PG administration on a dose dependent manner could provide an intervention

against STZ induced prefrontal cortex brain.
plaques and neurofibrillary tangles in the

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