

Research Article

Progesterone Treatment on Prefrontal Cortical Demyelination in Experimental Diabetes-induced Brain Injury

Adetunji Opeyemi Adebola

Department of Anatomy, College of Health and Medical Sciences, Benjamin Carson (SNR) School of Medicine, Babcock University, Ilisan-Remo, Ogun State, Nigeria & Department of Anatomy, College of Health Sciences, University of Ilorin, Kwara State, Nigeria.

I N F O

E-mail Id:

addturng1809@gmail.com

Orcid Id:

<https://orcid.org/0000-0001-5266-2354>

How to cite this article:

Adebola AO. Progesterone Treatment on Prefrontal Cortical Demyelination in Experimental Diabetes Induced Brain Injury. Int J Adv Res Gynaecol Obstet. 2023;1(1):26-33.

Date of Submission: 2022-11-28

Date of Acceptance: 2022-12-29

A B S T R A C T

Introduction: Clinical studies have been looking for an easy way to solve neurodegenerative diseases and testing of whether effect of progesterone can ameliorate the neurodegenerative effect caused by an induce streptozotocin.

Methods: Animals were randomly grouped into six (6) groups of eight (8) adult wistar rats each: Group CTR, Group LDP, Group HDP, Group STZ, Group STZ + LDP, and Group STZ + HDP. Progesterone was administered daily for seven days after hyperglycaemia is confirmed in groups (STZ + LDP and STZ + HDP), likewise administration of progesterone only is for seven (7) days in groups (LDP and HDP) and the doses of progesterone administration varies, low doses of 4 mg/kg/b.w/day to groups (LDP and STZ + LDP) and high doses of 8 mg/kg/b.w/day to groups (HDP and STZ + HDP), while groups (CTR and STZ) received none.

Results: Consumption of water and food in each group show an effect on the serum glucose, via an increase in body weight of diabetic animals in comparison with control rat's body weight which decreases. At the end of the experiment (18 weeks) the rats are sacrifice and microscopic study of neurotrophic and neuroprotective of rats, prefrontal cortex tissue sampling shows that cells in the molecular layer of the induce-hyperglycaemia groups rats (STZ, STZ + LDP & STZ + HDP) are reduced and the cortical layer are wider and contain fewer cells compared to the non-induced hyperglycaemia groups rats (CTR, LDP & HDP) that has thinner cortical layers and Loss of myelin sheath in the groups (STZ, LDP and STZ + LDP) in contrast to groups (CTR and STZ + HDP).

Conclusion: Involvement of myelinated axons in the morphologic impairment induced by an untreated hyperglycaemic state, an evident in the study, therefore doses of progesterone have an effect on the myelination of axon.

Keywords: Myelination, Progesterone, Prefrontal cortex, Streptozotocin

Introduction

Streptozotocin 2-Deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose (STZ) is a beta cytotoxic substance that has been implicated to cause diabetes mellitus type in rats and mice experiments for decades.^{24,29,33} STZ injection impairs cognitive function by the occurrence of dysfunction of glucose metabolism in the brain as well as suppressing the activation of essential enzymes to produce insulin resistance and corresponding processes by selectively reducing the autophosphorylation process of the insulin receptor.¹⁴ Decreased glucose and energy metabolism has been reported in STZ and has been found to induce diabetes mellitus and affect the dendritic morphology in the limbic structures, such as the prefrontal cortex, occipital cortex, and hippocampus, which are implicated in cognitive disorders.^{29,31} Diabetes mellitus, if not properly attended to, can result in a wide range of damage to organs and systems in the body.²⁹

STZ causes the death of pancreatic β -cells by alkylation of DNA resulting in reduced synthesis and release of insulin, and has been shown to be involved in the fragmentation of DNA through the production of reactive oxygen species.²⁷

Diabetes induced by STZ is associated with polydipsia and loss in body weight.²⁷ The sulfonyleurea, glibenclamide (GLB) and the semi-essential amino acid taurine (TR) have hypoglycaemic and hypocholesterolemic effects in animal models of diabetes.²⁷ Complications related to diabetes mellitus are associated with oxidative stress induced by the generation of free radicals.¹² Free radicals result in the consumption of antioxidant defences leading to disruption of cellular functions and oxidative damage to membranes and enhanced susceptibility to lipid peroxidation, increased generation of reactive oxygen species (ROS).³ Lipid peroxidation has been found to be involved in the pathogenesis of many diseases of known and unknown aetiology and in the toxic actions of many compounds.³

STZ treatment in the brain of rats has been associated with an increase in tau hyperphosphorylation and neuroinflammation, a disturbance of brain insulin signalling, reduced synaptic plasticity and amyloid β peptides have been reported.³⁷ Sub-diabetogenic doses of STZ have been shown to induce cognitive and brain cholinergic deficits,²¹ oxidative stress as well as decrement in brain glucose/energy metabolism,¹⁶ and insulin-resistant brain state.³⁰ There is a hypothesis of sporadic Alzheimer's disease (AD) being described as the brain type of non-insulin-dependent diabetes mellitus (DM) and the presence of insulin-resistant brain state in AD patients post-mortem,¹⁵ the expression of twenty (20) AD-related genes, including those involved in the processing of amyloid precursor protein, cytoskeleton, glucose metabolism, insulin signalling, synaptic function, protein kinases and apoptosis, was altered, suggesting that STZ disturbs multiple metabolic and cell signalling pathways

in the brain.³⁷ Cognitive dysfunction and dementia have recently been proven to be common complications of DM.¹⁷ Studies show that phenotypes associated with obesity and alterations in insulin homeostasis are at increased risk for developing cognitive decline and dementia.¹⁷ Both types 1 and 2 diabetes are risk factors for decreased performance in several neuropsychological functions, chronic hyperglycaemia and hyper-insulinaemia primarily stimulate the formation of Advanced Glucose End-products (AGEs), which leads to an overproduction of ROS.¹⁷

Protein glycation and increased oxidative stress are the two main mechanisms involved in biological ageing, both being also probably related to the aetiopathogeny of AD.¹⁷ AD patients were found to have lower than normal cerebrospinal fluid levels of insulin.¹⁷ Besides its traditional glucoregulatory importance, insulin has significant neurotrophic properties in the brain.¹⁷ Clinically hyperinsulinism is a factor for AD and lab experiments proved insulin to have a neurotrophic factor. These two apparent paradoxical findings may evoke the concept of insulin resistance. Insulin is neurotrophic at moderate concentrations, too much insulin in the brain is associated with reduced amyloid- β ($A\beta$) clearance as a result of competition for its common and main depurative mechanism - the Insulin-Degrading Enzyme (IDE), IDE is more selective for insulin than for $A\beta$, brain hyperinsulinism deprives $A\beta$ of its main clearance mechanism.¹⁷ Hyperglycaemia and hyperinsulinaemia seem to accelerate brain ageing also by inducing tau hyperphosphorylation and amyloid oligomerization, as well as by leading to widespread brain microangiopathy.¹⁷

The prefrontal cortex is commonly implicated in higher cognitive functions.³⁴ The prefrontal cortex (PFC) is one of the key cortical structures to monitor the internal state of the organism. The prefrontal cortex (PFC), commonly implicated in higher cognitive functions,³⁴ is one of the key cortical structures used to monitor the internal state of the organism and to initiate behavioural outputs accordingly. The PFC is implicated in many regulatory processes, including cognitive functions, attention, drive and motivation, decision making and working memory.^{9,35} In addition to being generally acknowledged as a substrate of higher cognitive function, the prefrontal cortex has been implicated in some of the most common and devastating neurological and psychiatric disorders, including age-related cognitive decline, attention-deficit hyperactivity disorder, Parkinson's disease, Huntington's disease, Wernicke-Korsakoff syndrome, unipolar depression, and schizophrenia.^{2,5,11,13,20,36}

Progesterone (PRO) possesses antioxidant properties that are involved in the scavenge ring of ROS in cancer cells²⁵ and increasing superoxide dismutase (SOD) activity in human endometrial stromal cells.¹⁹ Morrissy S et al. reported that

progesterone can induce antioxidant gene expression in cardiomyocyte cells and exert antioxidant and anti-apoptotic effects.²³ Progesterone's ability to reduce cerebral oedema associated with traumatic brain damage first became apparent when we observed that males had significantly more oedema than females after cortical contusion.²⁸ In addition, oedema was almost absent in pseudo-pregnant female rats, a condition in which progesterone levels are high relative to estrogen. Progesterone injections given after injury also reduced oedema and were equally effective in both males and females.²⁸ In addition, progesterone-treated rats were less impaired on a Morris water maze spatial navigation task than rats treated with the oil vehicle.²⁸ Progesterone-treated rats also showed less neuronal degeneration twenty-one (21) days after injury in the medial dorsal thalamic nucleus, a structure that has reciprocal connections with the contused area.²⁸ Progesterone reduced tau hyperphosphorylation when administered both alone and in combination with oestrogen.⁷ However, oestrogen and progesterone administered independently and interactively regulate AD-like neuropathology and suggest that optimised hormone therapy may be useful in reducing the risk of AD.⁷

Methodology

Animal Management

Forty-eight (48) male, two weeks (2 weeks) old Wistar rats were sourced from the Department of Zoology, University of Ilorin. The rats are reared and acclimatized for four months (16 weeks) in the experimental facility of the Faculty of Basic Medical Science Animal House, University of Ilorin, for 12-hour light: 12-hour dark cycle with a room temperature of 30 °C. All rats received standard laboratory animal chow and water ad-libitum during the whole period of the experiment.

The rats were divided into six (6) groups with eight (8) rats per cage of a group. Water was provided ad libitum, and animals were fed an irradiated rodent diet (Diet 2919 Teklad Global 35% Protein Rodent Diet), diet was selected preferentially over standard chow because of its slightly higher energy density compared with that of the conventional mouse diet (10.3 kcal/g). This higher energy level is advantageous in support of the diabetic state. Studies using these rats for assessment of islet cell preparations were approved by the University of Ilorin Institutional Animal Care and Use Committee, conducted in compliance with the Animal Welfare Act, and adhered to principles stated in the Guide for Care and Use of Laboratory Animals of the University of Ilorin Animal Ethics Committee in-line with the National Institute of Health (NIH) guidelines on the use of animals in experiment research.

Induction and Animal Monitoring

The rats were injected with double low doses of 30 mg/

kg b.w streptozotocin intraperitoneally which was given along with an oil vehicle of citrate buffer solution of 0.1 ml of 4.5 pH after fasting for 24 hours. The rats' body weight was monitored once before and daily after streptozotocin injection to groups (STZ, STZ + LDP and STZ + HDP) 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. Animals were randomly grouped into six (6) groups of eight (8) rats each: Group CTR, Group LDP, Group HDP, Group STZ, Group STZ + LDP and Group STZ + HDP. Progesterone was administered daily for seven days after hyperglycaemia was confirmed in groups (STZ + LDP and STZ + HDP), likewise, administration of Progesterone only was for seven (7) days in groups (LDP and HDP), and the doses and frequency of Progesterone administration range, low doses of 4 mg/kg/b.w/day to groups (2 and 5) and high doses of 8 mg/kg/b.w/day to groups (HDP and STZ + HDP), while groups (CTR and STZ) received none.

Animal Sacrifice and Sample Collection

At the end of the experiment (18 weeks), the animals were anaesthetized with an intermuscular injection of 20 mg/kg of ketamine, skin excised and transcardially perfused with saline and subsequently with 4% paraformaldehyde in 0.1M phosphate. The brains were removed and the prefrontal cortex was excised from the brain and put into 30% sucrose solution for biochemical assay and 4% paraformaldehyde fixative for histological analysis.

Estimation of Prefrontal Catalase (CAT) and Malondialdehyde (MDA) and Histological Staining

Prefrontal Catalase activity was estimated according to the method shown by Sinha³² and MDA was estimated by the thiobarbituric acid test.^{10,26} Prefrontal lobes of rats' brains were fixed in 4% paraformaldehyde and processed for paraffin embedding. Paraffin sections (5 µ) were stained with cresyl fast violet to study the morphology of the prefrontal neurons.⁴

Statistical Analysis

Data collected on body weight, CAT, and MDA, were analysed using Microsoft Excel and one-way analysis of variance (ANOVA) followed by Tukey's (HSD) multiple comparison test with the aid of SPSS V20. Data were presented as mean ± SEM (standard error of the mean) P value less than 0.05 (p < 0.05) was considered statistically significant.

Results

This study investigated the histological and histochemical patterns of the prefrontal cortex cells in untreated and treated rats; the protection and regeneration progesterone might cause to these cells, and the neuronal instability and oxidative stress caused by streptozotocin and progesterone.

Body Weight

Control (CTR); Low doses of Progesterone (LDP) of 4 mg/kg; High doses of progesterone (HDP) of 8 mg/kg; Double injection of streptozotocin (STZ); Double injection of streptozotocin followed by low doses of Progesterone of 4 mg/kg (STZ + LDP); Double injection of streptozotocin followed by high doses of progesterone of 8 mg/kg (STZ + HDP).

There are no significant differences in body weight across these groups before administration but there are significant differences after administration of Streptozotocin (STZ), and Streptozotocin plus progesterone (STZ + PRG) across the groups when compared to before administration in the course of the experiment. Values are presented as mean \pm SEM ($p < 0.05$).

Catalase level reduced in animals exposed to streptozotocin only (STZ group), though not statistically significant ($p > 0.05$) compared to control (Control group) and animals given different doses of progesterone (LDP and HDP groups) and increased in animals exposed to streptozotocin and treated with progesterone (STZ + LDP and STZ + HDP groups) in which, it shows statistically significant different ($\mu - p < 0.05$) by the level of catalase (CAT) increased significantly ($\mu - p < 0.05$) in animals treated with high doses of progesterone (HDP group) compared to animals exposed to streptozotocin (STZ group).

Histological Observation of the Photomicrograph

The population of cells in the molecular layer of the prefrontal cortex of the induce-hyperglycaemia group rats (STZ, STZ + LDP & STZ + HDP) are reduced and their

cortical layer is wider and contains fewer cells compared to the non-induced hyperglycaemia groups rats (CTR, LDP & HDP) that has thinner cortical layers.

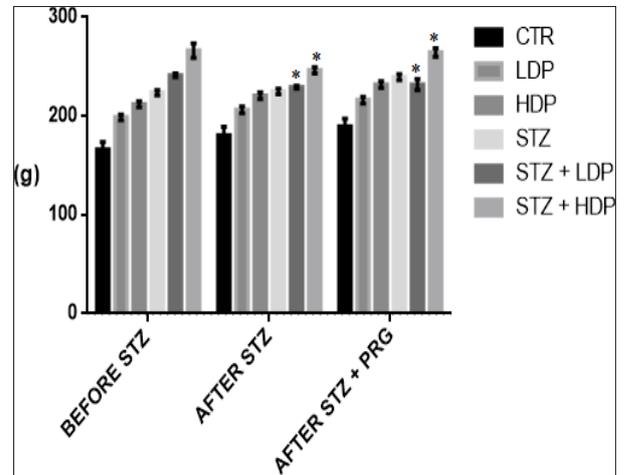


Figure 1. Graph showing the Animal Body weight across all Groups Before and After Administration of Streptozotocin and After Administration of Progesterone ($p < 0.05$)

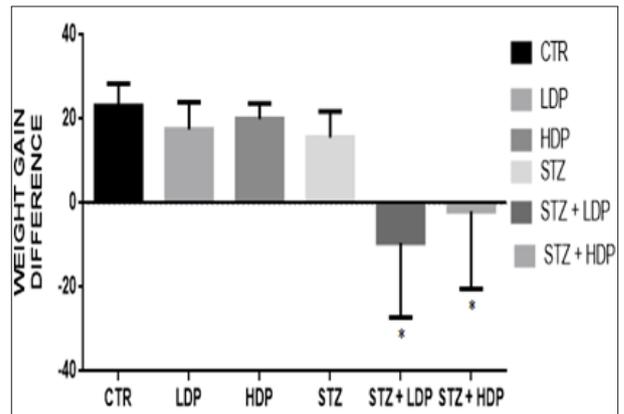


Figure 2. Graph showing the Animal Body Weight Difference across all Groups ($p < 0.05$)

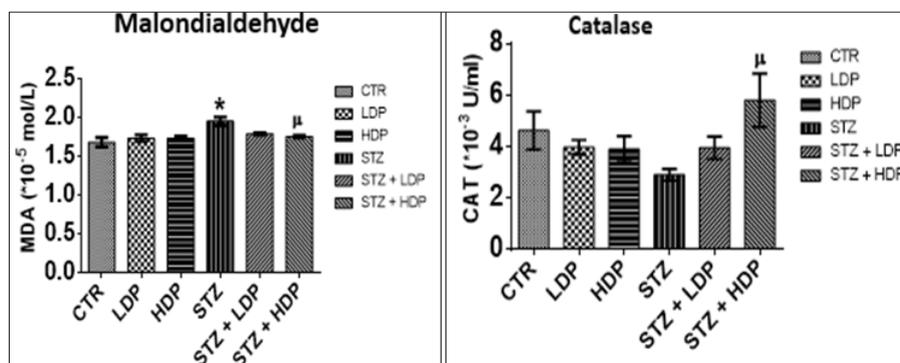


Figure 3. Graph showing Malondialdehyde Levels across all Groups ($p < 0.05$) Statistically Significant Difference compared to Control Animals (CTR), ($\mu - p < 0.05$) Statistically Significant Difference compared to Animals Exposed to Streptozotocin (STZ). Catalase Levels across all Groups, ($\mu - p < 0.05$) Statistically Significant Difference compared to Animals Exposed to Streptozotocin (STZ)

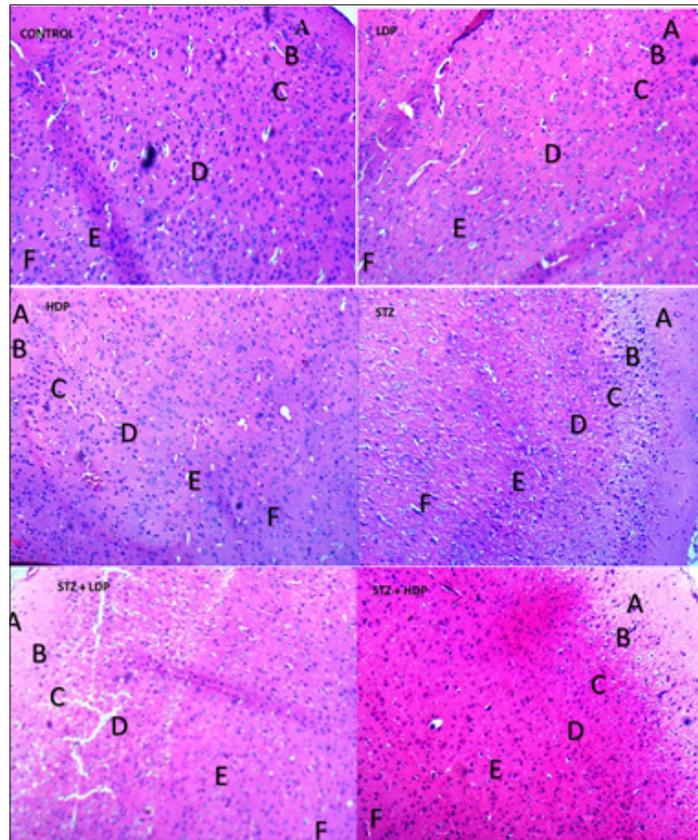


Figure 4. Photomicrograph showing the Cortical Layer Show by Tag A and Granular Layers (GL) 'Layer II - Tag B, III - Tag C, IV - Tag D, Layer V - Tag E, and Layer VI - Tag F' (H & E, X 100)

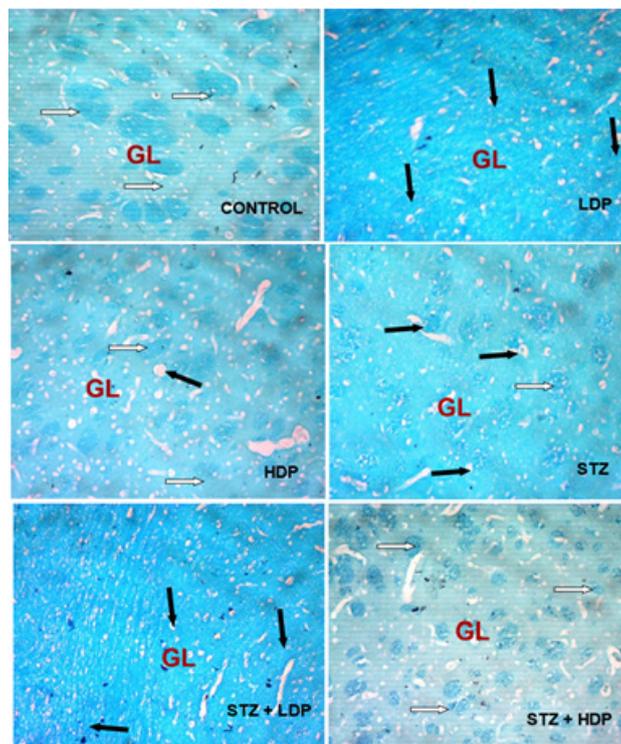


Figure 5. Photomicrograph showing the Pyramidal Cells of the Granular Layer (GL) of the Prefrontal Cortex of Adult Male Wistar Rats (Luxol Fast Blue Stain X 100). The black arrow shows a poorly myelinated (demyelinated) sheath and the white arrow shows an intact myelin sheath which represents the normal neurons.

The cortical layer contains few scattered neurons and glial cells, some cajal-retzius and spiny stellate cells, while the granular layers contain in layer II small pyramidal neurons and numerous stellate neurons, in layer III small and medium-size pyramidal neurons and vertically oriented non-pyramidal neurons, in layer IV different types of stellate and pyramidal neurons, in layer V large pyramidal neurons and beta cells, and in layer VI few large pyramidal neurons and many small spindle-like pyramidal and multiform neurons. This layer can also be referred to as I - marginal layer, II - cortical layer, III - subcortical layer, IV - intermediate layer, V - subventricular layer and VI - ventricular layer.

e prefrontal cortex marked by Luxol Fast Blue Stain in the study shows Loss of myelin sheath in the STZ group, LDP group, and STZ + LDP group, in contrast to the intact myelin sheath of the CONTROL group and STZ + HDP group. While there is a stint of loss of myelin in a minute in the HDP group.

Discussion

Progesterone reduces Stress Markers in the Prefrontal Cortex of Adult Male Wistar Rats

Progesterone might have an ameliorative effect in the group STZ + LDP, but in group STZ + HDP it serves a more protective role, in the prefrontal cortex of adult male Wistar rats which corresponds with the works of Carroll et al. and Chao et al.^{7,8} The activities of malondialdehyde level increased significantly (< 0.05) in animals exposed to streptozotocin (STZ group), compared to control (CTR group) and animals given different doses of progesterone (LDP and HDP groups); but the malondialdehyde (MDA) level reduced in animals exposed to streptozotocin and treated with different doses of progesterone (STZ + LDP and STZ + HDP groups), most significantly in animals exposed to streptozotocin and treated with a high dose of progesterone. This process involves a free radical chain reaction mechanism terminated either by the counter-effects of antioxidants or the production of mutagenic or carcinogenic reactive aldehydes such as malondialdehyde. The resulting chain reaction makes lipid peroxidation more damaging due to increased oxidative stress and it has been duly implicated in most neurodegenerative diseases.¹⁸ Lipid peroxidation results from the oxidative degradation of lipids and involves the stealing of electrons from the lipid cell membrane by free radicals resulting in cellular damage.²²

Action of Progesterone on Myelination of the Prefrontal Cortex

The involvement of myelinated axons in the morphologic impairment induced by an untreated hyperglycaemic state is evident in the study on plate 2. Loss of myelin sheath of axons was demonstrated by the LFB technique (STZ, LDP and STZ + LDP groups). A previous study using the Golgi

technique showed a significant reduction in the mean density of pyramidal neuron dendritic spines of the prefrontal cortex region after fourteen days of untreated hyperglycaemic (STZ group). The latter finding, and our observations in the present study, support the report of diabetes-induced brain lesion and injury by Akinola OB et al.¹ Therefore, the dosage of progesterone has neuroprotective and neuro-regeneration effects on the myelination of the axon.

Conclusion

The use of progesterone may reduce the morphological alterations and oxidative damage associated with streptozotocin-induced demyelination in the prefrontal cortex. This could offer clinical benefits in neurological associated dysfunction with may be ameliorated with the aid of progesterone.

Recommendation

There is some limitation in term of the immunological study of the STZ group and the ameliorative groups (STZ + LDP and STZ + HDP). Likewise, on the dose affirmation groups (LDP and HDP), I will recommend more research before finally deciding on clinical usage.

Source of Funding: None

Conflict of Interest: None

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