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Role of Progesterone Treatment on the Microanatomy of the Prefrontal Cortex of Streptozotocin - Induced Diabetic Wistar Rats

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Abstract: Elucidation of progesterone effect on the prefrontal cortex of a streptozotocin-induced diabetic Wistar rats via comparison of changes in theoxidative stress, cyto-architecture of the prefrontal cortex of rats, levels of glucose and on the dosages of progesterone administered intra-peritoneal. Streptozotocin (STZ, 30mg/kg/b.w) double dosages were injected to mimic diabetes by degenerating beta cells within third to seventh days in adult male rats. There are eight rats per group and treatments included (STZ, received two doses of 30mg/kg/b.w Streptozotocin; STZ+LDP treatment received 30 mg/kg/b.w Streptozotocin and low dose progesterone (LDP, 4 mg/kg/b.w/day); STZ+HDP treatment received 30 mg/kg/b.w Streptozotocin and high dose progesterone (HDP, 8 mg/kg/b.w/day); LDP received 4 mg/kg/b.w/day progesterone; and HDP received 8 mg/kg/b.w/day progesterone while the control (CTR) and STZ treatments received no progesterone. In the study, blood glucose level increased in the diabetic animals in comparison with normal animals. Metabolically and histologically, comprising between diabetic and untreated rats showed that Streptozotocin caused degeneration in astrocytes and induced experimental diabetes while progesterone dosage induced ameliorative effect. In conclusion, progesterone protects and keeps the neurons in the brain, likewise proved that streptozotocin have a neurodegenerative effect.

Key words: Progesterone · Streptozotocin · Prefrontal Cortex · Diabetes · Rats

INTRODUCTION

Primates are unique among mammals in possessing a region of prefrontal cortex with a well-developed internal granular layer. This region is commonly implicated in higher cognitive functions [1]. The prefrontal cortex (PFC) is one of the key cortical structures 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 [2, 3].

In addition to being generally acknowledged as a substrate of higher cognitive function, 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 [4-9].

Streptozotocin (STZ) injection impairs cognitive function by the activities of dysfunction of glucose metabolism in the brain as well as suppressing the activation of essential enzymes to produce insulin

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resistance and corresponding processes by selectively reducing the autophosphorylation process of the insulin receptor [10]. Decreased glucose and energy metabolism has been reported with STZ and has been found to induce diabetes mellitus. It affects the dendritic morphology in the limbic structures, such as prefrontal cortex, occipital cortex and hippocampus, which are implicated in cognitive disorders [11, 12].

The 2-deoxy-2-(3-(methyl-3-nitrosoureido)-Dglucopyranose) (STZ) is a betacytotoxic substance that has been implicated to cause diabetes mellitus type (1 and 2) in rats and mice experiment for decades [13, 14]. Some of these neurological disorders have been characterised in diabetic patient and diabetes is characterized by a constant stage of hyperglycemia, which if not well managed leads to severe damage to several systems in the animals or humans body [15], includes the pancreas, retina and central nervous system (CNS). Report says diabetes cause several brain conditions such as cerebral ischemia, macrovascular disease, micro-angiopathy, cognitive decline and brain atrophy [16]. Diabetes was characterized by increased lipid peroxidation, altered glutathione redox status, exacerbated levels of reactive oxygen species (ROS) and mitochondrial dysfunction [17].

Progesterone been implicated in brain functions, where it was synthesized and secreted by nerve tissues, active in both neuro-protective and regenerative potentials on the damaged neurons [18, 19]. Progesterone acts a neuro-protectant by suppressing neural cell apoptosis, promoting nerve growth and relieving inflammatory swelling [20], while acting as neuro-regeneration by increasing the concentration of macrophages and microglia at injured sites [21] thus, increasing the circulation of endothelia progenitor cells in the brain [22]. Progesterone has been stated to reduce the concentration of oxygen free radicals [23], therefore it might be able to contribute to neurogenesis. It's an endogenous steroid produced in the ovary and schwann cell (neurosteroid) and it is a metabolic intermediate in the production of other endogenous steroid and being responsible in regulation of pregnancy and menstrual cycle, likewise neurogenesis might result out of progesterone [24].

The prefrontal cortex (PFC), which covers the most frontal part of the mammalian brain contains Brodmann areas 9, 10, 11, 12, 46 and 47 and the basic activity of this brain region was considered to be the orchestration of thoughts and actions in accordance with internal goals such as executive function [25]. Executive function relates to abilities of an individual to differentiate among conflicting thoughts, determine good and bad, better and best, same and different, future consequences of current activities, working toward a defined goal, prediction of outcomes, expectation based on actions and social "control" (the ability to suppress urges that, if not suppressed, could lead to socially unacceptable outcomes) [26]. The PFC has also been associated with other functions like planning complex cognitive behaviour, personality expression, decision making and moderating social behaviour [27].

The dorsolateral prefrontal cortex (DLPFC) is an area in the prefrontal cortex lying in the middle frontal gyrus, around the principal sulcus (lateral to the Brodmann's area 9 and 46) of the brain of humans and primates, therefore DLPFC is not just an anatomical structure in the brain but a functional region that undergoes a prolonged period of maturation which lasts until adulthood [28, 29]. The DLPFC is connected to various regions of the brain including the orbitofrontal cortex, hippocampus, primary and secondary areas of the neocortex, basal ganglia and thalamus [30]. The primary function of the DLPFC is executive function, though not exclusively because all complex mental activity requires the additional cortical and subcortical circuits with which the DLPFC is connected. The executive functioning includes working memory, cognitive process, planning, inhibition and abstract reasoning [31, 32]. The DLPFC is also very much involved in motor planning, organization and regulation due to the association with the basal ganglia and motor areas of the brain [31].

The ventrolateral prefrontal cortex (VLPFC) is located on the inferior frontal gyrus, bounded by the inferior frontal sulcus superiorly and the lateral sulcus inferiorly. The VLPFC is attributed to Brodmann's area 47, 45 and 44 [33, 34]. The whole right VLPFC is active during motor inhibition, where it activates to stop or to override the motor activity in the cortex [35]. The right posterior VLPFC (BA 44) is active during the updating of action plans. The right middle VLPFC (BA 45) is responding to decision uncertainty (presumably in right-handed individuals) [35].

The ventromedial prefrontal cortex (VMPFC) is located in the frontal lobe at the bottom of the cerebral hemispheres. The VMPFC is interconnected with the tegmental area, amygdala, cingulate gyrus, temporal lobe, the olfactory system the hippocampal formation and the thalamus. The VMPFC though does not have a universally agreed demarcation; it is associated with Brodmann area 10, 14, 25 and 32, as well as portions of Brodmann area 11, 12 and 13 [36]. The VMPFC is implicated in processing risk and fear and also plays a role

in the inhibition of emotional responses and in the process of personal and social decision making [34, 37]. These sides of the prefrontal cortex of the brain have an implicit effect on age-like related measure [34].

MATERIALS AND METHODS

Animal Grouping: The procedure and experiment were done in the University of Ilorin Animal holding and was in-line with the National Institute of Health (NIH) guidelines on the use of animals in experiment research. Forty eight male Wistar rats (*Rattusnovergicus*) of two weeks old were procured and were kept in different wooden cages with wire gauze roofs. The rats were acclimatized under the light and dark cycle at room temperature (37°C) and fed on high fat diet (Veritus Livestock Feeds, Tanke, Ilorin) and distilled water throughout the duration of three months until the animal weight was within the range of 150-250g. The streptozotocin was purchased at sigma and progesterone injection was purchased from a reputable government pharmacy (Aromokeye pharmacy) in Ilorin, Nigeria. The Wistar rats were grouped based on their weights and starved overnight before administrating their treatments. This was carried out in the experimental facility house of the Faculty of Clinical Science, University of Ilorin, Kwara State. The animals were equally allotted into six groups.

Group 1: Control (CTR) received 2 ml of normal saline for 14 days; Group 2low dose progesterone: LDP received 4 mg/kg/day progesterone intra peritoneal for seven days; Group 3 high dose progesterone: HDP received 8 mg/kg/day progesterone intra peritoneal for seven days; Group 4 streptozotocin: STZ received double doses of 0.1M citrate buffer containing 30mg/kg streptozotocin in oil vehicle of pH 4.5;

Group 5: STZ+LDP received double dose of 0.1M citrate buffer containing 30mg/kg streptozotocin in oil vehicle of pH 4.5 on Day 1 and Day7 followed by the administration of 4mg/kg of progesterone for another seven days; Group 6: STZ+HDP received double doses of 0.1M citrate buffer containing 30mg/kg streptozotocin (Strone-50) in oil vehicle of pH 4.5 on Day 1 and Day 7 followed by administration of 8mg/kg of progesterone for another seven days.

The groups that received 30mg/kg streptozotocin were starved overnight before their first dose administration and the second dose was given to them the second day making it two days single dose termed (double doses) of 30 mg/kg/b.w. streptozotocin.

The body weight of the Wistar rats were measured before and during experiment period (three weeks). Glucometer was used to measure the blood glucose level across the groups before and doing (the third and seventh day after the administration of 30 mg/kg streptozotocin) the experiment. The final measurement was done one day before sacrificing the animal and after the administration of progesterone. Rats with a glucose concentration exceeding 150 mg/dL were considered diabetic.

Animal Sacrifice and Sample Collection: At the end of the experiment, some (four rats per group) animals were anaesthetized with intermuscular injection of 20 mg/kg of ketamine, skin excised and transcardially perfused with saline and subsequently with 4% paraformaldehyde in 0.1M phosphate, four rats were cervical dislocated in order to collect blood sample cardially for biochemical analysis. The brains were removed and the prefrontal cortex was excised from the brain and put into 4% paraformaldehyde fixative for subsequent histological (haematoxylin and eosin stain) analysis and 30% sucrose solution for biochemical assay.

Estimation of Malondialdehyde (MDA) and Glutathione Peroxidase (GPx) and Histological Staining: Malondialdehyde (MDA) was estimated by the thiobarbituric acid test [38]. Gpx activity was determined through Mishra and Fridovich method [39]. 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 [40] to study the morphology of the prefrontal neurons.

Statistical Analysis: Data collected 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 Version 20. Data were presented as means ± SEM (standard error of mean) P value less than 0.05 ($P<0.05$) was considered statistically significant.

RESULTS

Blood Glucose Level: Streptozotocin (STZ) administration to animals in groups STZ, STZ+LDP and STZ+HDP showed statistical insignificant ($P>0.0.05$) by an increase in the blood glucose compared to animals that were not exposed to streptozotocin. On the other hand, progesterone reduced the blood glucose levels significantly in animals exposed to streptozotocin compared to those received STZ, STZ+LDP and STZ+HDP (Figure 1).

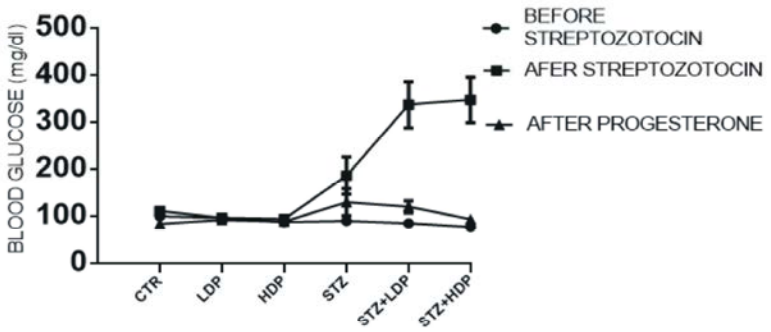
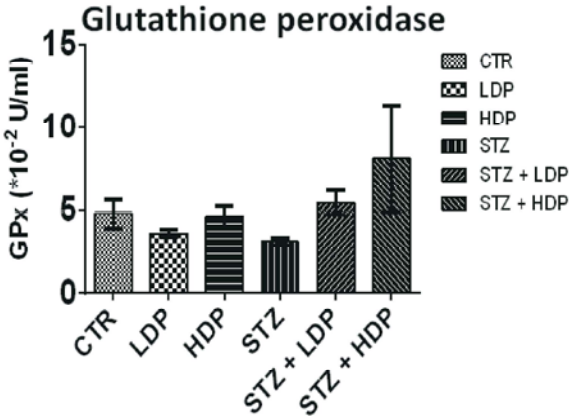


Fig. 1: Lines Graph showing the blood glucose levels across all groups before and after administration of streptozotocin and after administration of progesterone



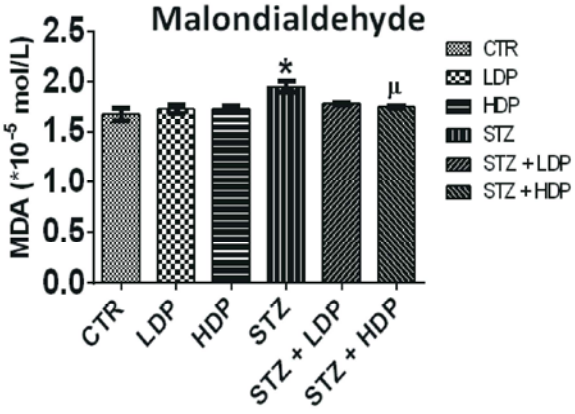


Fig. 2: Graph showing glutathione Peroxidase

Fig. 3: Mean malondialdehyde levels with error bars groups

Fig. 3 *- P<0.05 statistical significant difference compared to control animals (CTR). µ- P<0.05 statistical significant difference compared to animals exposed to streptozotocin (STZ)

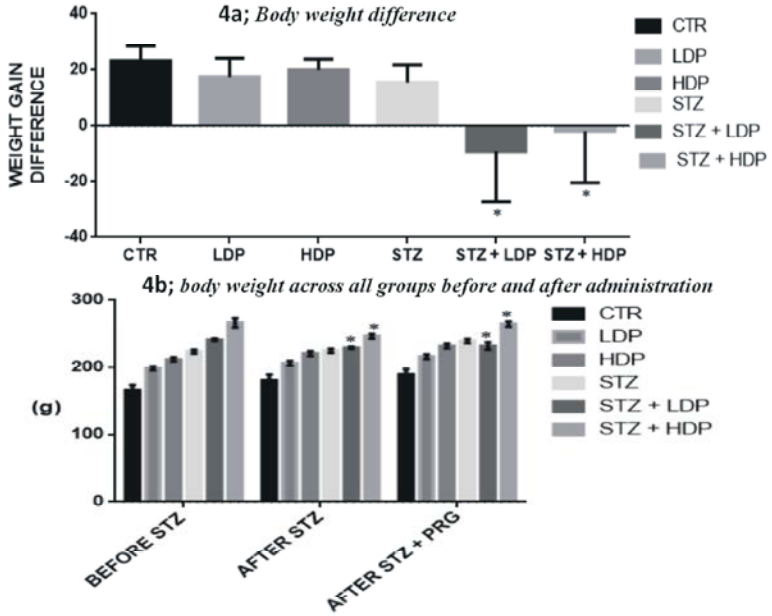


Fig. 4(a&b): Graph showing the animal body weight difference across all groups. P<0.05 and Graph showing the animal body weight across all groups before and after administration of streptozotocin and after administration of progesterone. P<0.05

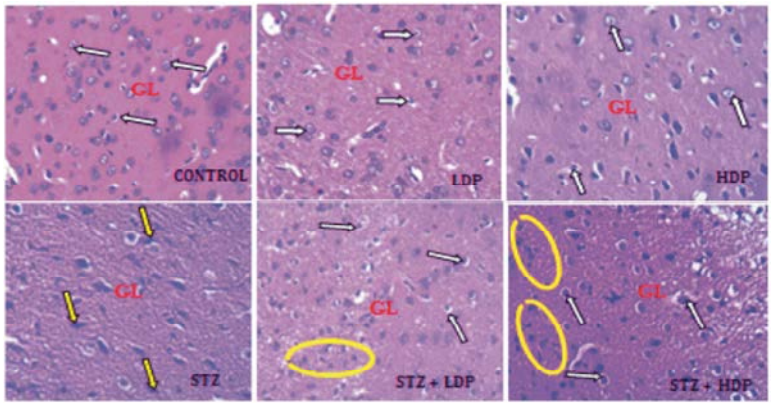


Fig. 3: Photomicrograph showing the pyramidal cells of the granular layer (GL) of the prefrontal cortex of adult male Wistar rats (Haematoxylin and Eosin stain x 400)

Biochemical Assay: Glutathione peroxidase level was reduced in animals exposed to streptozotocin, though not statistically significant (P>0.0.05) compared to control and in animals given different doses of progesterone. Malondialdehyde level increased significantly (P<0.05) in animals exposed to streptozotocin, compared to CTR and in animals given different doses of progesterone. However, the malondialdehyde (MDA) levels were reduced in animals treated with different doses of progesterone with and without streptozotocin and CTR.

Body Weight: Body weight before administration showed insignificant differences, whereas there were significant differences (50.76±0.4921) after administration of STZ and STZ+ PROG across the groups when compared. Values are presented as Mean ± SEM, □ P<0.05 (Figure 4).

Cyto-Architectural Result: CONTROL (CTR), LDP and HDP shows location of neuron with the intact nucleus (white arrow), this indicates normal cyto-architecture of the prefrontal cortex of adult male Wistar rats while the STZ, STZ+LDP and STZ+HDP shows location of abnormal normal neuron (yellow arrow); showing extrusion of the nucleus from the neuronal cytoplasm which implies the process of apoptosis.

DISCUSSION

Investigation of the histological structure of the prefrontal cortex, serum malondialdehyde and glutathione peroxidase levels of the rats; showed that streptozotocin and progesterone implicit neuronal instability and oxidative stress and progesterone is responsible for the protection and regeneration of cells. Progesterone is known to influence neurons survival and aid in neural protection in traumatic brain injury [20]. Studies suggested that progesterone increase the concentration of circulating endothelia progenitor cells responsible for neural regeneration and vascular remodelling play a role in the regeneration of injured neurons in the brain therefore circulating endothelial progenitor cells are major actors in the remodelling process. Progesterone protect the neuron by decreasing cellular apoptosis, cerebral edema and inhibits inflammation, thus promoting neuro-protective effects in rat's brain [20]. In this research, the effect of progesterone was further studied in the rat models using STZ, a neurotoxin which acts or affects the prefrontal cortex and limbic system. The mechanism of action by which it exerts its effect is not well understood. This study also investigated the histological and histochemical patterns of the prefrontal cortex cells in the untreated and treated rats; the protection and regeneration progesterone might cause to these cells and the neuronal instability and the oxidative stress caused by STZ and progesterone.

The weights of the rats that took only normal saline increased along the weeks of administration, the weights of the rats that collected double dose of STZ alone increased in the first two weeks of administration and later decrease in the third weeks. The rats that collected only progesterone increased in weight along the weeks of administration, while the rats that collected STZ start followed by progesterone increase in weight after a week of administration and reduction in weight was noticed in the second week and remained the same through period of the administration. The weights of the rats that collected low dosage progesterone (LDP) had weight increment along the weeks of administration while the rats that collected high dosage progesterone (HDP) had a constant weight throughout the administration period.

The glutathione level reduced in animals exposed to streptozotocin (STZ), though not statistically significant (P>0.0.05) compared to control (CTR) and animals given different doses of progesterone groups (LDP and HDP). The glutathione peroxidase level of the streptozotocin group was not statistically different compared to control and the progesterone groups. Neurodegenerative progressively leads to ataxia and dementia, mitochondria (Mt) dysfunctions, excitotoxicity and apoptosis of the neurons; redox metal catalyzedoxidative stress and free radical generation which play critical role in regulating redox reactions *in-vivo* contributing to reactive nitrogen species (RNS) and reactive oxygen species (ROS). Oxidative stress arises due to disturbed equilibrium between pro-oxidant/antioxidant homeostasis that further takes part in generation of ROS and free radicals which are potentially toxic for neuronal cells. Enzymatic antioxidants are exogenous or endogenous molecules that act against any form of oxidative stress. They neutralize ROS and other kinds of free radicals produced as consequence of oxygen species (OS).

The activities of Malondialdehyde level increased significantly (*P<0.05) in animals exposed to streptozotocin (STZ), compared to control (CTR) and animals given different doses of progesterone groups (LDP and HDP); but the malondialdehyde (MDA) level reduced in animals exposed to streptozotocin and treated with different doses of progesterone groups (STZ+LDP and STZ+HDP), most significantly in animals exposed to streptozotocin and group treated with high dose of progesterone (STZ+HDP). Malondialdehyde activity in the Prefrontal cortex of Adult Male Wistar Rats 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, while the resulting chain reaction makes lipid peroxidation more damaging due to increased oxidative stress and it has been duly implicated in most neurodegenerative diseases [41]. Lipid peroxidation results from oxidative degradation of lipids and involves the movement of electrons from the lipid cell membrane by free radicals resulting in cellular damage [42].

The pyramidal neurons of the groups that received progesterone alone (LDP and HDP), have their cytoplasm intact while groups that received streptozotocin followed by progesterone (STZ+LDP and STZ+HDP), revealed some of the nuclei extruded and many intact in the cytoplasm of the pyramidal neurons while the STZ group have majority of its nuclei extruded it membrane. The neuron exposure to neurotoxin has been implicated in pyknotic nuclei as a result of DNA fragmentation and chromatin condensation that affects the morphology and integrity of the cytoplasm of a neuron [43, 44]. Pyramidal neurons, are found in forebrain structures such as the cerebral cortex, hippocampus and amygdala, they are also known as pyramidal cells. The basal dendrites emerge from the base and the apical dendrites from the apex of the pyramidal cell body [45]. These are excitatory cell types of the cortical playingan advanced cognitive functional role. The perikaryon nucleus of pyramidal neurons house the genes, consisting of DNA which contains the cell history (the basic information to manufacture all the proteins characteristic of that cell). The nucleus synthesizes RNA from DNA and moves it through its pores to the cytoplasm in-line with protein synthesis. The degenerative changes observed in this study as a result of exposure to streptozotocin, underlie the ability of streptozotocin to initiate cellular apoptosis and cause damage to the pyramidal neurons of the prefrontal cortex of rats. This is in line with the studies of Baykara, *et al.* [43], Akinola, *et al.* [44], Alrefaie and Alhayani [45], Kolb and Nonneman [46], which suggested extensive damage to the pyramidal neurons of the prefrontal cortex of rats after exposure to streptozotocin. Data indicate that progesterone has multiple non-reproductive functions in the central nervous system to regulate cognition, mood, inflammation, mitochondrial function, neurogenesis and regeneration, myelination and recovery from traumatic brain injury [47]. The recovery and protection noticed in the pyramidal cells of the rat's prefrontal cortex were as a result of the influence of progesterone given after the Streptozotocin in the experimental groups. And in the sectional tropical region these correspond with the findings of Li, *et al.* [20], Carroll, *et al.* [48], Stein [49], He, Hoffman and Stein [50].

CONCLUSION

This study suggests that intra-peritoneal administration of progesterone can protect the cyto-architecture of prefrontal cortex cells against the process of apoptosis induced by streptozotocin and progesterone proved to have the ability to protect the prefrontal cortex, therefore progesterone play protective and restorative role on cyto-architecture of some pyramidal cells in regions of the prefrontal cortex of adult male Wistar rats, when administered alone and after STZ respectively. And limitation is prone to dosages of progesterone.

REFERENCES

1. Todd Preuss, M., 1995. Do Rats Have Prefrontal Cortex? The Rose-Woolsey-Akert Program Reconsidered. Journal of Cognitive Neuroscience, 7(1): 1-24.
2. Dalley, J.W., R.N. Cardinal and T.W. Robbins, 2004. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neuroscience and Biobehavioral Reviews, 28: 771-784.
3. Watanabe, M., 1996. Reward expectancy in primate prefrontal neurons. Nature., 382: 629-632.
4. Amsten, A.F.T. and P.S. Goldman-Rakic, 1985. α2- Adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. Science, 230: 1273-1276.
5. Berman, K.E. and K.E. Weinberger, 1990. The prefrontal cortex in schizophrenia and other neuropsychiatric diseases: In vivo physiological correlates of cognitive deficits. In Uylings, B.M., Eden, G.V., DeBruin, P.C., Corner, M.A. and Feenstra, G.P. (Eds.). The prefrontal cortex: Its structure, Functions and pathology. Progress in Brain Research, 85: 521-'537. Amsterdam. Elsevier.
6. Drevets, D.C., T.O. Videen, S.H. Presskorn, J.L. Price, S.T. Carmichael and M.E. Raichle, 1992. A functional anatomical study of unipolar depression. Journal of Neuroscience, 12: 3628-3641.
7. Goldman-Rakic, P.S., 1987. Circuitry of primate prefrontal cortex and the regulation of behaviour by representational memory. The Nervous System, 5: 373-417.
8. Mattes, J.A., 1980. The role of frontal lobe dysfunction in childhood hyperkinesis. Comprehensive Psychiatry, 21: 358-369.
9. Weinberger, D.R., 1993. A connectionist approach to the prefrontal cortex. Journal of Neuropsychiatry and Clinical Neuroscience, 5: 241-253.
10. Grünblatt, E., M. Salkovic-Petrisic, J. Osmanovic, P. Riederer and S. Hoyer, 2007. Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein. Journal of Neurochemistry, 101(3): 757-770.
11. Shoham, S., C. Bejar, E. Kovalev, D. Schorer-Apelbaum and M. Weinstock, 2006. Ladostigil prevents gliosis, oxidative-nitrative stress and memory deficits induced by intracerebroventricular injection of streptozotocin in rats. Neuropharmacology, 52: 836-843
12. Rubelia, M., Ma De Jesús Gómez-Villalobos and F. Gonzalo, 2005. Alteration in dendritic morphology of cortical neurons in rats with diabetes mellitus induced by streptozotocin. Brain Research Volume 1048, Issues 1-2, 28 June 2005, Pages 108-115.
13. National Toxicology Program, 2005. Streptozotocin CAS No. 18883-66-4. National Institute of Environmental Health Sciences. 11th Ed Report on Carcinogenesis, pp: 760.
14. Szkudelski, T., 2001. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiology Research, 50: 336-346.
15. Alberti, K.G. and P.Z. Zimmet, 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic Medicine, 15(7): 539-553.
16. Moreira, P.I., M.S. Santos, A.M. Moreno, T. Proenca, R. Seica and C.R. De Oliveira, 2004. Effects of streptozotocin-induced diabetes on rat brain mitochondria. Journal of Neuroendocrinology, 16(1): 32-38.
17. Ortiz-Avila, O., E. Mauricio, E.O. Berenice, S. Alfredo, R.R. Alain and C. Christian, 2015. Avocado Oil Improves Mitochondrial Function and Decreases Oxidative Stress in Brain of Diabetic Rats. Journal of Diabetes Research. Vol. 2015. Article ID 485759; 9.
18. Zhang, Z., R. Yang, R. Zhou, L. Li, M. Sokabe and L. Chen, 2010. Progesterone promotes the survival of newborn neurons in the dentate gyrus of adult male mice. Hippocampus, 20(3): 402-412
19. Petersen , S.L., K.A. Intlekofer, P.J. Moura-Conlon, D.N. Brewer, J. Del Pino Sans and J.A. Lopez, 2013. Nonclassical progesterone signalling molecules in the nervous system. J. Neuroendocrinol., 25(11): 991-1001.
20. Li, Z., B. Wang, Z. Kan, B. Zhang, Z. Yang, J. Chen, D. Wang, H. Wei, J. Zhang and R. Jiang, 2012. Progesterone Increases Circulating Endothelial Progenitor Cells and Induces Neural Regeneration after Traumatic Brain Injury in Aged Rats. Journal of Neurotrauma, 29(2): 343-353
21. Schneider, J.S., M.K. Stone, K.E. Wynne-Edwards, T.H. Horton, J. Lydon, B. O'Malley and J.E. Levine, 2003. "Progesterone receptors mediate male aggression toward infants". Proceedings in National Academy of Science USA, 100 (5): 2951-2956.
22. Espinoza, T.R. and D.W. Wright, 2011. "The role of progesterone in traumatic brain injury". Journal of Head Trauma Rehabilitation, 26(6): 497-499.

23. Sriram, D., 2007. Medicinal Chemistry. New Delhi: Dorling Kindersley India Pvt. Ltd., pp: 432.
24. King, T.L. and M.C. Brucker, 2010. Pharmacology for Women's Health. Jones & Bartlett Publishers, pp: 372-373
25. Miller, E.K., D.J. Freedman and J.D. Wallis, 2002. The prefrontal cortex: categories, concepts and cognition. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 357(1424): 1123-36.
26. Badre, D., A.S. Kayser and M. D'Esposito, 2010. Frontal cortex and the discovery of abstract action rules. Neuron, 66(2): 315-26.
27. Yang, Y. and A. Raine, 2009. Prefrontal structural and functional brain imaging findings in antisocial, violent and psychopathic individuals: a meta-analysis. Psychiatry Research, 174(2): 81-8.
28. Luciana, M., 2001. Handbook of developmental cognitive neuroscience. Ed. by Charles A. Nelson. Cambridge, Mass. [u.a.]: MIT Press. ISBN 0-262-14073-X: 12-17.
29. Hoshi, E., 2001. Functional specialization within the dorsolateral prefrontal cortex: a review of anatomical and physiological studies of non-human primates. Neuroscience Research, 54(2): 73-84.
30. Moss, S., 2013. Dorsolateral Prefrontal Cortex. Psychlopedia. Retrieved 11 November.
31. James, B.H. and A.F. Catherine, 2004. School neuropsychology: A Practitioner's Handbook. Guilford Press, pp: 64-65. ISBN 1593850115.
32. Bruce, L.M. and L.C. Jeffrey, 2007. The Human Frontal Lobes: Functions and Disorders. The Guilford Press. p. 355. ISBN 1-59385; 329-337.
33. Katrin, A., M. Karsten, B. Claudia, V.R. Paraskevi, K. Jürgen, B. Inga, V. Arno and S. Julia, 2015. Progesterone mediates brain functional connectivity changes during the menstrual cycle-a pilot resting state MRI study. Frontiers in Neuroscience/ Neuroendocrine Science.
34. Bechara, A., D. Tranel and H. Damasio, 2000. Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. Brain, 123(11): 2189-2202.
35. Motzkin, J.C., J.P. Newman, K.A. Kiehl and M. Koenigs, 2011. Reduced prefrontal connectivity in psychopathy. The Journal of Neuroscience, 31(48): 17348-17357.
36. Nicolle, A. and V. Goel, 2013. What is the role of ventromedial prefrontal cortex in emotional influences on reason? In I. Blanchette (Ed.), Emotion and Reasoning. Psychology Press: 5-24.
37. Ongur, D., 2000. The Organization of Networks within the Orbital and Medial Prefrontal Cortex of Rats, Monkeys and Humans. Cerebral Cortex, 10(3): 206-219.
38. Davey, M.W., E. Stals, B. Panis, J. Keulemans and R.L. Swennen, 2005. High-throughput determination of malondialdehyde in plant tissues. Analytical Biochemistry, 347(2): 201-207.
39. Mishra, P.H. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.
40. Bancroft D. John and Alan Stevens, 1982. Theory and Practice of Histological Techniques. Churchill Livingstone, Edinburgh (1982).
41. Keller, J.N. and M.P. Mattson, 1998. Roles of lipid peroxidation in modulation of cellular signalling pathways, cell dysfunction and death in the nervous system. Review of Neuroscience, 9: 105.
42. Michel, F., D. Bonnefont-Rousselot, E. Mas, J. Drai and P. Thérond, 2008. Biomarkers of lipid peroxidation: analytical aspects; Annals of Biological and Clinical Science, 66(6): 605-20.
43. Baykara, B., I. Aksu, E. Buyuk, M. Kiray, A.R. Sisman, B. Baykara, A. Dayi, A. Tas, D. Ozdemir, M.N. Arda and N. Uysal, 2013. Progesterone treatment decreases traumatic brain injury induced anxiety and is correlated with increased serum IGF-1 levels; prefrontal cortex, amygdala, hippocampus neuron density; and reduced serum corticosterone levels in immature rats. Biotechnic & Histochemistry, 88(5): 250-257.
44. Akinola, O.B., O.G. Omotoso, O.O. Dosumu, O.S. Akinola and F. Olotufore, 2011. Diabetes-Induced prefrontal Nissl substance deficit and the effects of Neem-Bitter leaf extract treatment. International Journal Morphology, 29(3): 850-856.
45. Alrefaie, Z. and A. Alhayani, 2015. Vitamin D3 improves decline in cognitive function and cholinergic transmission in prefrontal cortex of Streptozotocin induced diabetic rats. Behavioural Brain Research, 287: 156-162.
46. Kolb, B. and A.J. Nonneman, 1975. Prefrontal cortex and the regulation of food intake in the rat. Journal of Comparative & Physiological Psychology, 88: 806-815.
47. Brinton, R.D., R.F. Thompson, M.R. Foy, M. Baudry, J.M. Wang, C.E. Finch, T.E. Morgan, F.Z. Stanczyk, C.J. Pike and J. Nilsen, 2008. Progesterone Receptors: Form and Function in Brain. Journal of Neuroendocrinology, 29(2): 313-339.

48. Carroll, J.C., E.R. Rosario, L. Chang, F.Z. Stanczyk, S. Oddo, F.M. LaFerla and C.J. Pike, 2007. Progesterone and Estrogen Regulate Alzheimer-Like Neuropathology in Female 3xTg-AD Mice. The Journal of Neuroscience, 27(48): 13357-13365.
49. Stein, D.G., 2008. Progesterone exerts neuroprotective effects after brain injury. Brain Research Reviews, 57: 386-397.
50. He, J., S.W. Hoffman and D.G. Stein, 2004. Allopregnanolone, a progesterone metabolite, enhances behavioral recovery and decreases neuronal loss after traumatic brain injury. Restorative Neurology and Neuroscience, 22: 19-31.