Original Article Research Journal of Health Sciences

Ascorbic acid modulates prefrontal cortex cellular changes in androgen deprived rats

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Abstract

Background: Testosterone deficiency has been implicated in numerous neurodegenerative diseases such as Alzheimer's, Parkinson and Huntington's disease. We used a model of androgen deprived rats to determine the effects of ascorbic acid on prefrontal cortex (PFC) cellular changes associated with a subset population of androgen deprived patients.

Methodology: Chemical castration (using testosterone antagonist) as well as orchiectomy can induce androgen deprivation. Twenty-one (21) adult male Wistar rats with an average weight of 170g±10g were randomly assigned into three groups with each group containing seven (7) rats. Group A was control group, Group B= Orchiectomy + Flutamide (11 mg/kg body weight), and group C= Orchiectomy + Flutamide (11 mg/kg body weight)+Ascorbic acid (100 mg/kg body weight). Treatment lasted for 30 days. Nitric oxide and Malondialdehyde levels were assessed; while serum testosterone level was assayed. Histological, Histochemical, and immunohistochemical investigations were performed using Hematoxylin & Eosin, Cresyl fast violet, and Bielschowsky stains respectively.

Result: Our results showed increased expression of Nitric Oxide (NO), and increased lipid peroxidation (MDA) in the PFC of orchiectomized animals with altered cytoarchitectural morphology evidenced by decreased Nissl staining polarity in neuronal axons and aggregation of neurofibrillary tangles. Oxidative and nitrosative stress were well modulated in animals treated with ascorbic acid with unaltered prefrontal cortex morphology.

Conclusion: The results indicated that decline in brain androgen activities caused nitrosative and oxidative stress-driven pathology in the prefrontal cortex while supplementing endogenous ascorbic acid offered therapeutic value by scavenging free radicals in the prefrontal cortex

Keywords: Ascorbic acid, Orchiectomy, Prefrontal cortex, Testosterone deficiency, nitrosative and oxidative stress.

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Received: June 30, 2021 *Accepted: January 7, 2022* *Published: June 30, 2022*

http://dx.doi.org/10.4314/rejhs.v10i2.3

Article original

Research Journal of Health Sciences

Acide ascorbique module les changements cellulaires du cortex préfrontal chez les rats privés d'androgenes

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Résumé

Contexte général de l'étude : La déficience en testostérone a été impliquée dans de nombreuses maladies neurodégénératives telles que la maladie d'Alzheimer, de Parkinson et de Huntington. Nous avons utilisé un modèle de rats privés d'androgènes pour déterminer les effets de l'acide ascorbique sur les changements cellulaires du cortex préfrontal (PFC) associés à un sous-ensemble de patients privés d'androgènes.

Méthode de l'étude : La castration chimique (utilisant un antagoniste de la testostérone) ainsi que l'orchidectomie peuvent induire une privation d'androgènes. Vingt et un (21) rats Wistar mâles adultes d'un poids moyen de 170 g ± 10 g ont été répartis au hasard en trois groupes, chaque groupe contenant sept (7) rats. Le groupe A était le groupe témoin, le groupe B = orchidectomie + flutamide (11 mg/kg de poids corporel) et le groupe C = orchidectomie + flutamide (11 mg/kg de poids corporel) + acide ascorbique (100 mg/kg de poids corporel). Le traitement a duré 30 jours. Les niveaux d'oxyde nitrique et de malondialdéhyde ont été évalués ; tandis que le niveau de testostérone sérique a été dosé. Des investigations histologiques, histochimiques et immunohistochimiques ont été réalisées en utilisant respectivement les colorations à l'hématoxyline et à l'éosine, au violet rapide de crésyle et à Bielschowsky.

Résultat de l'étude : Nos résultats ont montré une expression accrue de l'oxyde nitrique (NO) et une augmentation de la peroxydation lipidique (MDA) dans le PFC d'animaux orchiectomisés avec une morphologie cytoarchitecturale altérée , mise en évidence par une diminution de la polarité de la coloration de Nissl dans les axones neuronaux et l'agrégation des enchevêtrements neurofibrillaires. Le stress oxydatif et nitrosatif était bien modulé chez les animaux traités avec de l'acide ascorbique avec une morphologie du cortex préfrontal inchangée.

Conclusion : Les résultats ont indiqué que le déclin des activités androgènes cérébrales provoquait une pathologie induite par le stress nitrosatif et oxydatif dans le cortex préfrontal, tandis que la supplémentation en acide ascorbique endogène offrait une valeur thérapeutique en éliminant les radicaux libres dans le cortex préfrontal.

Mots-clés : Acide ascorbique, orchidectomie, cortex préfrontal, déficit en testostérone, Stress nitrosatif et oxydatif

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Received: June 30, 2021 *Accepted: January 7, 2022* *Published: June 30, 2022*

http://dx.doi.org/10.4314/rejhs.v10i1.3

INTRODUCTION

Testosterone is an androgenic hormone that regulates reproduction, sexual and aggressive behaviors (1,2). Testosterone exerts important function in the central nervous system (CNS) development. It has been reported to prevent neuronal death in experimental models of neuronal injury, and is linked to an increase in neuron somal size, neuritic growth, plasticity and synaptogenesis. An interruption of this hormone may trigger the formation of apoptosis in neural tissues (3,4). Testosterone administration has been found to demonstrate preventive activity against oxidative stress (5) as well as also prevented tissue damage and improved antioxidant enzyme levels (6,7). Furthermore, accumulating evidence suggest that testosterone and androgenic receptors play important roles in brain regions associated with learning and memory (8,9).

Orchiectomy is performed for varying conditions such as prostatic and testicular cancer (10); and trauma in males. Despite achievements from this method, one of its reported concern is in relation to the effects of such artificial manipulation on the endocrine functions and the resultant effects on cortico-hippocampal (learning and cognitive) functions in the affected male population (11). Bilateral orchiectomy reduces the level of circulating testosterone which leads to alterations in physical, social, sexual and brain functions (12). Moreover, studies have shown that as men with healthy gonads advance in age, testosterone levels decline (13).

Numerous studies have implicated testosterone deficiency in many neurodegenerative diseases such as Alzheimer's disease, Parkinson and Huntington's disease (14, 15). This is due to the fact that testosterone deficit as seen in aging males triggers free radical species production which eventually leads to oxidative stress (16) as well as increased inflammation in both humans and animal studies (17). Orchiectomy, a form of surgical induction of testosterone deficiency, elevates the susceptibility of brain tissue to oxidative stress. Low testosterone levels in diabetic men have been linked to enhanced inflammation. The vital basic processes involved in neurodegeneration includes deposition of amyloids, neuroinflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress (18, 19). Among these, oxidative stress is well documented as an early and important pathogenic operator in brain aging and neurodegeneration (18) as implicated in testosterone depletion.

Flutamide is a nonsteroidal androgen receptor antagonist used in the management of metastatic prostate cancer and in the treatment of hirsutism in women. It is a pure androgen antagonist and produces no androgenic or other steroidal effects. Flutamide, have been employed to augment the effectiveness of the standard androgen deprivation therapies, surgical castration, in the treatment of advanced prostate cancer. Antiandrogens are also gaining popularity as potential monotherapy for select prostate cancer patients (19). Although monotherapy rarely results in reduction of PSA to undetectable levels, survival outcome has not been found to be significantly different to that observed after castration in men with locally advanced, nonmetastatic disease (20).

Ascorbic acid (AA), an essential micronutrient with many biological roles; possesses powerful antioxidant properties as it can directly scavenge ROS and indirectly regenerate other antioxidant systems (21). It has been established to revert oxidative stress impacts on the cellular system. ROS and other kinds of free radicals produced as a result of oxidative stress are neutralized by vitamin C and hence attention of research has been focused on it due to its therapeutic potential (21). Herein, the bidirectional relationship in the cellular and molecular mechanisms associated with testosterone deprivation and cognitive perturbations were evaluated. Also, we examined the putative role of ascorbic acid in modulating prefrontal cortex pathology arising from testosterone depletion.

METHODOLOGY

Chemicals and its Reconstitution

Flutamide (Cat. NO: 13311-84-7) was obtained from Akol Pharmaceutical store, Osogbo while ascorbic acid (Cat. NO: 50-81-7) was procured from Sigma-Aurich (USA). These chemicals were reconstituted with distilled water obtained from Department of Anatomy, Osun State University.

Animal Care and Ethical Approval

Twenty-one (21) adult male Wistar rats with an average weight of 180g±10g were used for this study. The animals being housed in the Animal facility of Osun State University, had access to rat chow and water *ad libitum*. The protocols on animal handling strictly followed the guidelines of Institutional Animal Care and Use Committee (IACUC) and was approved by

the UNIOSUN Health Research Ethics Committee, with approval number (UNIOSUNHREC 2021/020).

Animal Grouping and Orchidectomy procedure

The animals were randomized into three groups with n= 7;
Group A: control received distilled water
Group B: Orchiectomy + Flutamide (11 mg/kg).
Group C: Orchiectomy + Flutamide (11 mg/kg) + Ascorbic acid (100 mg/kg).
Animals were bilaterally orchiectomized according to the method described by Idris, (22). Summarily, anaesthesia was induced by intraperitoneal injection of ketamine hydrochloride (50 mg/kg). The fur over the ventral side of the scrotum was shaved to expose skin, swabbed with 70% ethanol followed by sterile PBS. An incision was made on the ventral side of the scrotum (1.6 cm) using sterile scalpel. The caudal epididymis and caput epididymis were severed from the testis after which the testis was removed gently by severing blood vessels with a small scissors. The remaining content of the testicular sac were carefully replaced and the skin was closed using metal clips. Intraperitoneal injection of atipamezole hydrochloride (1 mg/kg) was given to reverse anaesthesia and animals were allowed to recover for 4 days, before commencement of ascorbic acid and flutamide treatments. The control group animals were subjected to the same operation without removal of the testis. Flutamide was administered to block testosterone secretion from other sources. Ascorbic acid and flutamide were administered daily at 09:00hrs via oral gavage using oropharyngeal cannula for a period of 30 days (23).

Animal Sacrifice

After 24 hours of last dose of treatments, the animals were anaesthetized with Isoflurane anesthesia. Venous blood was taken from a large vein in the hind limb of the rats for hormonal assay. Animals were decapitated and the prefrontal cortex (4.2 mm from Bregma) was quickly excised from the brain. The prefrontal cortices were fixed in 10% neutral buffered formalin and used for histology; and rinsed in 0.25 M sucrose and preserved in 0.1M of PBS for biochemical analysis.

Determination of Testosterone in the Serum

Venous blood collected from a vein in the hind limb into heparinized bottle was centrifuged immediately at 4000 rpm at 4°C, serum was aspirated into plain bottles and stored at -20°C. Serum levels of testosterone was determined using Testosterone ELISA kit (Testosterone Cat #: 3633-300) obtained from Monobind Inc. Lake forest, CA, USA, according to the manufacturer's procedure. Summarily, 25µL of samples, standards and control were added in triplicate to the wells. 100µL Testosterone-HRP Conjugate was added and incubated at 37ºC. Thereafter, the contents of each well was decanted and washed with wash buffer thrice. This was followed by the addition of TMB substrate solution, after which each well was incubated at room temperature. 100µL Stop Solution was then added into all wells with color change from blue to yellow. Absorbance was read spectrophotometric at a wavelength of 450 nm (24).

Determination of Malondialdehyde (MDA) Levels

Malondialdehyde enzyme activities was determined by measuring thiobarbituric acid reactive substances present in the supernatant of the prefrontal cortex tissue homogenate according to the modified method of Buege and Aust, (25). Briefly, 0.25g of prefrontal cortex sample was homogenized in 2.5ml of 0.15M potassium chloride, centrifuged at 1000g and the supernatant collected in plain bottle. 2ml of the reagent comprised of trichloroacetic acid, thiobarbituric acid and hydrochloric acid (TCA/TBA/HCl) at ratio 1:1:1 was combined with 1ml of the supernatant. This was vigorously mixed with a spatula, boiled for 15 min, and cooled on ice. Thereafter it was centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was read at 532 nm against a blank. MDA concentration was estimated using the molar extinction coefficient of 1.55×10^6 M^{-1} cm^{-1}.

Determination of Nitric Oxide

Nitric oxide level was quantified in the tissue homogenate by measuring the accumulation of its stable degradation products, nitrate and nitrite. Briefly, the isolated prefrontal cortex was homogenized and transferred to a 96-well plate. In this assay, nitrate reductase (NaR) is employed in the enzymatic conversion of nitrate to nitrite, followed by quantitation of nitrite using Griess Reagent (1% sulfanilamide, 0.1% N-(1-naphthyl) ethylenediamine hydrochloride, 2.5% H3PO4). Absorbance was read at 540 nm using a spectrophotometer. Nitric oxide assay kit (Cat #:

EMSNO) procured from Thermo Fish Scientific, Austria was used according to manufacturer's protocol contained in the assay kit (26).

Histological Assessment

After dehydration, clearing and embedding, the paraffin block containing the tissue was sectioned by a rotary microtome at 4µm thickness. Histological staining was performed using Hematoxylin and Eosin as described by Bancroft and Layton, (27). Demonstration of Nissl substances was performed by modified method of Kádár et al. (28). Photomicrograph of the stained sections were captured using Olympus binocular research microscope (Olympus, New Jersey, USA) which was connected to a 5.0 MP Amscope. The staining polarity were measured with Image J software.

Bielschowsky Silver Stain

Prefrontal serial 10µm thick sections were rinsed in distilled water. Sections were covered with silver A (20% Silver nitrate) for 2 hours in 37°C in a moist chamber. Thereafter, they were *washed in a pot* of reducer A for 3–5 min. After proper rinsing, sections were placed in silver B for 30s. Sections were rinsed a second time and *washed in a pot* of reducer B for 2–5 min. Toning was then carried out by placing the sections in 0.2% gold chloride for 3 min. This was followed by fixing in 5% sodium thiosulphate for 5 mins. Finally, sections were washed properly in distilled water, dehydrated, cleared, and mounted in DPX (29).

RESULTS

Orchiectomy and flutamide treatment associated with decreased serum testosterone levels.

In order to confirm testosterone deficiency induced by orchiectomy and flutamide treatment, serum testosterone concentration was measured in the experimental animals. The Orchiectomized rats (group B) showed significantly decreased level of testosterone in comparison with the control group ($P< 0.05$). Ascorbic acid treated animals (group C) also had significantly ($P< 0.05$) low testosterone levels in contrast to the control group. (Figure 1)

Ascorbic acid modulated lipid peroxidation in the prefrontal cortex induced by testosterone depletion

As shown in figure 2, we assessed the level of lipid peroxidation in the tissue by measuring MDA concentration in the prefrontal cortex of the animals. Orchiectomized rats (group B) showed significant increased concentration of MDA in contrast to the control animals (group A) ($p<0.05$); whereas the MDA concentration in animals treated with ascorbic acid (group C) revealed a reduced tissue concentration of MDA which was significant ($p< 0.05$) when compared to animals in group B.

Testosterone deficient rats reveals Nitrosative Stress

Concentration of nitric oxide in the prefrontal cortex increased significantly ($P<0.05$) in animals that underwent orchiectomy (group B) in contrast to control (A) group. Animals administered with ascorbic acid regime had reduced concentration of NO which was significant ($P<0.05$) when compared to animals in group B (Orchi+flutamide). This suggests that testosterone deficiency triggered nitric oxide overexpression while ascorbic acid modulated the concentration of nitric oxide in the PFC. (Figure 3)

Ascorbic acid regime maintained the Histomorphological integrity of the PFC

Figures 4 show representative micrographs of the general cytoarchitecture of the PFC in Wistar rats **(H&E x*400*)**. The PFC of the control and ascorbic acid treated (A and C respectively) groups revealed numerous normal neurons scattered within the pyramidal layer (yellow arrow). Orchi+Flutamide (group B) showed clusters of pyknotic and necrotic pyramidal neurons with condensed nuclei within soma (red arrow).

Testosterone depletion altered prefrontal cortex Nissl profile

As shown in the chart and photomicrograph, normal morphological presentation was observed in the control and Orchi+Flutamide+Ascorbic acid (groups A and C respectively) animals, characterized with normal and densely populated Nissl proteins, well stained and outlined neurons (yellow arrows). Orchi+Flutamide (group B) caused chromatolytic changes and some pyknotic changes in both the pyramidal and granule cell layers with a significant reduction in the cytoplasmic Nissl proteins (red arrows). Figure 5

Ascorbic acid prevented the aggregation of neurofibrillary tangles in the PFC following Orchiectomy

In figure 6, photomicrograph of experimental animals in orchiectomised group showed increased aggregation of neurofibriliary tangles (red dotted circle). The Ascorbic acid treated group had similar morphological presentation as the control group.

DISCUSSION

In this study, a rat model was investigated to characterize prefrontal cortex structural and functional changes that might be implicated in a subset of testosterone deprived patients. Lipids are mostly affected amongst the various biological targets of oxidative stress (30). Malondialdehyde, one of the secondary products of lipid oxidation is the principal outcome of peroxidation of polyunsaturated fatty acid in the cells. MDA is highly toxic and a common oxidative stress marker (31, 32). Presence of free radicals generate lipid peroxidation process in an organism.

In our present study, orchidectomy and flutamide administration resulted to depleted serum testosterone levels. Studies have shown that testosterone depletion results in increased risk of dysfunction in the brain. Recent evidence indicates that one deleterious effect of testosterone loss in men is increased risk for Alzheimer's disease (AD). Oxidative stress-induced neuronal death is a key signature in AD. Our findings suggest that loss of testosterone triggered lipid peroxidation processes evidenced by increased malondiadehyde concentration in the PFC. This finding was consistent with previous studies (33-35).Though, ascorbic acid treatment was unable to restore serum testosterone back to its normal levels as observed in the control group, it was able to reduce lipid peroxidation process in the PFC tissue. This is basically due to the antioxidative property of ascorbic acid which enhanced the tissue antioxidant status. Previous studies reveals that fruits and vegetables rich in antioxidants reduced lipid peroxidation status of orchiectomized rats (36).

Proper neuronal function requires minimal physiological concentrations of nitric oxide (NO) in the central nervous system (CNS), with sustained high NO levels leading to detrimental effects when it reacts with superoxide anion to form peroxynitrite (37). Upregulation of brain NO concentration following orchidectomy as seen in our findings could lead to inflammatory processes which contributes to inflammatory cytokine release (38). Excessive inflammatory response characterize increase in mitochondrial dysfunction, free radicals, and nitric oxide (NO). Consequently, there may be damage to the systemic vascular endothelium, redox-glutathione depletion, and mitochondrial respiratory dysfunction causing reduction in ATP consumption (37). In our study, prefrontal cortex histopathology can be linked to the impact of testosterone depletion in exacerbating reactive oxygen species neural levels which elicits oxidative stress and neuroinflammation (39, 40). Inflammatory cascade is a key mediator in the pathogenesis of neurodegenerative diseases (41). Ascorbic acid has been found to naturally have a powerful anti-inflammatory response that is comparable with both steroidal drugs and nonsteroidal drugs, which have undesirable effects. Its anti-inflammatory effect is mediated by inhibiting the activation of transcription factors like NF-κB, and AP-1, induction of iNOS, COX-2 and production of cytokines such as interferon and tumor necrosis factor (34, 42).

According to our findings, the histoarchitecture of the PFC in control and ascorbic acid-treated rats (group C) was characterized by an evenly layered neural morphology with neurons arranged in typical arrays across cortical layers. In contrast to animals treated with ascorbic acid following orchiectomy, PFC morphology in orchiectomized rats showed a significant increase in the deposition of pyknotic cells with poorly stained nuclei through the fragmented cortical layer. The Nissl profile of the PFC of control and ascorbic acid treated groups present with well-stained Nissl substance in the soma of the neuronal cells. On the other hand, testosterone depletion induced chromatolysis, characterized by reduced staining intensity in the central portion of neuronal cells in rats that underwent orchiectomy and flutamide treatment. We also reported that ascorbic acid prevented the aggregation of neurofibrillary tangles following androgen depletion resulting from orchiectomy and flutamide administration. Studies have shown that ascorbic acid does not only function to restore immune homeostasis, chelates iron, induces anti-oxidant response elements, enhance clearance of toxic aggregates, scavenges free radicals, but also binds to and limits aggregation of amyloid sheets which is characteristic of some neurodegenerative diseases (43,44). Ascorbic acid can bind Aβ and reduce toxic aggregates by modulating aggregation of neurofibrillary

tangles (45).

CONCLUSION

Findings from this study reveals that ascorbic acid has potent antioxidant and anti-inflammatory properties that can modulate the adverse effects of testosterone depletion in neural tissue. Ascorbic acid effectively modulated nitric oxide and malondialdehyde levels in the prefrontal cortex of orchiectomized rats and also prevented the aggregation of neurofibrillary tangles and neuronal death.

Conflict of Interest: Authors report no actual or potential conflict of interest

Acknowlegdements: We acknowledge Mr Yamson Emmanuel for his assistance in the Orchiectomy procedure

REFERENCES

1. Nyby JG. Reflexive testosterone release: a model system for studying the nongenomic effects of testosterone upon male behavior. Frontiers in neuroendocrinology. 2008;29(2):199-210.
2. Farrell SF, McGinnis MY. Effects of pubertal anabolic-androgenic steroid (AAS) administration on reproductive and aggressive behaviors in male rats. Behavioral neuroscience. 2003;117(5):904.
3. Fargo KN, Galbiati M, Foecking EM, Poletti A, Jones KJ. Androgen regulation of axon growth and neurite extension in motoneurons. Hormones and behavior. 2008;53(5):716-28.
4. Taylor GT, Maloney S, Dearborn J, Weiss J. Hormones in the mentally disturbed brain: steroids and peptides in the development and treatment of psychopathology. Central Nervous System Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Central Nervous System Agents). 2009;9(4):331-60.
5. Eleawa SM, Sakr HF, Hussein AM, Assiri AS, Bayoumy NM, Alkhateeb M. Effect of testosterone replacement therapy on cardiac performance and oxidative stress in orchidectomized rats. Acta physiologica. 2013;209(2):136-47.
6. Azevedo RB, Lacava ZG, Miyasaka CK, Chaves SB, Curi R. Regulation of antioxidant enzyme activities in male and female rat macrophages by sex steroids. Brazilian Journal of Medical and Biological Research. 2001 May;34(5):683-7.
7. Mancini A, Leone E, Festa R, Grande G, Silvestrini A, De Marinis L, et al., Effects of testosterone on antioxidant systems in male secondary hypogonadism. Journal of andrology. 2008;29(6):622-9.
8. Hajszan T, MacLusky NJ, Leranth C. Role of androgens and the androgen receptor in remodeling of spine synapses in limbic brain areas. Hormones and behavior. 2008;53(5):638-46.
9. Ota H, Akishita M, Akiyoshi T, Kahyo T, Setou M, Ogawa S, et al., Testosterone deficiency accelerates neuronal and vascular aging of SAMP8 mice: protective role of eNOS and SIRT1. PloS one. 2012;7(1):e29598.
10. Akinola OB, Gabriel MO. Neuroanatomical and molecular correlates of cognitive and behavioural outcomes in hypogonadal males. Metabolic brain disease. 2018;33(2):491-505.
11. Mitra S, Behbahani H, Eriksdotter M. Innovative therapy for Alzheimer's disease-with focus on biodelivery of NGF. Frontiers in neuroscience. 2019 Feb 5;13:38.
12. Kluger J, Roy A, Chao HH. Androgen deprivation therapy and cognitive function in prostate cancer. Current Oncology Reports. 2020;22(3):24.
13. Anand-Ivell R, Wohlgemuth J, Haren MT, Hope PJ, Hatzinikolas G, Wittert G, et al., Peripheral INSL3 concentrations decline with age in a large population of Australian men. International journal of andrology. 2006;29(6):618-26.
14. Bianchi VE, Rizzi L, Bresciani E, Omeljaniuk RJ, Torsello A. Androgen therapy in neurodegenerative diseases. Journal of the Endocrine Society. 2020;4(11):bvaa120.
15. Bansal R, Singh R. Exploring the potential of natural and synthetic neuroprotective steroids against neurodegenerative disorders: A literature review. Medicinal research reviews. 2018;38(4):1126-58.
16. Son SW, Lee JS, Kim HG, Kim DW, Ahn YC, Son CG. Testosterone depletion increases the susceptibility of brain tissue to oxidative damage in a restraint stress mouse model. Journal of neurochemistry. 2016;136(1):106-17.
17. Toro-Urrego N, Garcia-Segura LM, Echeverria V, Barreto GE. Testosterone protects mitochondrial function and regulates neuroglobin expression in astrocytic cells exposed to glucose deprivation. Frontiers in aging neuroscience. 2016;8:152.
18. Agostinho P, A Cunha R, Oliveira C. Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Current pharmaceutical design. 2010;16(25):2766-78.
19. Anderson J. The role of antiandrogen monotherapy in the treatment of prostate cancer. BJU international. 2003;91(5):455-61.
20. Iversen P, Tyrrell CJ, Kaisary AV, Anderson JB, Van Poppel HE, Tammela TL, et al., Bicalutamide monotherapy compared with castration in patients with nonmetastatic locally advanced prostate cancer: 6.3 years of followup. The Journal of urology. 2000;164(5):1579-82.
21. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, et al., Vitamin C as an antioxidant: evaluation of its role in disease prevention. Journal of the American college of Nutrition. 2003;22(1):18-35.

22. Idris AI. Ovariectomy/orchidectomy in rodents. InBone Research Protocols 2012 (pp. 545-551). Humana Press, Totowa, NJ.
23. Yawson EO, Akinola OB. Hippocampal cellular changes in androgen deprived insulin resistant rats. Metabolic Brain Disease. 2021;36(5):1037-48.
24. Matsumoto AM, Bremner WJ. Serum testosterone assays—accuracy matters. The Journal of Clinical Endocrinology & Metabolism. 2004;89(2):520-4.
25. Buege JA, Aust SD. [30] Microsomal lipid peroxidation. In Methods in enzymology 1978;52:302-310. Academic press.
26. Nims RW, Darbyshire JF, Saavedra JE, Christodoulou D, Hanbauer I, Cox GW, Grisham MB, Laval F, Cook JA, Krishna MC, Wink DA. Colorimetric methods for the determination of nitric oxide concentration in neutral aqueous solutions. Methods. 1995;7(1):48-54.
27. Bancroft JD, Layton C. The hematoxylins and eosin. Bancroft's theory and practice of histological techniques. 2012:173-86.
28. Kádár A, Wittmann G, Liposits Z, Fekete C. Improved method for combination of immunocytochemistry and Nissl staining. Journal of neuroscience methods. 2009;184(1):115-8.
29. Litchfield S, Nagy Z. New temperature modification makes the Bielschowsky silver stain reproducible. Acta neuropathologica. 2001;101(1):17-21.
30. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutrition, metabolism and cardiovascular diseases. 2005;15(4):316-28.
31. Padurariu M, Ciobica A, Lefter R, Lacramioara Serban I, Stefanescu C, Chirita R. The oxidative stress hypothesis in Alzheimer's disease. Psychiatria Danubina. 2013;25(4):0-409.
32. Patlolla AK, Barnes C, Yedjou C, Velma VR, Tchounwou PB. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. Environmental Toxicology: An International Journal. 2009;24(1):66-73.
33. Rosa SD, Cirillo P, Paglia A, Sasso L, Palma VD, Chiariello M. Reactive oxygen species and antioxidants in the pathophysiology of cardiovascular disease: does the actual knowledge justify a clinical approach? Current vascular pharmacology. 2010;8(2):259-75.
34. Abayomi TA, Tokunbo OS, Adebisi BT, Fadare MU, Gbadamosi IT, Akinwale JO, et al., Neurobehavioral assessment of the impact of vitamins C and E following acute exposure to sodium azide-induced neurotoxicity. Journal of Environmental Toxicology and Public Health. 2019;4:15-20.
35. Avontuur JA, Bruining HA, Ince C. Sepsis and nitric oxide. In Oxygen Transport to Tissue XVII 1996 (pp. 551-567). Springer, Boston, MA.
36. Deyhim F, Lopez E, Gonzalez J, Garcia M, Patil BS. Citrus juice modulates antioxidant enzymes and lipid profiles in orchidectomized rats. Journal of medicinal food. 2006;9(3):422-6.
37. Beck KF, Eberhardt W, Walpen S, Apel M, Pfeilschifter J. Potentiation of nitric oxide synthase expression by superoxide in interleukin 1β-stimulated rat mesangial cells. FEBS letters. 1998;435(1):35-8.
38. Traish A, Bolanos J, Nair S, Saad F, Morgentaler A. Do androgens modulate the pathophysiological pathways of inflammation? Appraising the contemporary evidence. Journal of clinical medicine. 2018;7(12):549.
39. May JM. How does ascorbic acid prevent endothelial dysfunction?. Free radical biology and medicine. 2000;28(9):1421-9.
40. Solerte SB, Fioravanti M, Schifino N, Cuzzoni G, Fontana I, Vignati G, et al., . Dehydroepiandrosterone sulfate decreases the interleukin-2-mediated overactivity of the natural killer cell compartment in senile dementia of the Alzheimer type. Dementia and geriatric cognitive disorders. 1999;10(1):21-7.
41. Hsieh HL, Yang CM. Role of redox signaling in neuroinflammation and neurodegenerative diseases. BioMed research international. 2013;2013-2226.
42. Hazewindus M, Haenen GR, Weseler AR, Bast A. The anti-inflammatory effect of lycopene complements the antioxidant action of ascorbic acid and α-tocopherol. Food Chemistry. 2012;132(2):954-8.
43. Solerte SB, Fioravanti M, Schifino N, Cuzzoni G, Fontana I, Vignati G, et al., . Dehydroepiandrosterone sulfate decreases the interleukin-2-mediated overactivity of the natural killer cell compartment in senile dementia of the Alzheimer type. Dementia and geriatric cognitive disorders. 1999;10(1):21-7.
44. Jahić A, Žnidarič MT, Pintar S, Berbić S, Žerovnik E. The effect of three polyphenols and some other antioxidant substances on amyloid fibril formation by Human cystatin C. Neurochemistry international. 2020;140:104806.
45. Barten DM, Albright CF. Therapeutic strategies for Alzheimer's disease. Molecular neurobiology. 2008;37(2):171-86.

How to cite this article:
Abayomi, T.A., Tokunbo, O.S., Saka, T.O., Abayomi, O.A., Osuntokun, O.S., Ajilore, B.S. Ascorbic acid modulates prefrontal cortex cellular changes in androgen deprived rats. Research Journal of Health Sciences, 2022, 10(1): 90-100

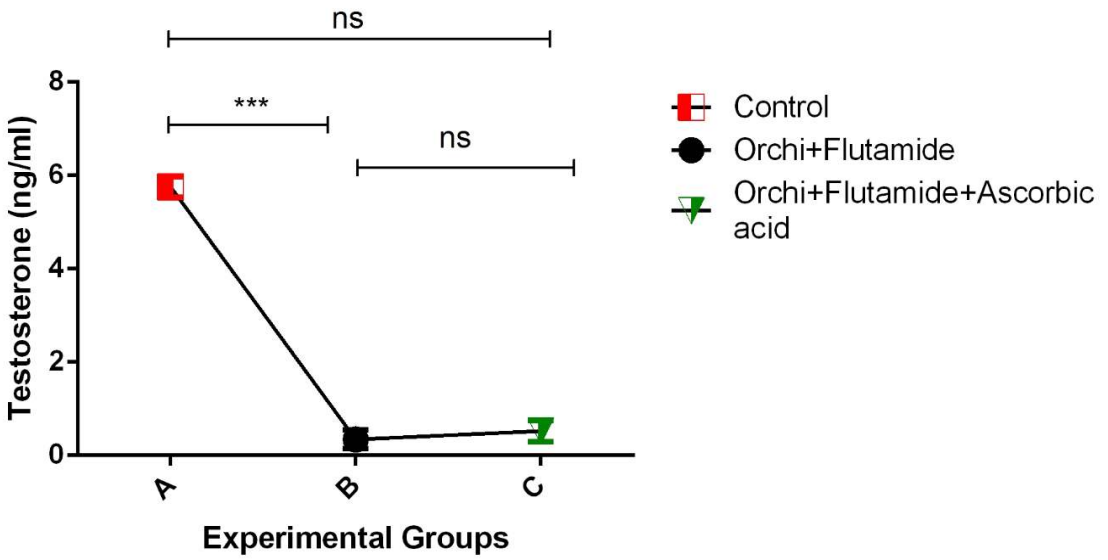

Figure 1: Concentration of serum testosterone level across the experimental animals. * is the significant level of difference in comparison to the control group (Group A) while + is the significant level of difference in comparison with group B. **ns** means not significant. */+ p< 0.05; **/++ p<0.01; ***/+++ p< 0.001.

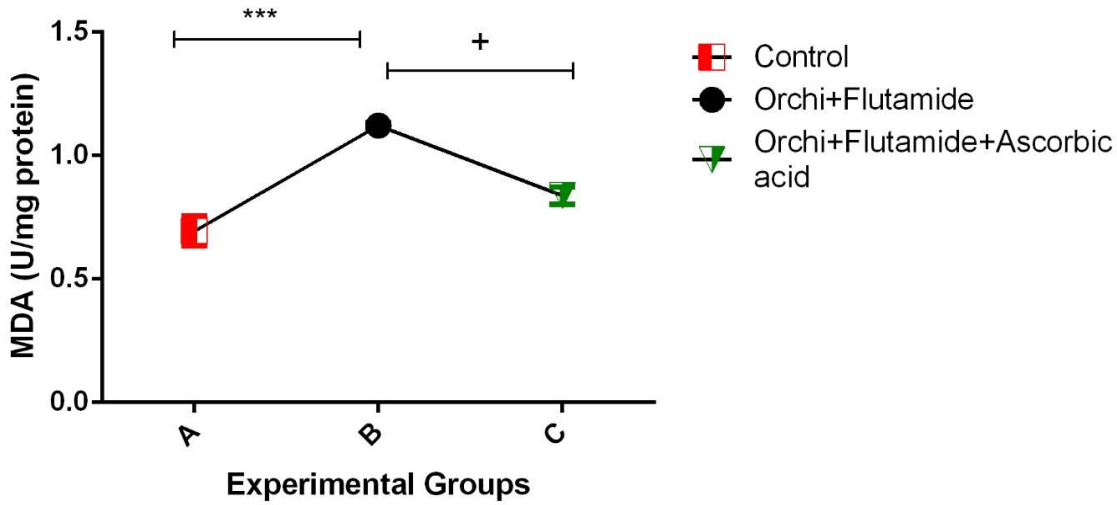

Figure 2: Malondialdehyde levels in the PFC tissue. * is the significant level of difference in comparison to the control group (Group A) while + is the significant level of difference in comparison with group B. **ns** means not significant. */+ p< 0.05; **/++ p<0.01; ***/+++ p< 0.001.

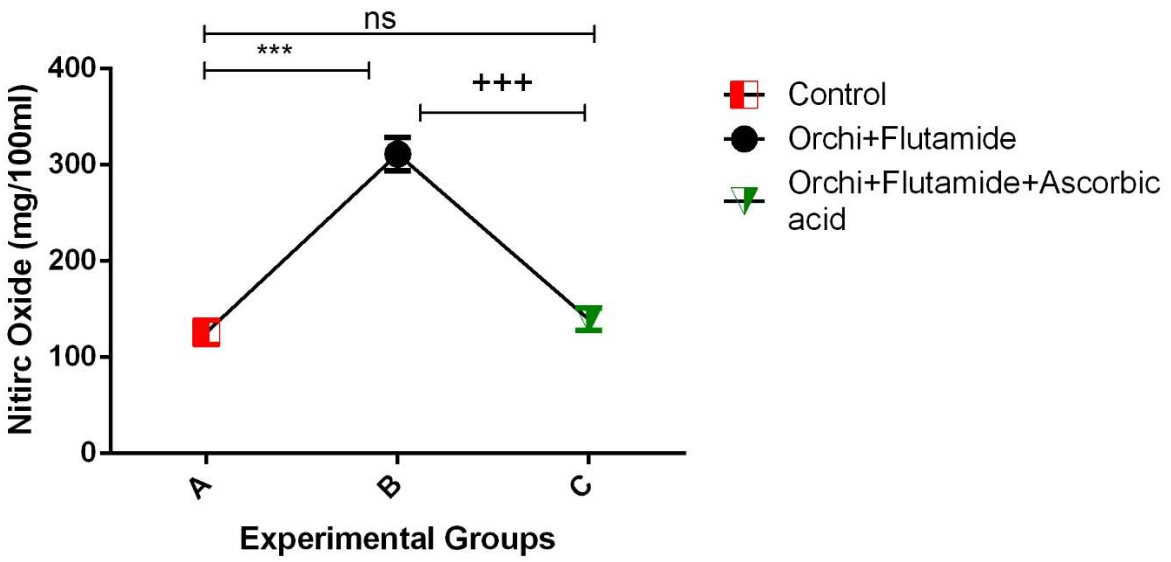

Figure 3: Nitric Oxide levels in the PFC tissue. * is the significant level of difference in comparison to the control group (Group A) while + is the significant level of difference in comparison with group B. **ns** means not significant. */+ p< 0.05; **/++ p<0.01; ***/+++ p< 0.001.

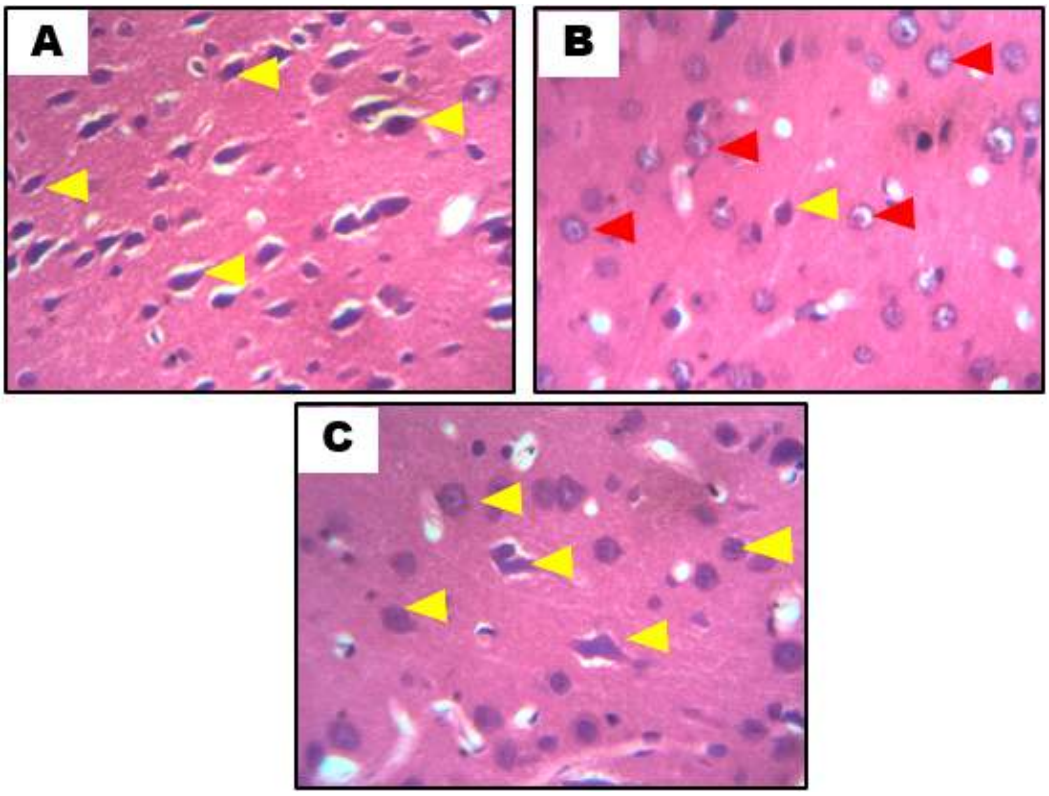

Figure 4. Photomicrographs showing prefrontal cortex general histomorphological presentations in Wistar rats across the study groups. Hematoxylin and Eosin stain (x400). A = control, B = Orchi+Flutamid, C = Orchi+Flutamid+Ascorbic acid

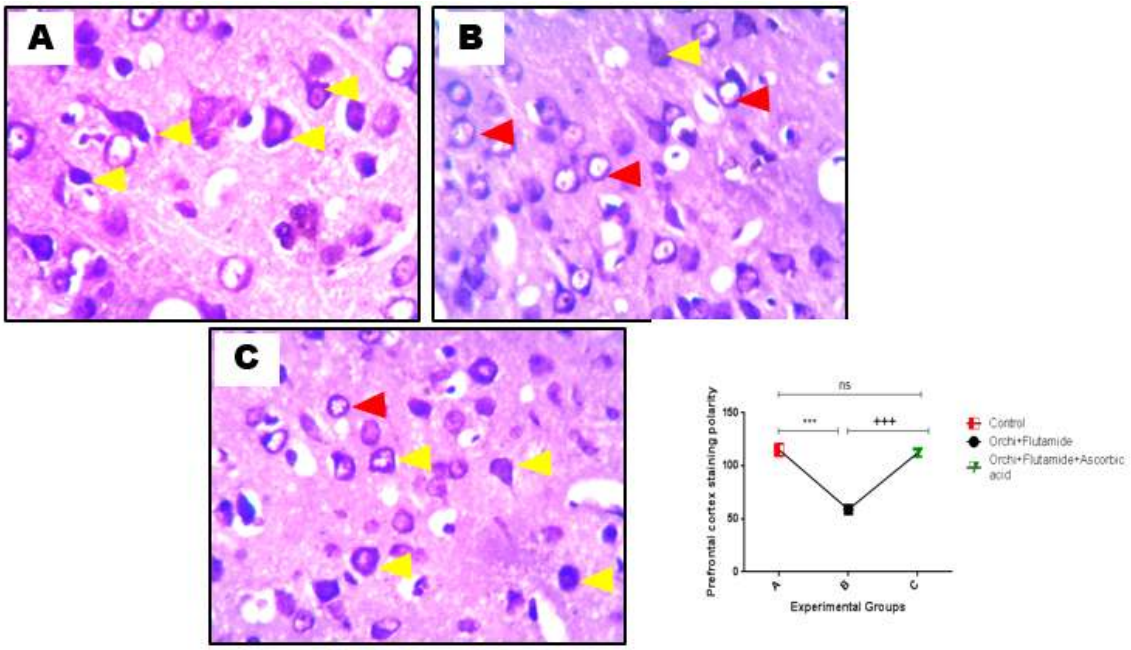

Figure 5: Representative photomicrograph of PFC showing the Nissl substances. (x400). A = control, B = Orchi+Flutamid, C = Orchi+Flutamid+Ascorbic acid. Staining polarity showed significantly reduced nissl substance intensity in orchiectomised group in contrast to control and ascorbic acid treated groups.

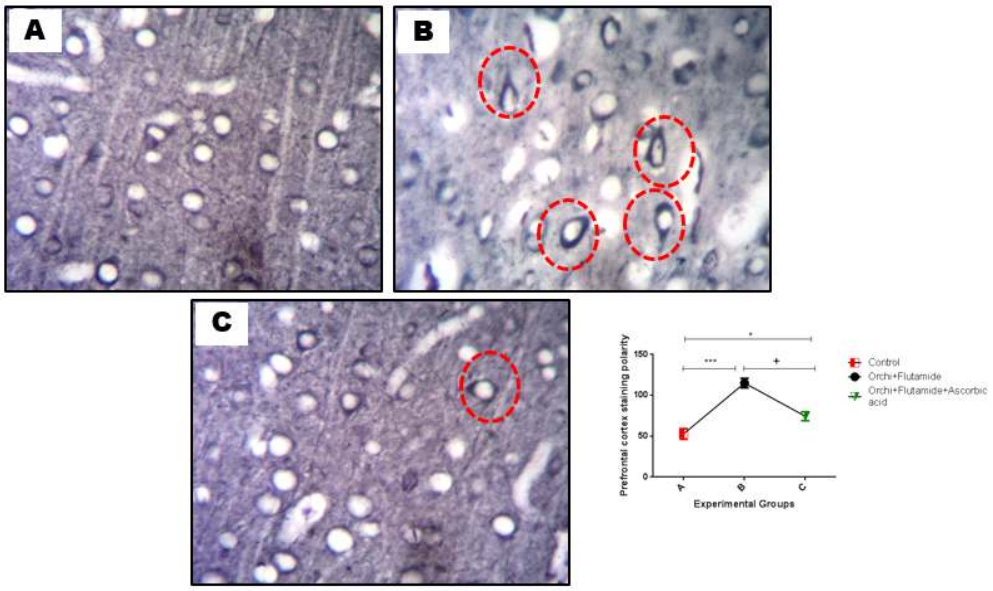

Figure 6: Representative photomicrograph of the PFC showing neurofibrillary tangles (x400). A = control, B = Orchi+Flutamid, C = Orchi+Flutamid+Ascorbic acid. Staining polarity showed significantly increased neurofibrillary tangles in orchiectomised group in contrast to control and ascorbic acid treated groups.