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PROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF ALOE VERA ON THE PREFRONTAL CORTEX OF WISTAR RAT WITH NICOTINE INDUCED OXIDATIVE STRESS

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ABSTRACT

Background: This study assessed the protective effect of aloe vera gel on the prefrontal cortex of wistar rats with nicotine induced oxidative stress. **Methodology:** Thirty adult wistar rats were divided into six experimental groups, n=5. Group I (normal control) received food only, Group II received 1.5mg/kg nicotine, Group III was treated with 120mg/kg Aloe-Vera, Group IV was treated with 60mg/kg of Aloe-Vera and 1.5mg/kg nicotine, Group V was treated with 120mg/kg of Aloe-vera and 1.5mg/kg nicotine, while Group VI (Imipramine) was treated with 25mg/kg of Imipramine and 1.5mg/kg nicotine. After the experiment, the rats were sacrificed and both blood samples and brain tissues were gotten for the biochemical and histological analysis respectively. **Results:** There is a statistically significant increase ($p < 0.05$) in the serum level of MDA with decreased SOD, Catalase in the nicotine treated animals without aloe vera intervention when compared with post-treatment with lauric acid and donepezil after inducing neuroinflammation and oxidative stress. There is a statistically significant increase ($p < 0.05$) in the activity of malondialdehyde in group II, slight decrease activity of malonaldehyde in group IV and a significant decrease of malonaldehyde in group V and group VI compared to with the control group. Also there is a close relationship in activity of malonaldehyde in group III and control group. The histological section of the negative control group showed scattered atrophic neurons, few neurons with well-developed vacuolated cytoplasm and blood vessels with dilated perivascular spaces. It also presents normal neurofillary network and coagulative necrosis while the treated group was cured. **Conclusion:** Aqueous extract of aloe vera has a protective effect in wistar rats with nicotine induced oxidative stress and prefrontal cortex injury.

Keywords: Antioxidative, Aloe Vera, Wistar rat, Nicotine, Prefrontal Cortex

INTRODUCTION

Background of the study

Smoking is hazardous to health; it exposes an individual to carcinogens, cytotoxins such as carbon monoxide and cadmium as well as a neuromoderatory molecule, nicotine, which is a major chemical component responsible for addiction in tobacco products (Zahran and Emam, 2018; Control, 2010; Cahill et al., 2013; Arany et al., 2016). It has been argued that the risk of nicotine addiction depends on the dose delivered and the route of delivery (Benowitz and Burbank, 2016; Card and Mitchell, 2010). The cessation of smoking in addicts, results in withdrawal symptoms caused by nicotine dependence (Changeux, 2011; Chattopadhyay et al., 2018). Experimental data have shown that nicotine has neuromodulatory effect on both cognitive and behavioral functions, some of which are positive while others are negative (Omotoso et al., 2013; Gbadamosi et al., 2016). In fact, there are data stating that nicotine has no significant effect on the central nervous system (Conceicao et al., 2015; Fagerstrom and Eissenberg, 2010). One notable controversy on nicotine studies is the effect that it has on reactive oxygen and nitrogen species (Gbadamosi et al., 2016). It was previously reported that nicotine induces oxidative stress in the frontal cortex by upregulating lipid peroxidation through the excessive generation of reactive oxygen species (Gbadamosi et al., 2016; Melroy-Greif et al., 2016). Another study also found that nicotine compromises the intrinsic antioxidant enzyme activity and increase

lipid peroxidation (Benowitz and Jacob, 2002).

Aloe vera has been used medically for several thousands of years with a long and illustrious history (Shen et al., 2017). The gel of aloe vera contains about 99 to 99.5% water with PH in the range of 4.4 to 4.7. The major components in the gel are glucomannans, acemannan, minerals, flavonoids, tannic acid, alprogen, C-glucosyl chromone, etc (Al-Shinnawy et al., 2014). The solid material contains about 45 different ingredients including vitamins, minerals, enzymes, sugars, anthraquinone or phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid. The plant is reported to contain an anti-allergic glycol protein and C-glycosyl chromone, a novel anti-inflammatory compound (Bashir et al., 2011; Bashir et al., 2015). The plant is reported to possess numerous pharmacological properties like abortifacient effect, adjuvant activity, analgesic activity, anticlastogenic, etc (Rehman et al., 2017). Several works are recently reported on the effect of consumption of extracts of Aloe vera gel on gastric ulcer, gastric micro circulatory changes, anti-inflammatory, hepatoprotective, clinical treatment of sepsis, etc (Rajeswari, 2012; Azmi et al., 2018). Aloe vera gel has extracted for antioxidant potential, and exhibits radical scavenging activity (72.2%) which is higher than that of BHT (70.5%) and α -tocopherol (65.65%) (Baby and Justin, 2010; Dechanet et al., 2010). The human bioavailability of vitamins C and E are enhanced with Aloe Vera preparations (Bashir et al., 2015). However, effect of the gel extract on

chemically induced toxicity and oxidative stress is sparse.

Nicotine is reported to generate the extremely reactive hydroxyl radical inducing oxidative stress (Terry and Clarke, 2012; Shen et al., 2017; Stabile et al., 2018) that participates in peroxidation of the membrane lipids leading to the increased reactive oxygen specie formation (Shen et al., 2017).

Due to the addictive nature of nicotine containing products, nicotine has become a substance of abuse, (Benowitz and Jacob, 2002; Grainge et al., 2010). The recreational use of tobacco remains one of the principal causes of its abuse which causes chronic ill health and early death worldwide, (Slotkin, 2010; Liu et al., 2017; Guo et al., 2017; Guo et al., 2016). The tobacco epidemic was largely reflected in more affluent Western countries but, increasingly, the illness associated with tobacco use have spread to the developing world (Alkam, et al., 2017; Halder et al., 2015). Cigarettes are considered to be the most harmful tobacco product although other forms of tobacco used recreationally may also result in harm to the user (Ashford et al., 2018; Benedict et al., 2011; Marinucci et al., 2017; Rubinstein et al., 2011). However, synthetic drugs used for oxidative stress and morphological injury (i.e. prefrontal cortex injury) treatment have shown serious adverse effect to the body (Miguel et al., 2017; Barth et al., 2016; Brennan et al. 2013; Das and Prochaska, 2017). Therefore, the need to seek for cheap and natural remedies with little or no side effect arises, hence this study.

The result of this study will encourage the use of natural plant products in treating nicotine induced oxidative stress caused disorders. Most current therapeutic approach for prefrontal cortex injury and oxidative stress has side effects and are quite expensive, (Ozen and Rezaki, 2017; Corcoles- Parada et al., 2017). This study looked on providing safe and affordable therapeutic remedy for nicotine induced oxidative stress caused disorders.

MATERIAL AND METHODS

3.1: PLANT PRECUREMENT

Mature fresh Aloe Vera leave was obtained from aloe vera garden at Nsukka, Enugu State, Nigeria.

3.2: PREPARATION OF EXTRACT

The mature and healthy aloe vera plants were harvested from aloe vera garden in Nsukka, Enugu state, Nigeria. A taxonomist in the Department of Plant Science and Technology, University of Nigeria Nsukka, identified the plant and a voucher specimen (Ref No: 2847) was deposited in the herbarium for reference. The plants were washed in water, and the thick epidermis was peeled off. The gel was then scooped with a spatula and blended in a blender. The homogenate was concentrated and freeze-dried with a lyophilizer. The yield was put in a desiccator, and later refrigerated, and ready for the use in the experiment.

3.3: PRECUREMENT OF RATS

Thirty (30) adult male wistar rats (185 to 222g) were used for the study. The rats were procured from the animal house of

the Department of Veterinary Medicine, University of Nigeria, Nsukka. The rats were handled according to the guideline of the committee for the purpose of control and supervision of experiments on animals.

3.4: TREATMENT PROTOCOL

The rats were divided into six groups of five animals (n=5) each. Group I (normal control) received food only, Group II received 1.5mg/kg nicotine, Group III was treated with 120mg/kg Aloe-Vera , Group

IV was treated with 60mg/kg of Aloe-Vera and 1.5mg/kg nicotine, Group V was treated with 120mg/kg of Aloe-vera and 1.5mg/kg nicotine, while Group VI (standard group) was treated with 25mg/kg of Imipramine and 1.5mg/kg nicotine. After the experiment, the rats were sacrificed and both blood samples and brain tissues were gotten for the biochemical and histological analysis respectively.

Table 3.1: Drug administration:

GROUPS	ADMINISTRATION
I	Normal saline
II	1.5mg/kg nicotine daily from 15th day to 21 st day (1 week)
III	120mg/kg Aloe vera daily from 1 st day to 14th day (2 weeks)
IV	60mg/kg Aloe vera daily from 1 st day to 14th day (2 weeks) + 1.5mg/kg nicotine daily from 15th day to 21 st day (1 week)
V	120mg/kg Aloe vera daily from 1 st day to 14th day (2 weeks) + 1.5mg/kg nicotine daily from 15th day to 21 st day (1 week)
VI	25mg/kg of Imipramine daily from 1 st day to 14th day (2 weeks) + 1.5mg/kg nicotine daily from 15th day to 21 st day (1 week)

3.5: SACRIFICE OF EXPERIMENTAL ANIMALS

After twenty four hours of the last administration for various groups, the rats were sacrificed via cervical dislocation. Blood samples were collected with capillary tube via orbital puncture into plain specimen bottle, and taken to a laboratory for test on the oxidative and anti-oxidative stress activities. Perfusion was done, and the 'whole' brain tissues

were removed from the skull, and fixed in 10% formal saline for histological studies on the prefrontal cortex. Superoxide dismutase (SOD) and Catalase (CAT) was estimated by (Bancroft and Gamble, 2008) method and Malondialdehyde (MDA) was estimated by Thiobarbituric acid reaction method (Bancroft and Gamble, 2008).

3.6 OXIDATIVE STRESS STUDIES

Determination of superoxide dismutase activity

Superoxide Dismutase (SOD) activity was determined by Colorimetry, a method described by (Fridovich, 1989; Ezugwu et al., 2022).

Determination of catalase activity

Catalase activity was determined using the method described by (Sinha, 1972; Ezugwu et al., 2022). In this procedure, 5% Potassium heptaoxochromate (VI) $K_2Cr_2O_7$ was mixed with glacial acetic acid in the ratio 1:3, and stored in brown bottle at room temperature, after which 0.9 ml of distilled water was added to 0.1 ml of sera and mixed thoroughly.

Determination of Malondialdehyde (MDA) activity

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acid peroxidation in cells and is commonly known as a biomarker of oxidative stress.

3.7: HISTOLOGICAL STUDIES PROCEDURES

The standard tissue processing protocols involves the following procedures below;

Fixation: The tissues were received in a container with 10% formal saline after perfusion to prevent autolysis; improve staining quality and aid optical differentiation of cell.

Dehydration: The tissues were dehydrated with different grades of alcohol ranging from 70% to absolute alcohol for 30minutes each.

Clearing/Dealcoholization: The dehydrated tissue was cleared by removing the alcohol from the tissue by immersing it through 3 changes of xylene for 30minutes each.

Wax impregnation/infiltration: The cleared tissue was impregnated and infiltrated to remove the clearing agent (xylene) in the hot oven temperature of 60°C by passing it through three changes of molten paraffin wax in a hot air oven for 30minutes.

Embedding: The infiltrated tissue was attached with molten paraffin wax in an embedded mold and allowed to solidify.

Mounting on wooding block: The paraffin block of tissue was attached to a wooding block with the aid of a hot spatula held in between wood block and paraffin wax, the spatula melts the wax which solidifies when spatula was removed.

Microtomy: The block of tissues was sectioned using rotary microtome; it was trimmed to obtain the cutting surface of the tissue at 15 micron and was sectioned at 5micron, and dry in hot plate for staining.

3.7.1: Haematoxylin and Eosin (H&E) staining Procedure:

The tissue is dewaxed in xylene for 30 minutes; they were removed from xylene by rising in absolute alcohol graded, 90%, and 70% and 50% for two seconds each. They were washed in 2 changes of water and stained in 1% acid. They were again washed in water and counter stained in Eosin for 5minutes, after which they were again washed in water and then dried and cleared in xylene, mounted in D.P.X for micrographed and interpreted.

3.8: Data Analysis

Data obtained were expressed as mean \pm SEM (standard error of mean). One-way analysis of variance (ANOVA) was used to compare the mean differences. Tukey's

post hoc test was done where the result was significant. P-value less than to 0.05 was considered statistically significant. All results were analyzed using the Statistical

Package for Social Sciences (SPSS version 22).

4.0 RESULT

Table 4.1: **Oxidative Stress Biomarkers**

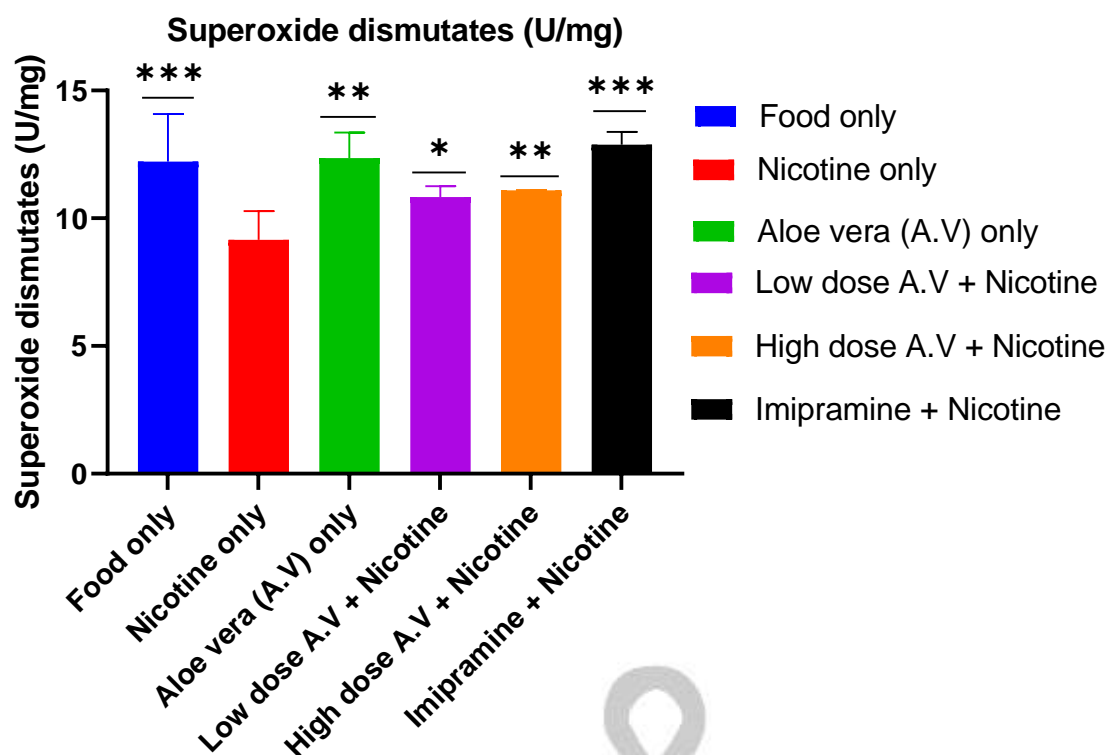
GROUP	TREATMENT	SOD(U/mg)	CAT (U/mg)	MDA(mg/dl)
1	Food only	12.2150 $\pm 1.8650^{***}$	0.5150 $\pm 0.012021^{**}$	3.8200 $\pm 0.6500^{***}$
2	Nicotine only	9.1550 ± 1.1250	0.4350 ± 0.05172	19.0450 ± 2.2850
3	Aloe vera (A.V) only	12.3600 $\pm 1.0000^{**}$	0.5260 $\pm 0.011425^{***}$	4.9450 $\pm 0.8250^{***}$
4	Low dose A.V+ Nicotine	10.8300 $\pm 0.4200^{*}$	0.4950 ± 0.04162	18.7600 ± 0.3100
5	High dose A.V + Nicotine	11.0950 $\pm 0.0150^{**}$	0.5050 $\pm 0.004549^{*}$	13.0600 $\pm 2.3900^{**}$
6	Imipramine+ Nicotine	12.8800 $\pm 0.5000^{***}$	0.5100 $\pm 0.010306^{**}$	9.0450 $\pm 1.2350^{*}$

Values are mean \pm SEM; n = 5 in each group, (P < 0.05) = Statistically significant.

* Significant when compared to the control at P < 0.05

** Significant when compared to the control at P < 0.01

*** Significant when compared to the control at P < 0.001



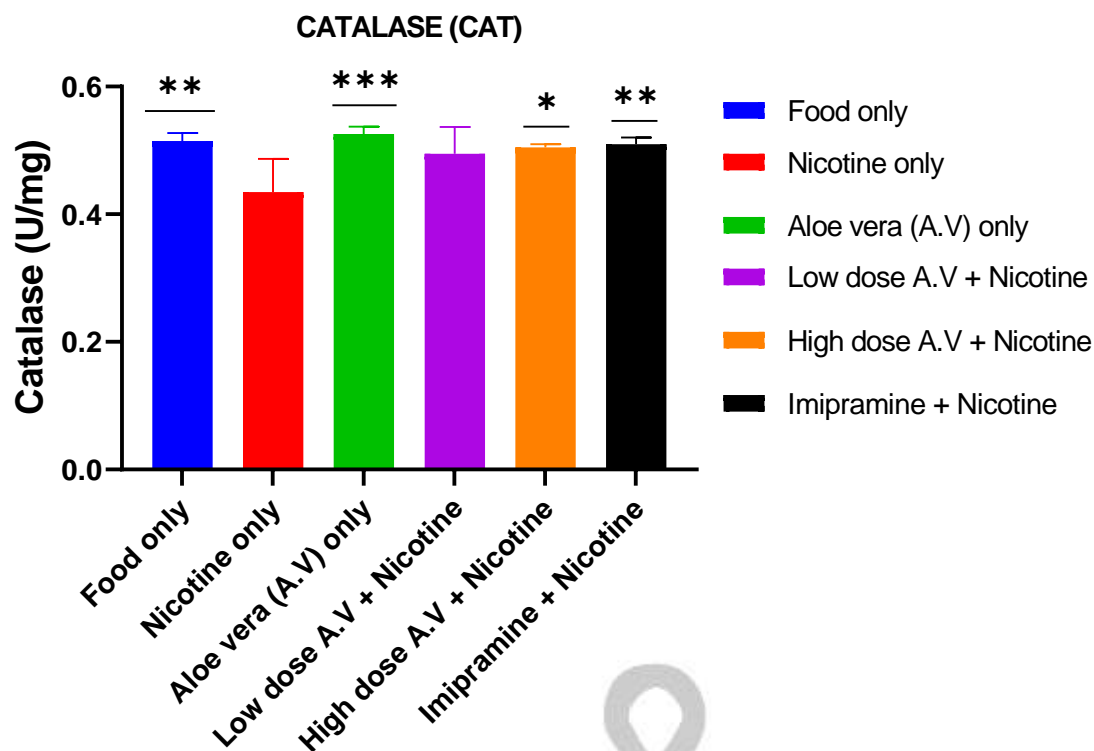
Values are mean \pm SEM; n = 5 in each group, (P < 0.05) = Statistically significant.

* Significant when compared to the control at P < 0.05

** Significant when compared to the control at P < 0.01

*** Significant when compared to the control at P < 0.001

Figure 4.3.2: Component bar graph showing oxidative stress level for Superoxide dismutase SOD



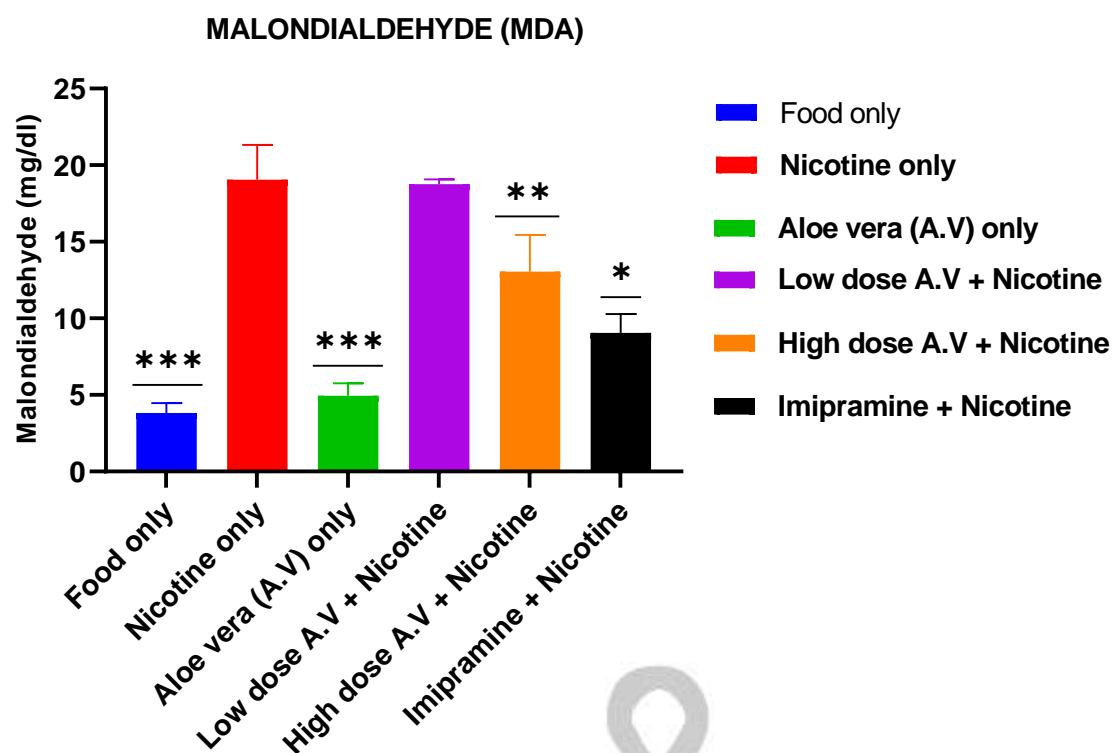
Values are mean \pm SEM; n = 5 in each group, ($P < 0.05$) = Statistically significant.

* Significant when compared to the control at $P < 0.05$

** Significant when compared to the control at $P < 0.01$

*** Significant when compared to the control at $P < 0.001$

Figure 4.3.3: Component bar graph showing oxidative stress level for Catalase (CAT)



Values are mean \pm SEM; n = 5 in each group, ($P < 0.05$) = Statistically significant.

* Significant when compared to the control at $P < 0.05$

** Significant when compared to the control at $P < 0.01$

*** Significant when compared to the control at $P < 0.001$

Figure 4.3.4: Component bar graph showing oxidative stress level for Malondialdehyde (MDA)

4.2: HISTOLOGICAL RESULT

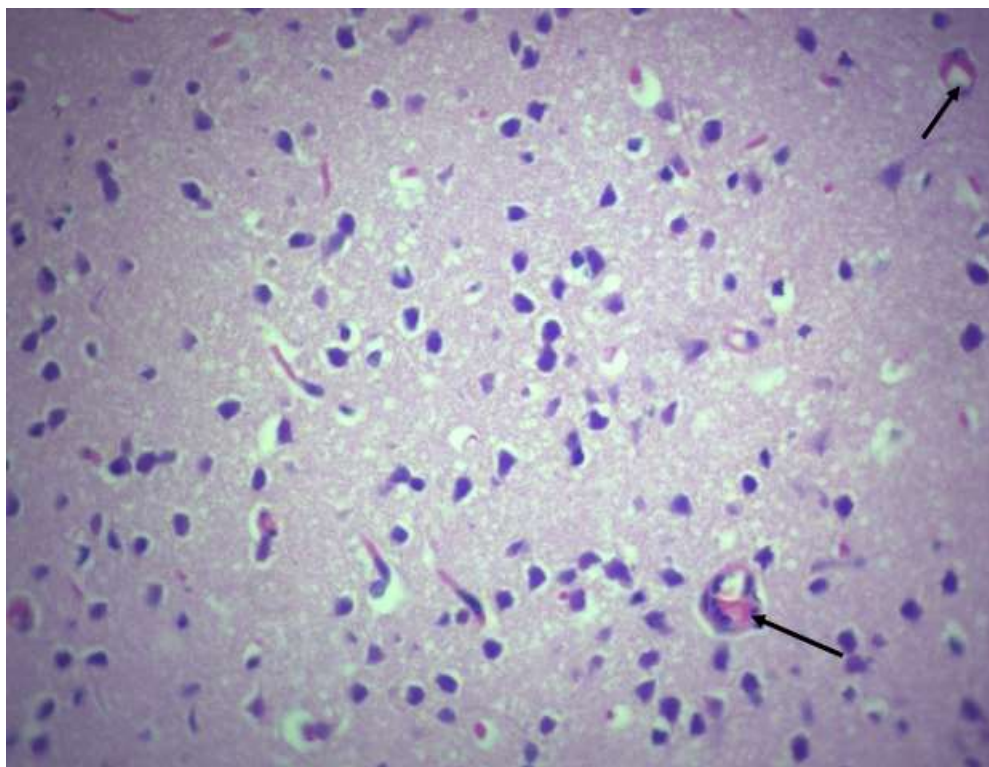


PLATE IA: Photomicrograph of a section of the prefrontal cortex of the group administered with food and water showed normal neuronal cells, numerous glia cells and a normal condensed neurofibrillary network. Blood vessels (Arrow) H&E.X400

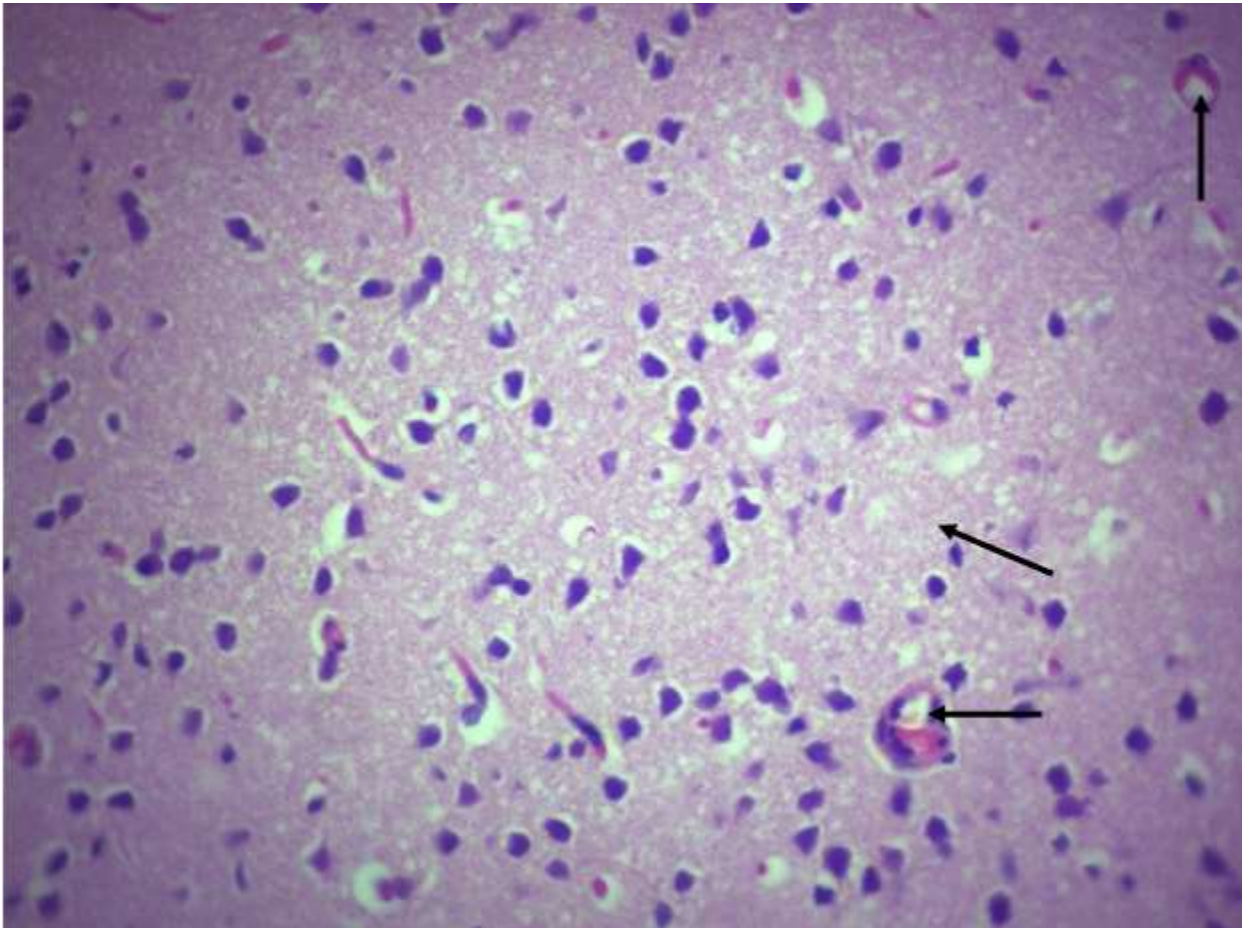


PLATE IB: Photomicrograph of a section of the prefrontal cortex of the group administered with food and water showed normal neuronal cells, numerous glia cells and a normal condensed neurofibrillary network. Blood vessels (Arrow) H&E.X400

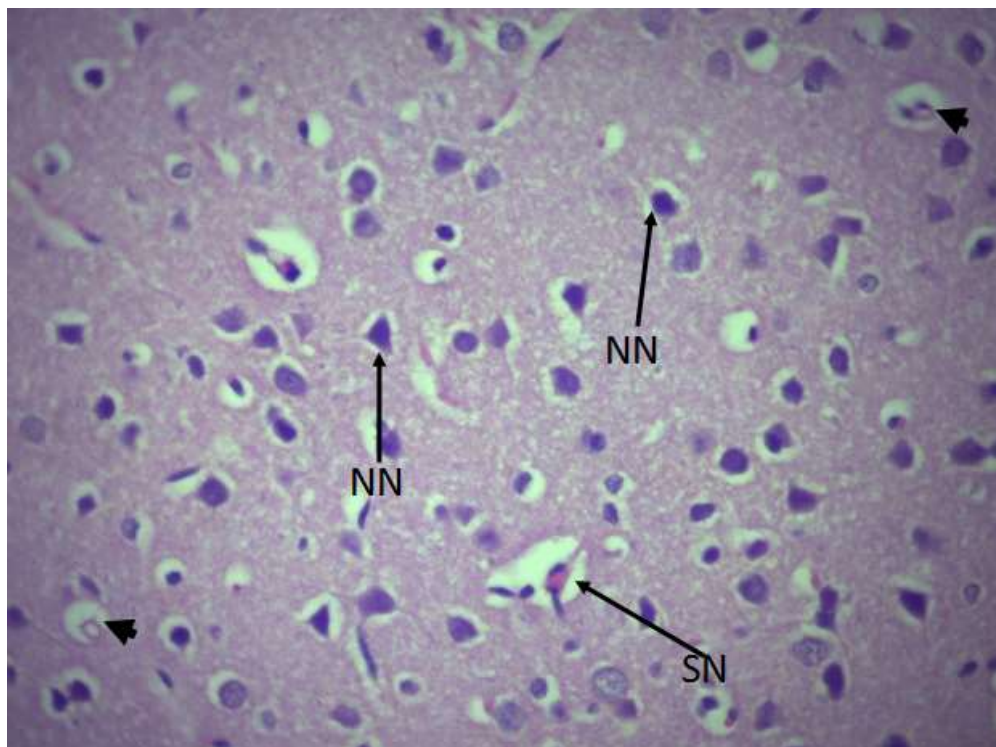


PLATE IIA: Photomicrograph of a section of the prefrontal cortex of the group administered with 1.5mg/kg of nicotine only showing normal neurons (NN) and a few shrinking neurons (SN) with vacuolated cytoplasm. Normal neurofibrillary network. H&E.X400

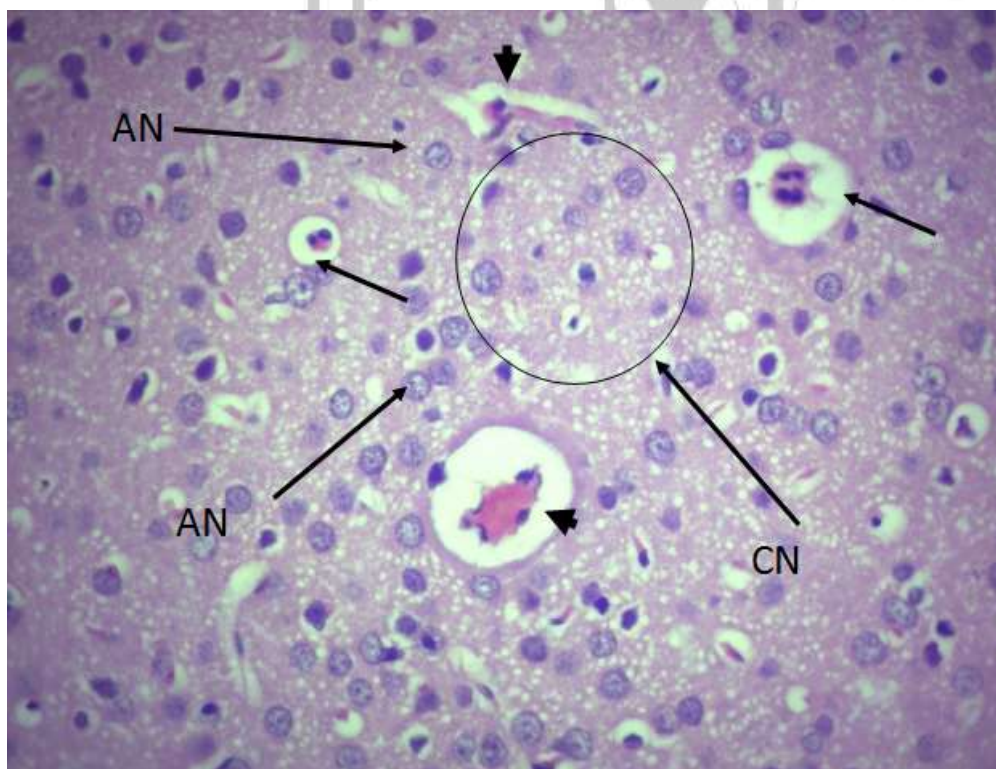


PLATE IIB: Photomicrograph of a section of the prefrontal cortex of the group administered with 1.5mg/kg of nicotine only showing scattered atrophic neurons (AN) a few neurons with well-developed vacuolated cytoplasm (Arrow) and blood vessels with dilated perivascular spaces (Arrow heads). It also presents Normal neurofibrillary network and co-agulative necrosis (CN). H&E.X400

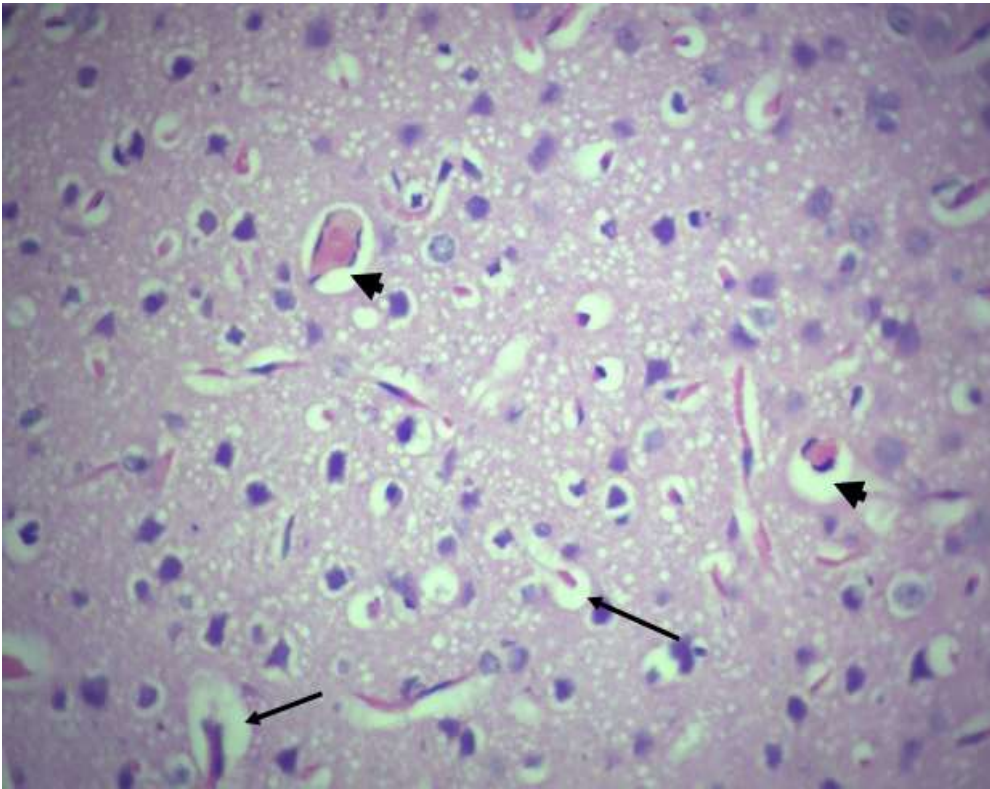


PLATE IIIA: Photomicrograph of a section of the prefrontal cortex of the group that was treated with 120mg/kg Aloe-Vera only showing a few neurons with well-developed vacuolated cytoplasm (Arrow) and blood vessels with dilated perivascular spaces (Arrow heads). Normal neurofibrillary network. H&E.X400

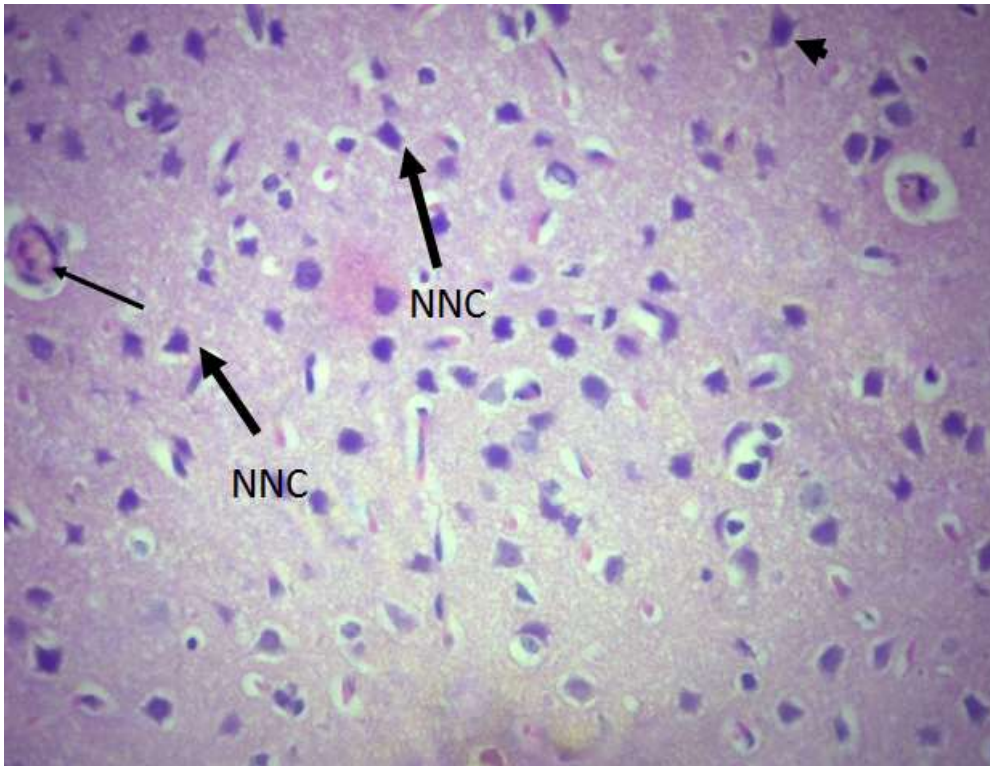


PLATE IIIB: Photomicrograph of a section of the prefrontal cortex of the group that was treated with 120mg/kg of Aloe-Vera only showing normal neuronal cells (NNC) and numerous glia cells with a normal condensed neurofibrillary network. Blood vessels (Arrow) H&E.X400

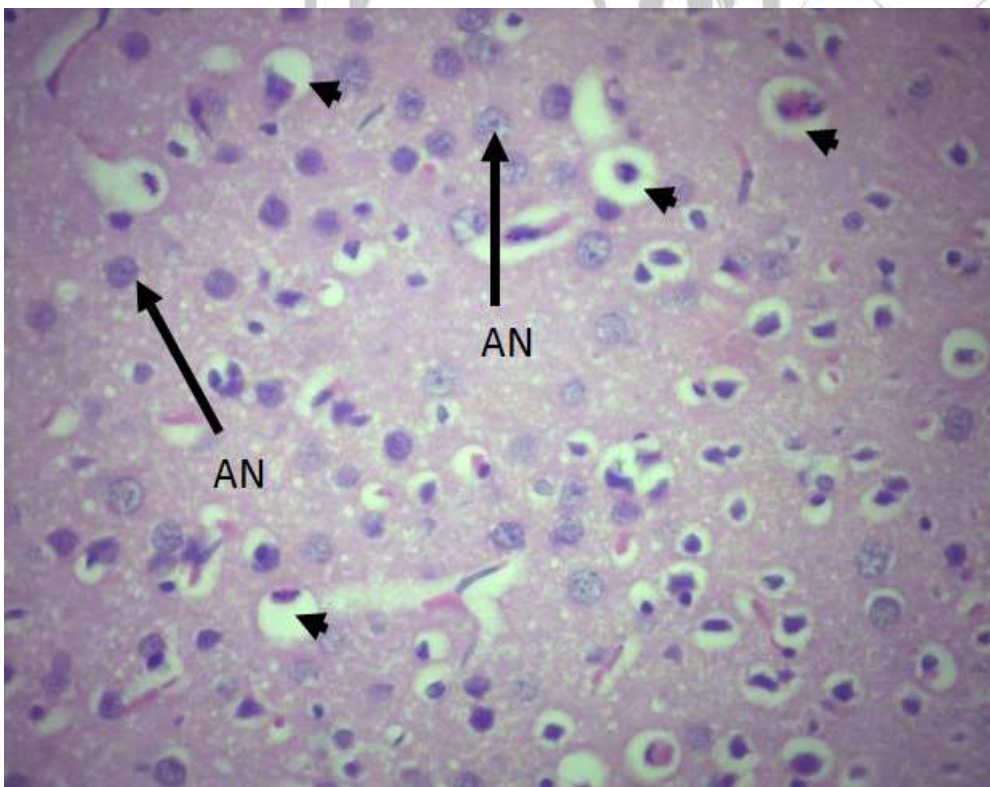


PLATE IVA: Photomicrograph of a section of the prefrontal cortex of the group treated with 60mg/kg of Aloe-Vera and 1.5mg/kg of nicotine showing cortical neurons with mild and well developed vacuolated cytoplasm (Arrow head) and atrophic neurons (AN) Normal neurofibrillary network. H&E.X400

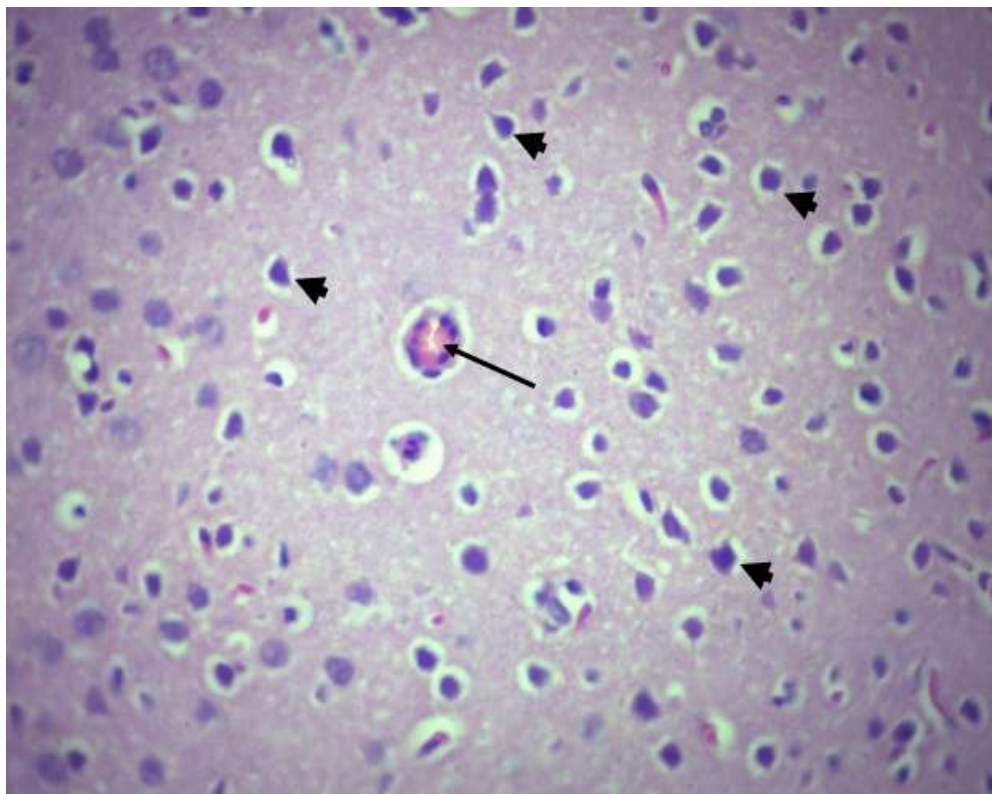


PLATE IVB: Photomicrograph of a section of the prefrontal cortex of the group treated with 60mg/kg of Aloe-Vera and 1.5mg/kg of nicotine showing normal neuronal cells, (Arrow head) and numerous glia cells with a normal condensed neurofibrillary network. Blood vessels (Arrow) H&E.X400.

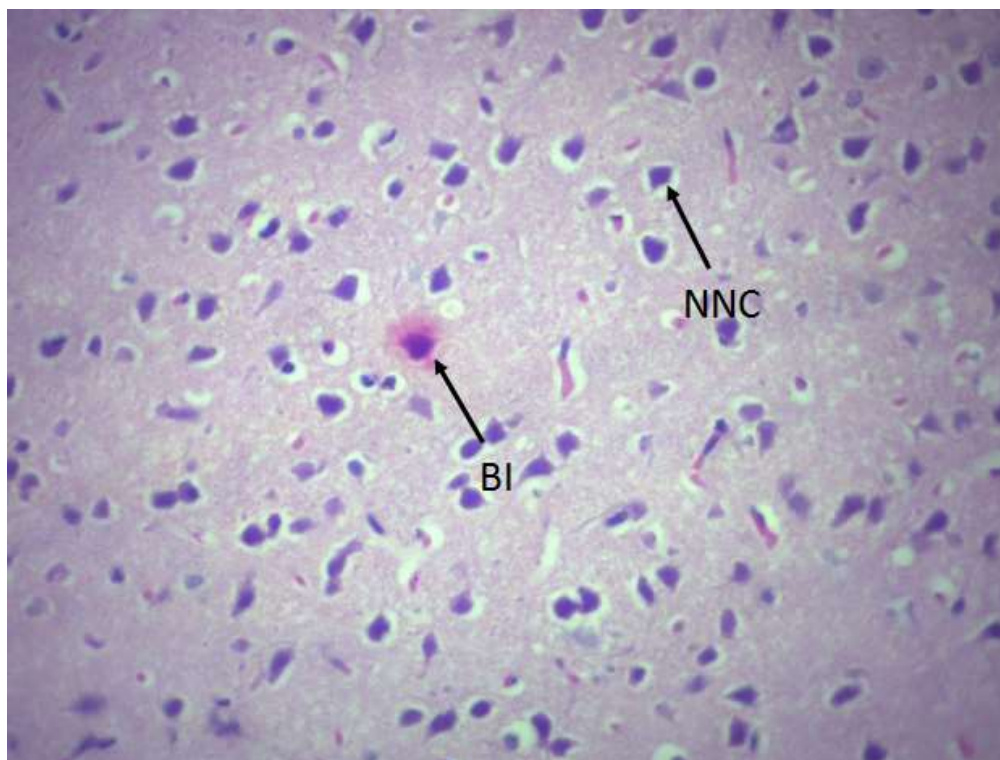


PLATE VA: Photomicrograph of a section of the prefrontal cortex of the group treated with 120mg/kg of Aloe-vera and 1.5mg/kg nicotine showing normal neuronal cells (NNC), and basophilic inclusion (BI) with numerous glia cells and a normal condensed neurofibrillary network. H&E.X400.

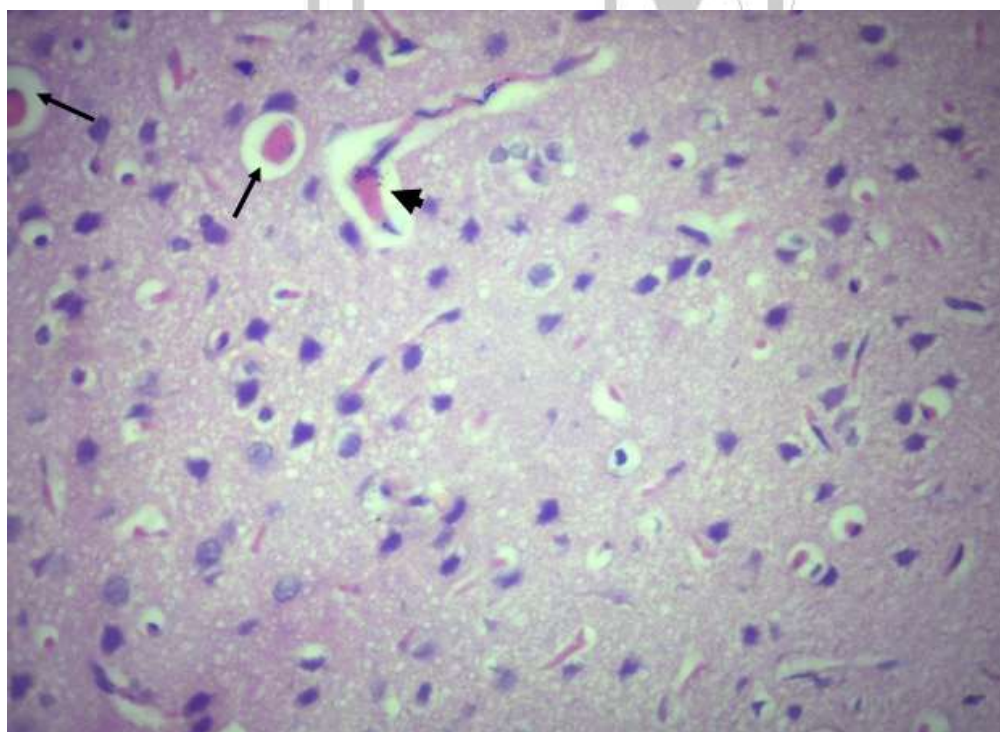


PLATE VB: Photomicrograph of a section of the prefrontal cortex of the group treated with 120mg/kg of Aloe-vera and 1.5mg/kg nicotine showing a few neurons with mild developed vacuolated cytoplasm (Arrow) and blood vessels with dilated perivascular spaces (Arrow heads). Normal neurofibrillary network. H&E.X400.

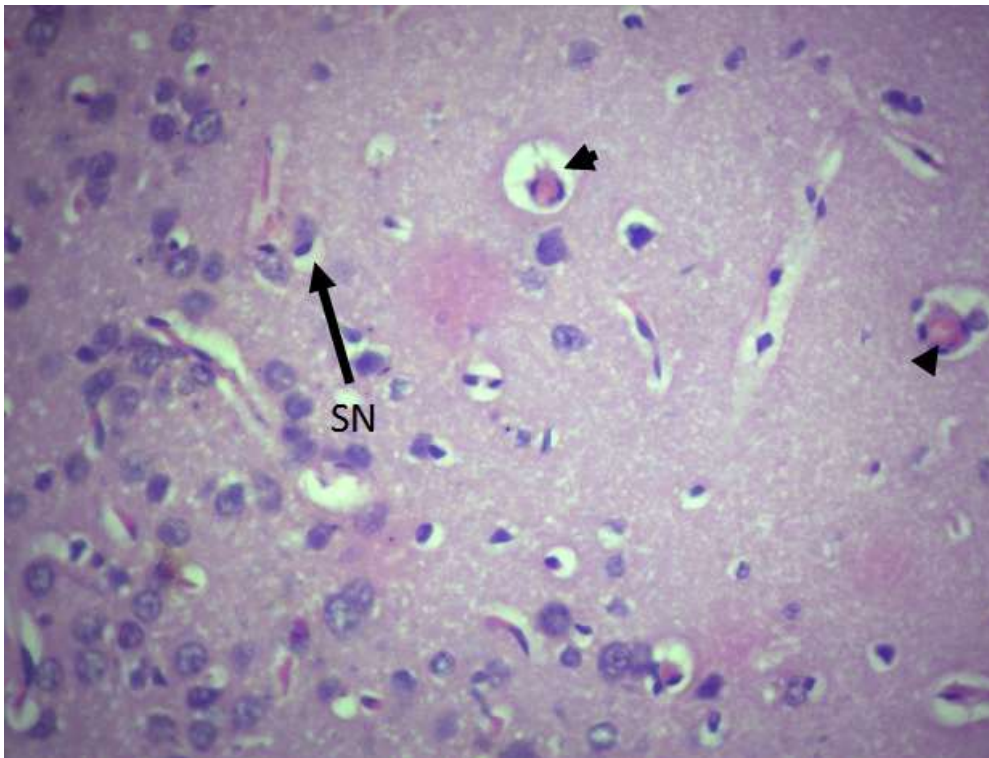


PLATE VIA: Photomicrograph of a section of the prefrontal cortex of the group treated with 25mg/kg of Imipramine and 1.5mg/kg of nicotine showing few shrinking neuronal cells (SN), numerous glia cells and a normal condensed neurofibrillary network. However, dilated perivascular spaces (Arrow heads) are present. H&E.X400.

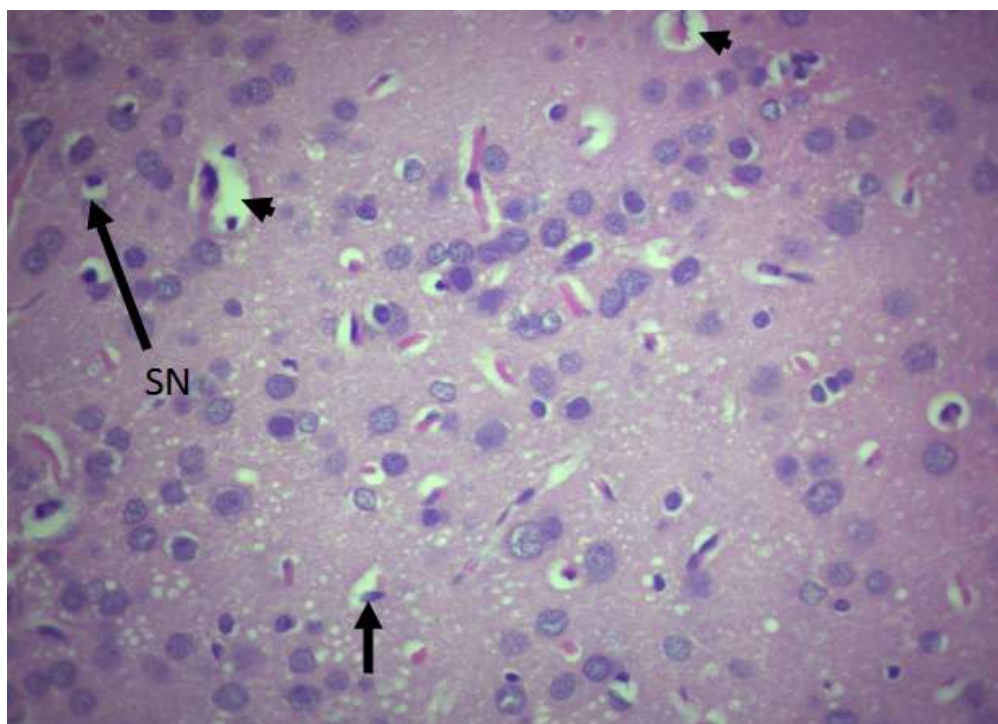


PLATE VIB: Photomicrograph of a section of the prefrontal cortex of the group treated with 25mg/kg of imipramine and 1.5mg/kg of nicotine showing few scattered shrinking neuronal cells (SN), numerous glia cells and a normal condensed neurofibrillary network. However, a few cortical neurons with mild vacuolated cytoplasm (Arrow head) were noticed. H&E.X40.

5.1 DISCUSSION

This study investigated the antioxidant effect of aloe vera gel on the prefrontal cortex of wistar rats with nicotine induced oxidative stress. Our results provide additional evidence of cognitive alteration and increased oxidative stress in the nicotine induced neurotoxicity rat model, which were protected by 60mg/kg/day and 120mg/kg/day of aloe vera for 14 days. Moreover, we found a significant correlation between most of the histological results and the levels of oxidative stress markers, from the prefrontal cortex, which is known to be the most susceptible cortical area to reactive oxygen species (Zhu et al. 2012). Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen

species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Nicotine is known to increase oxidative stress (Conceicao et al., 2015), and causes significant reduction in body weight of animals. Orr and Sohal, 2017 reported that the level of catalase (CAT) and superoxide dismutase (SOD) significantly ($p < 0.05$) decreased in the animals with nicotine induced oxidative stress as a result of exposure to nicotine. Also the significant decrease ($p < 0.05$) in the level of the malondialdehyde (MDA) in the aloe vera treated rats with no mortality when compared to the nicotine induced oxidative stress rats indicates that the aloe vera gel has the potential that may have protected the prefrontal cortex from the

neurotoxicity, which is linked to its anti-oxidative ability. This is in accordance with Al-Shinnawy et al., 2017 report on the anti-oxidative activity of aloe vera gel. Grindlay and Reynolds, 2010; Patidar et al., 2012 reported that aloe vera gel has antioxidant potential, and exhibits radical anti-inflammatory activity (72%) which is higher than that of BHT (70.5%) and α -tocopherol (65.65%). The close level of the MDA in the group treated with a higher dose of Aloe vera gel (i.e group V) when compared to the group treated with imipramine (group VI) also indicates that the Aloe vera gel has the potential/efficacy that may have protected the prefrontal cortex from the oxidative stress problem, (Bernard et al., 2013). A significantly ($p < 0.05$) increase in the level of SOD was also observed in aloe vera treated rats compared with the nicotine treated rats. The reduced level of SOD in animals that received the combination of Aloe vera gel and nicotine, though not significant ($p < 0.05$), could be ascribed to the compensating actions of aloe vera against nicotine. This is in accordance with the Zhu et al., 2012 study on aloe vera. This observation also is in agreement with previous reports by, Patidar et al., 2012 that nicotine treatment significantly decreased the level of the antioxidant enzyme activities such as SOD, Patidar et al., 2012. Also when only aloe vera treated rats (i.e. group III) is compared to the rats given only normal saline (i.e. group I), the significantly ($p < 0.05$) range shows that the aloe vera gel has potential/efficacy that may have protected the prefrontal cortex of the wistar rats from oxidative stress injury. This

supports Chudasama, 2011 models of prefrontal executive function. The administration of aloe vera gel significantly ($p < 0.05$) decreased malondialdehyde (MDA), thereby protecting the prefrontal cortex of the rats from the nicotine injury.

Anti-oxidative property of aloe vera gel has been traced to Manose-6-phosphate actually, gluconomannan and plant development hormone gibberellins connects with development variable receptor of fibroblast and invigorate its action and proliferation for expansion collagen blend its topical and oral organization of Aloe vera, Al-Shinnawy, 2014.

The photomicrograph of the group that was treated with a higher dose of Aloe vera gel (i.e. group V) showed normal neuronal cells (NNS), and basophilic inclusion (BI) with numerous glia cells and a normal condensed neurofibrillary network when compared with the standard group (i.e. group VI) which shows few shrinking neuronal cells (SN), numerous glia cells and a numerous condensed neurofibrillary network. We observed that the aloe vera treated groups possess more protective properties than the standard group. Also when comparing the group that was treated with Aloe vera alone (i.e group III) and the negative control group (i.e. group I), we observed similar appearance of their tissues which were normal. This also confirms the report made by Hayes (Grindlay and Reynolds, 2010) about the wound healing property that aloe vera gel possess. The results of this experiment also support that same report that aloe vera gel has a strong wound healing property

against the prefrontal cortex injury which was induced by nicotine (Bashir et al., 2011).

5.2. Conclusion

Administration of nicotine caused neurotoxicity which was further established by quantitative analysis of the oxidative activities, which is supported by the hypertrophy of the prefrontal cortex in the micrograph (Plate I to VI). These features were probably enhanced by the drug receptor interaction of nicotine with nAChRs in brain tissue. But the use of aloe vera extract may have reduced the hypertrophy in the prefrontal cortex injury and oxidative stress associated with nicotine-induced neurotoxicity in this region of the brain. This study could offer clinical benefits in neurological dysfunctions associated with consumption of nicotine-containing substances.

5.3. Recommendation

Further findings are required to confirm the exact mechanism underlining the antioxidant potential and wound healing property of aloe vera, and identify the chemical constituents responsible for this effect.

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