

AMELIORATIVE POTENTIALS OF *PSIDIUM GUAJAVA* (GUAVA) AQUEOUS LEAF EXTRACT ON THE HISTOLOGY OF THE HIPPOCAMPUS IN RAT MODELS OF NICOTINE-INDUCED NEUROTOXICITY

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Abstract: Background: Nicotine consumption has been linked to many diseases and the negative effects especially on the brain as concerned to memory and emotion. *Psidium guajava* (Guava) is a medicinal plant with a long history of use for its pharmacologic properties. Aim: This study aims at investigating the ameliorative effects of *Psidium guajava* aqueous leaf extract (PGALE) on the histology of the hippocampus in nicotine-induced neurotoxicity in wistar rats. Methodology: Thirty (30) adult male wistar rats (150-200g) were divided into six (6) groups (n=5). Group A (control group) received only food and water *ad libitum*. Group B (Untreated) was injected subcutaneously with 0.1ml of 1% nicotine every 48 hours till the end of the experiment. Group C, D, E and F received same but were treated daily with 200, 400, 600mg/kg of PGALE and 100mg/kg of Vitamin E respectively. The experiment lasted 28 days. The animals were sacrificed 24 hours after last treatment under ketamine (100mg/ml) as anesthesia. Neatly harvested brains were fixed for histological studies stained with H&E. Results: Nicotine injections caused severe neurodegenerative injuries on the histology of the hippocampus characterized by marked degeneration of neurons, cytoplasmic vacuolations, decreased cellular population and pyknosis of pyramidal cells in untreated group. Treatment with the PGALE and Vitamin E ameliorated these effects as they demonstrated only mild to moderate degenerative injuries relative to the untreated groups. Conclusion: *Psidium Guajava* demonstrated a strong dose-dependent ameliorative potential.

Keywords: *Psidium Guajava*, Neurotoxicity, Hippocampus, Nicotine.

1. INTRODUCTION

Tobacco smoking is a very common practice globally and has existed since ancient times. Nicotine is one of its major components and is responsible for the addiction associated with smoking tobacco products (Zeid *et al.*, 2018). Currently, the use of tobacco-free products that still contain nicotine such as e-cigarettes is rapidly gaining popularity among young people including pregnant and breast-feeding mothers and individuals undergo nicotine replacement therapy alternatives to replace the use of tobacco (Dwyer *et al.*, 2008; England *et al.*, 2017). Nicotine has been known to be toxic and is associated with the generation of reactive oxygen species (ROS) within tissues; a condition known as oxidative stress and leads to injuries at cellular levels and possibly organ damage (Swami *et al.*, 2006; Chowdury and Walker, 2008). The use

and exposure to products containing nicotine is known to cause toxic effects on the brain especially when consumed from a young age (Dwyer *et al.*, 2009).

The hippocampus is a structure situated within the temporal lobe of the cerebrum of the brain. Its function is strongly linked with memory, emotion, and adult neurogenesis (Phillips and LeDoux, 1995). It is a very sensitive region of the brain that reacts quickly and distinctively to drug abuse and its sensitivity is theorized to play an essential role in the advancement and continuation of addictions (Kutlu and Gould, 2016). Exposure to nicotine directly and actively affects the hippocampus via the activation of hippocampal nicotinic acetylcholine receptors (nAChRs), related to ionotropic neuronal receptors that regulate hippocampal activity throughout one's lifetime (Fabian-Fine *et al.*, 2001).

Psidium guajava (Guava) is a medicinal plant with a long history of use for its pharmacologic properties (Nwinyi *et al.*, 2008; Deguchi and Miyazaki, 2010). Numerous chemical and pharmaceutical companies are also known to utilize different parts of guava tree (Joseph and Priya, 2011).

The guava leaf has been phytochemically analysed and is known to be strongly antioxidant; containing phytochemicals like alkaloids, carotenoids, triterpenes, anthocyanins, vitamin-C and vitamin-A (Ghosh *et al.*, 2010; Metwally *et al.*, 2010; Chen *et al.*, 2010; Das, 2011). Alcoholic extracts from *P. guajava* leaf have also been seen to possess anti-inflammatory and analgesic capabilities (Muruganandan *et al.*, 2001) and also a strong anti-diabetic activity (Rai *et al.*, 2007; Mukhtar *et al.*, 2006).

2. MATERIALS AND METHODS

Plant Material and Extraction

Matured leaves of *Psidium guajava* were plucked from a Guava tree in a residential apartment at Independence layout of the Enugu metropolis of Enugu State. Identification and authentication was carried out at the Faculty of Agriculture, Enugu State University of Science and Technology.

The leaves were washed and air-dried at room temperature for 7 days. The dried leaves were grinded using an electronic grinder and the aqueous extraction was done according to the methods of Kanu *et al.*, (2022). The resultant crude extract was stored at -4°C until required for use.

Experimental Animals

Thirty adult male wistar rats weighing 150-200g were used for this study. This study was carried out in the Animal facility of the Enugu State University of Science and Technology College of Medicine, Parklane, Enugu. All animals were provided easy access to water and standard poultry pellets as food. The animals were kept and maintained under standard laboratory conditions and handling was done following international ethics guidelines on the use of experimental animals. Nigeria Limited) as food. Ethical approval was gotten from the university's ethical clearance committee with the ethical right permission number: ESUCOM/FBMS/ETR/23/006.

Experimental Design

The rats were randomly divided into six (6) groups (n=5). Group A (control group) received only food and water *ad libitum*. Group B (Nicotine-only group) received subcutaneous injection of 0.1ml of 1% nicotine every 48 hours till the end of the experiment. This dosage was adopted from Mamdouh *et al.*, (2003). Group C, D, E and F received the same administration of Nicotine as animal group B but were treated daily with 200, 400, 600mg/kg of *Psidium Guajava* Aqueous Leaf Extract (PGALE) and 100mg/kg of Vitamin E respectively. Extract doses were adopted from Kanu *et al.*, (2022). The experiment lasted 28 days.

Animal Sacrifice and Histological Study

The animals were sacrificed 24 hours after their last administration under ketamine (100mg/ml) as anesthesia. The brains were neatly harvested and fixed immediately with 10% formaldehyde solution for 72 hours. Their various hippocampi were isolated and processed using the standard protocols for histological tissue processing and stained with hematoxylin and eosin for histological studies. Photomicrographs were taken at x100 magnification.

Histological Findings

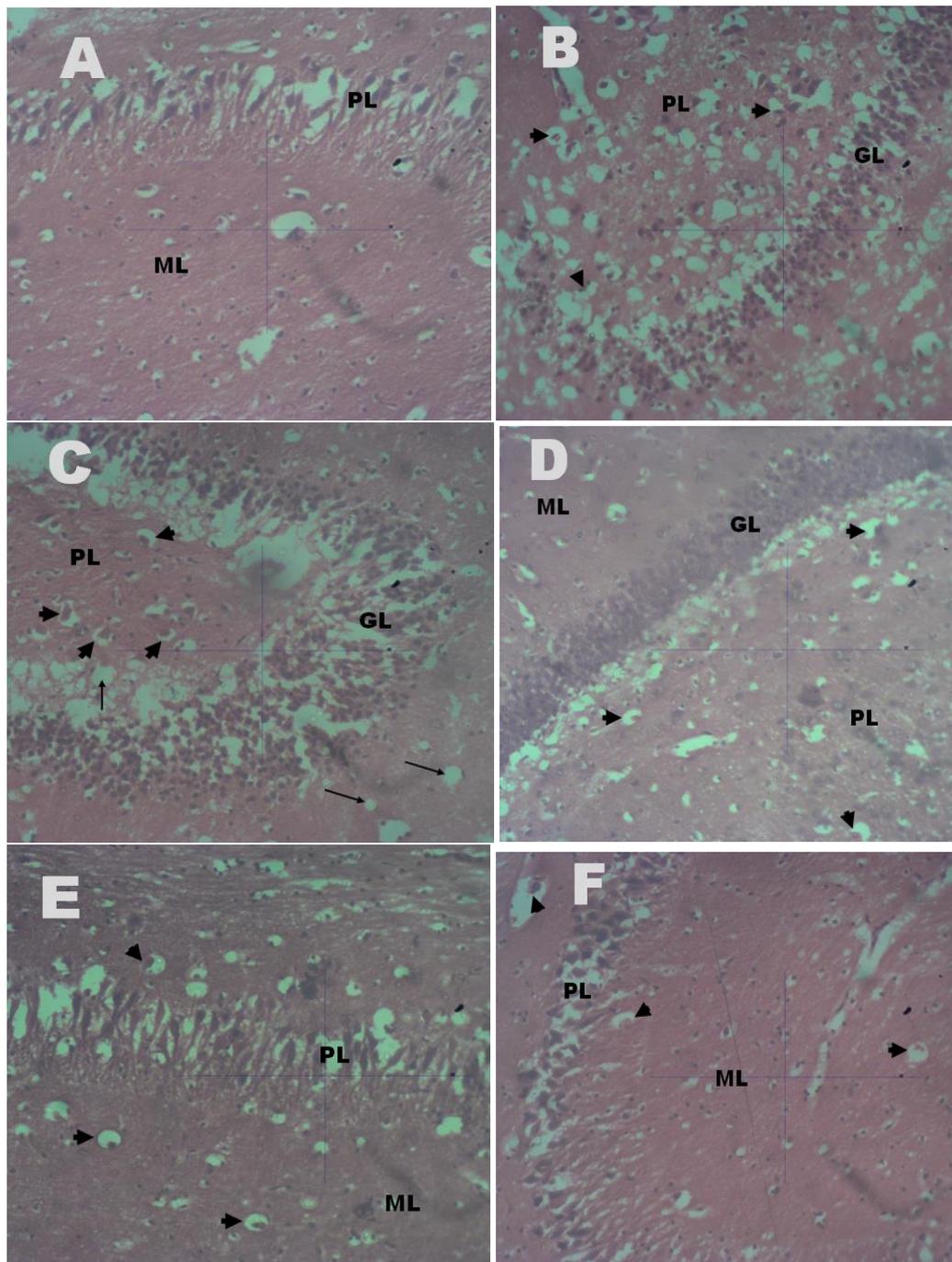


Figure A: Control groups showing the normal histo-architecture of the hippocampal neurons with compact polymorphic layer (PL) with large pyramidal cells. Normal neurons were also observed on the molecular layer (ML). **Figure B:** Untreated Nicotine group showing marked degeneration of hippocampal neurons, widespread cytoplasmic vacuolations, decreased cellular population within the granular layer (GL) and pyknosis of pyramidal cells (arrow head). **Figure C:** Nicotine + 200mg/kg of PGALE. Mild cytoplasmic vacuolations (arrow) within the hippocampal neurons and shrinkage (arrow head) of pyramidal cells. **Figure D:** Nicotine + 400mg/kg of PGALE. Histo-architecture appears normal with little mild neuronal shrinkage (arrow head). **Figure E:** Nicotine + 600mg/kg of PGALE. Near normal microstructural appearance with mild vacuolated cytoplasm (arrow head) observed in the molecular layer (ML). **Figure F:** Nicotine + 100mg/kg of Vit-E. Polymorphic layer (PL) appears normal. A few necrotic neurons (arrow head) seen in the Molecular layer (ML). H&E. x100.

3. DISCUSSION

Nicotine consumption has been linked to many diseases and the negative effects, causing oxidative stress and possibly organ damage (Swami *et al.*, 2006; Chowdury and Walker, 2008; Hralová *et al.*, 2010). Local herbs when consumed by man have proven to be a natural supply of therapeutic materials in terms of health improvement as well as in the prevention and treatment of diseases (Mamta *et al.*, 2013). This study investigated the ameliorative effect of *Psidium guajava* aqueous leaf extract (PGALE) on the histology of the hippocampus in nicotine induced neurotoxicity.

Subcutaneous administration of Nicotine caused severe neurodegenerative injuries on the histology of the hippocampus in untreated animal groups characterized by marked degeneration of hippocampal neurons, widespread cytoplasmic vacuolations, decreased cellular population and pyknosis of pyramidal cells (Figure B). This degenerative ability of Nicotine has been documented by previous studies and is linked to the oxidative stress induced by Nicotine treatment on the body tissues (Chowdury and Walker, 2008; Kadir *et al.*, 2015; Zeid *et al.*, 2018)

Treatment with the PCALE and Vitamin E ameliorated the effects of nicotine administration on the histology of the hippocampus as they demonstrated only mild to moderate degenerative injuries relative to the untreated groups with severe complications.

As seen in figure D and E, treatment with 400 and 600mg/kg of PCALE demonstrated a near normal microstructural appearance of the hippocampus. This can also be linked to the antioxidant properties of the plant as documented by previous studies which stating that *Psidium guajava* contain increased levels of antioxidant substances including vitamin A and C (Khan and Ahmad, 1985; Das, 2011). This study also advocates that this mild to moderate prevention of hippocampal damage can be improved with increased dosage. Iliyasu *et al.*, (2015) recorded that 1000mg/kg of PCALE for 14 days demonstrated strong ameliorative capacity on the hippocampal histology after lead acetate induced neurotoxicity. Meanwhile, vitamin E is known to be a very potent antioxidant and has been documented to reverse nicotine-induced oxidative stress within tissues (Mohamed *et al.*, 2010).

4. CONCLUSION

In recent times, products that contain Nicotine are largely consumed for their psychoactive abilities and have demonstrated to be addictive. This study, however, displayed the severe neurodegenerative complications of nicotine administration on the histology of the hippocampus. Treatment with *Psidium guajava* demonstrated strong dose-dependent ameliorative potentials on the histology of the hippocampus after nicotine-induced neurotoxicity.

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