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Doi: doi.org/10.4314/jeca.v22i1.19

Original Article

Effect of methanol leaf extract of *Nicotiana tabacum* (tobacco) on long-term memory in Wistar rats

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ABSTRACT

Background and aim: The dentate gyrus, an important part of hippocampal formation responsible for the formation of episodic memory as well as exploration of new environments. *Nicotiana tabacum* is a neurotoxic plant but its effects on long-term memory have not been fully explored. This study assessed the effects of Methanol Leaf Extract of *Nicotiana tabacum* (MLNT) on long-term memory using elevated plus maze (EPM) and step-down inhibitory apparatus (SDIA).

Methodology: Twenty male rats were divided into four groups of five animals each. Group A was the control which was administered with distilled water. Groups B to D were treated with 150, 300 and 600 mg/kg body weight of MLNT respectively. Treatment was done orally for twenty-eight days, following which animals were exposed to the EPM and SDIA. At the end of the experiment, the animals were sacrificed. The brain was dissected and fixed in Bouin's fluid for histochemical routine. The data obtained for behavioral studies were analyzed using Graph Pad Prism Version 20.

Results: The results obtained for both EPM AND SDIA showed no significant difference in the MLNT treated groups when compared to the control. However, histochemical analysis of the dentate gyrus revealed neurodegeneration in all treated groups when compared to the control.

Conclusion: The outcomes of the study revealed that MLNT is a potential neurotoxicant through the histoarchitectural distortion of the dentate gyrus of the Wistar rats.

Keywords:
Dentate gyrus; Neurotoxic; Long-term memory; Elevated Plus Maze; Step-Down Inhibitory Apparatus

Submitted: 22nd July, 2024
Revised: 25th March, 2025
Accepted: 29th March, 2025
Published: 31st March, 2025

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INTRODUCTION

The dentate gyrus is an integral region of hippocampal formation which is responsible for the formation of episodic memory as well as exploration of new environments. It is, therefore, part of the system that gives us autobiographical and episodic memory (Shahab, 2023). Eposidic memory refers to as the memory for an event that holds spatio-temporal relations (Tulving, 1983). Long-term memory is conceptually, the process by which events, skill, procedures and concepts are stored indefinitely in the mind. That is not to say forgetting is impossible, just that there is no precisely defined point when that will happen (Greene, 1987). The model most people are familiar with involves the movement of concepts from short term or working memory into long term memory with rehearsal or practice (Atkinson and Shiffrin 1968). It is thought that information moving through short term memory is encoded into long term memory through a process called synaptic consolidation which leads to the formation of a permanent change in the brain called Engram (Dudai, 2004; Liu *et al*., 2012). An engram also refers to as the enduring offline physical or chemical changes (presumably in the neural tissue) that were elicited by learning and underlie the newly formed memory in the brain (Josselyn, 2020).

Nicotiana tabacum is the largest genus of tobacco plant *Nicotiana* consist of over sixty species in the Solanaceae family (Charlton, 2004). It is a double-edged sword plant. Tobacco smoke causes many harmful disease to the body, while researches on *Nicotiana tabacum* leaves extract show that tobacco has many medical uses, as antibacterial, anti-inflammatory, antioxidant, cardiovascular effect and anticancer (Aminata *et al*., 2012; Zaidi *et al*., 2012; Zhou and Lin,2006). The ethnomedical uses include the use of the decoction of leaves as antispasmodics, diuretics, emetics, expectorants, sedatives, and in rheumatic swellings, anesthetics, antibacterial, antimicrobial, anthelmintic, anticonvulsants and for

How to cite this article: Wahab A.O., Ochai J., Yusha'u Y., Adeyemi S., Muhammad S.B., Okunlola M.A., Ezekiel A.S., Ugbir S.I., Omoniyi A.A., Abubakar S.M. and Quawiyah A.O. Effect of methanol leaf extract of *Nicotiana tabacum* (tobacco) on long-term memory in Wistar rats. *J Exp Clin Anat* 2025; 22(1):141-147.
https://dx.doi.org/10.4314/jeca.v22i1.19

anti-fungal activities. Tobacco has also been used for the treatment of asthma by Indians, treatment of worms in East Africa, treatment of wounds in Columbia and treatment of dysmenorrheal in Cuba among others. The plant *Nicotiana tabacum* have great activities on peripheral nervous system, central nervous system, cardiovascular system, gastrointestinal tract, exocrine glands, haematopoietic system, algesia, Alzheimers and on body weight (Kishore, 2014).

Tobacco leaves consist of many organic alkaloid compounds which found in all plant parts. The organic compounds exist in tobacco are Nicotine, Nicotinine, Nicotelline, Nornicotine, Nicotyrine, resin, myosmine, albumin and glycoprotein. Tobacco leaves contain solanesol, a long chain terpenoids alcohol (Zhou and Lin, 2006), polyphenols and carotinoids (Leffingwell, 1999), tocotrienols (Matringe *et al*., 2008). Rodu and Ou, (2000), demonstrated that tobacco products had a range of antioxidant activity from moderate to high, a significant linear relationship was observed between the overall antioxidant capacity and the total phenolic content. Jiang *et al*., (2001) founds that chlorogenic compound in tobacco leaves induces cytotoxic effect of cancer formation. The high antioxidant explains why low cancer risk is associated with long term use of these products. Therefore, the aim of the study was to investigate the effect of MLNT on long-term memory in Wistar rats.

MATERIALS AND METHODS

Ethical Approval

The approval for this research was given by Ahmadu Bello University Ethical Review Committee with approval number ABUCAUC/2021/120. This research was conducted in accordance to the National Institute of Health Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Plant Material

Fresh *Nicotiana tabacum* (tobacco) leaves were obtained from a local farm in Zaria, Kaduna State, Nigeria and authenticated and deposited in the Herbarium Unit of Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria with a Voucher Specimen Number of ABU 054.

Plant Extract Preparation

The preparation of methanol leaf extract of *Nicotiana tabacum* was conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The method of maceration as reported by Wahab *et al*., 2023 was adopted.

Briefly, the leaves were air dried in an enclosed environment and pulverized using laboratory mortar and pestle. A total of 600 g of the powdered material was soaked in absolute methanol and was allowed to stand for a period of eight days after which the suspension was filtered and funnel. The filtrate was poured into evaporating dish which was allowed to stand for three days so as to allow the solvent to evaporate. The percentage yield obtained was 16.66%.

Experimental Animals

A total of twenty apparently healthy male Wistar rats (150 to 200 g) were obtained from Animal House of the Department of Pharmacology and Therapeutic, Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria and housed in plastic cages (40cm x 35 cm) of five Wistar rats each. The Wistar rats were acclimatized for two (2) weeks prior to the commencement of the experiments. They were fed with grower pelletized feed and the rats were provided with tap water *ad libitum*.

Drugs

Tween 80 manufactured by Hail hang Industry, China was obtained from Department of Human Anatomy, Ahmadu Bello University, Zaria and was used for dissolution of MLNT.

Ketamine manufactured by Tag pharmaceutical limited, India was obtained from reputable pharmaceutical shop at Zaria and was used as an anesthesia.

Experimental Design

A total of twenty Wistar rats were divided into four groups of five animals each. Group A was the 2ml/kg of distilled water which served as vehicle for dissolution of MLNT. Groups B to D were treated with 150, 300 and 600 mg/kg body weight of MLNT respectively. The administration was done orally for twenty eight days

Elevated Plus Maze

Elevated plus maze test is used to evaluate anxiety related spatial memory (Itol *et al*., 1990). The elevated plus maze consists of four arms at right angles to each other as described by Handley and Mithani, 1984. The two open arms lie across from each other measuring 25 x 5 x5 cm and perpendicular to two closed arms measuring 25 x 5 x 16 cm with a center platform (5 x 5 x 0.5 cm). The closed arms have a high wall (16 cm) to enclose the arms whereas the open arms have no side wall. The platform and the floor were made from wood and the lateral walls of closed arms arm was made of wood painted black. The maze was elevated 38 cm above the floor. On the first day (training), each Wistar rat was placed at the end of one open arm, facing away from the central platform. The latency of the Wistar rat to move from one open arm to the enclosed arms was recorded within 90s. Following entry into the arm, the mice were allowed to explore the apparatus for 30s. Twenty-four hours later, the second trial (retention test) was performed and the rat was observed for ninety seconds. After each trial, the maze was cleaned with a cotton wool dipped in 70 % ethyl ethanol to remove any olfactory odour.

Step-down Inhibitory Apparatus

Step-down inhibitory apparatus is used to evaluate aversive memory and learning. The step-down inhibitory apparatus

consists of a 50 x 25 x 30 cm poly (methyacrylate) box as described by Borba-Filho *et al* (2015). This box has a 48 x 30 cm transparent acrylic window, a 5 cm- high, 12 cm-wide and 25 cm-long platform on the left facing a grid of a series of 20 pairs of stain steel bars (2mm diameter) spaced 2mm with bars and each pair spaced 1 cm apart. Each pair of bars makes an electrical dipole where the polarity inverted after each pulse. All the bars have insulating layer on the sides and, bottom. In the first phase which represent learning, an electric current of 0.35mA at frequency of 62 Hz was used. Each animal was gently placed on the platform facing the rear left corner of the inhibitory avoidance apparatus (training session). When the animal step down on the grid with the four paws, it received the foot shock (stimulus) for three seconds. As soon as the animal received the foot shock, it run to the escape platform and the animal was allowed to rest on the platform. After twenty four hours (test session) the second trials (test for memory) was done and each animal is placed on the platform again. In this session no shock was applied when the animal step down on the grid. A scores is taken which represent as the number of times at which the animal stepdown from the plat form to the grid is recorded in both training as an index of cognition. The step down latencies were cut-off at sixty second in the training session and one hundred and eighty seconds in the test session.

Animal sacrifice

At the end of the, the experiment, rats from each group were humanly sacrificed using ketamine and whole brain tissue was fixed in Bouin's fluid for histochemical analyses.

Statistical Analysis

All neurobehavioral data were analyzed with one way analysis of variance (ANOVA) followed by Tukey's *post hoc* tests using Graph Pad Prism Version 8.0.2 carried out to determine the source of a significant effect. Results were expressed as Mean ± S.E.M., $p<0.05$ was taken as level of significant difference from control.

Tissue Processing

The fixed brain tissues were removed from the Bouin's fluid and dehydrated using ascending grades of alcohol. The dehydrated tissues were then cleared in two changes chloroform for two hours, the clearing helped in removing opacity from the dehydrated tissues thereby making them transparent. The cleared tissues were then infiltrated by immersion in molten paraffin wax and allowed to solidify. The embedded tissues were blocked in a rectangular block and then sagittal sections were cut using rotary microtome at five micrometer per section. The tissue sections were allowed to float in water bath at three hundred degree census to help the spreading of the paraffin ribbons. The clean slides were used to pick the tissue from warm water bath (Drury *et al*., 1967).

Cresyl Fast Violet Staining

The tissue sections were deparaffinized and hydrated to distilled water and then stained for 5 minutes in Cresyl violet solution. The stained sections were rinsed in two changes of distilled water and placed in 95% alcohol for 30 seconds. Sections were transferred to absolute alcohol for 30 seconds and then placed in xylene for 1 minute and 2 minutes sequentially. Differentiations were made in absolute alcohol, two changes for 10 and 30 seconds each. The sections were then taken through several change of xylene and mounted with synthetic resin (Drury *et al*., 1967). Sections photomichrographs were taking using digital camera Amscope (MD 900) fitted to light microscope (Leica Microsystem Inc. Tokyo, Japan).

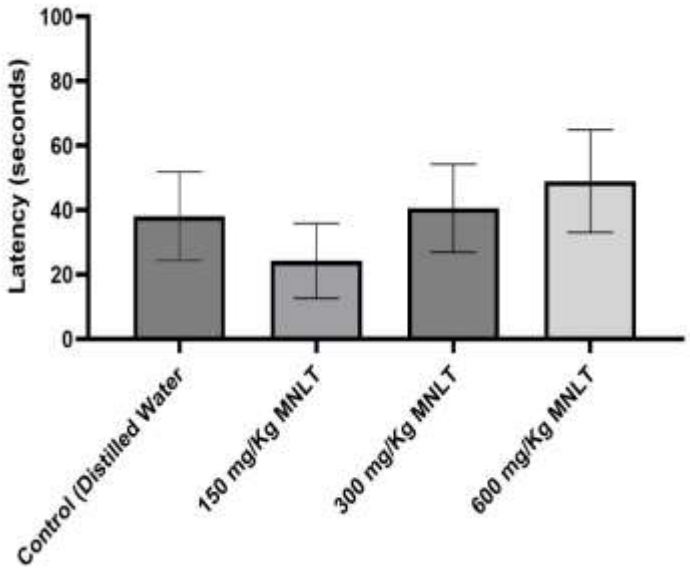

Figure 1: Effect of MLNT on the Latency in second of the Acquisition Phase of the Wistar rats. n= 5; mean± SEM, one-way ANOVA, *Tukey's post hoc* test (MLNT= Methanol Leaf Extract of *Nicotiana tabacum*)

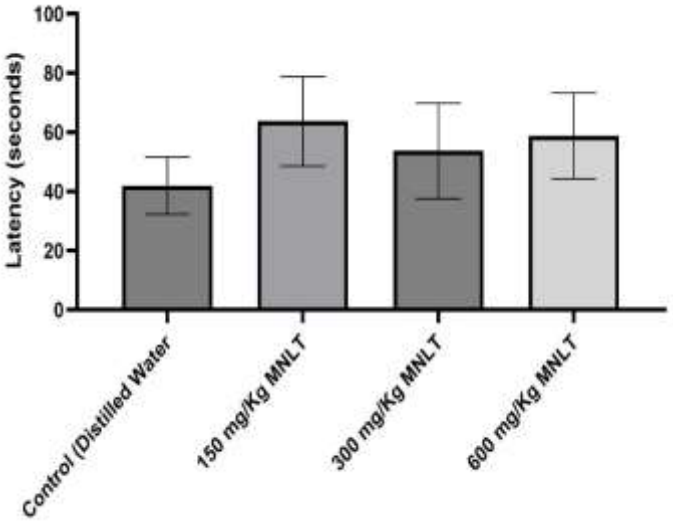

Figure 2: Effect of MLNT on the Latency in seconds of the Retension Phase of the Wistar rats. n= 5; mean± SEM, one-way ANOVA, *Tukey's post hoc* test (MLNT= Methanol Leaf Extract of *Nicotiana tabacum*)

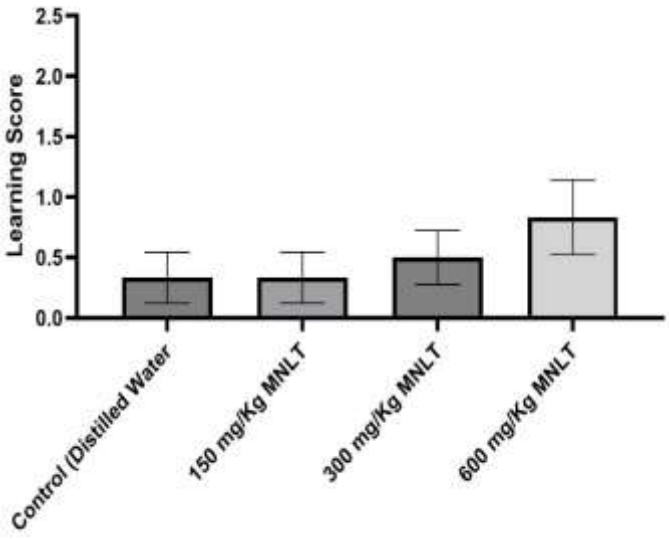

Figure 3: Effect of MLNT on learning score in SDIA of the Wistar rats. n= 5; mean± SEM, one-way ANOVA *Tukey's post hoc* test. (MLNT= Methanol Leaf Extract of *Nicotiana tabacum*, SDIA= Step-Down Inhibitory Apparatus).

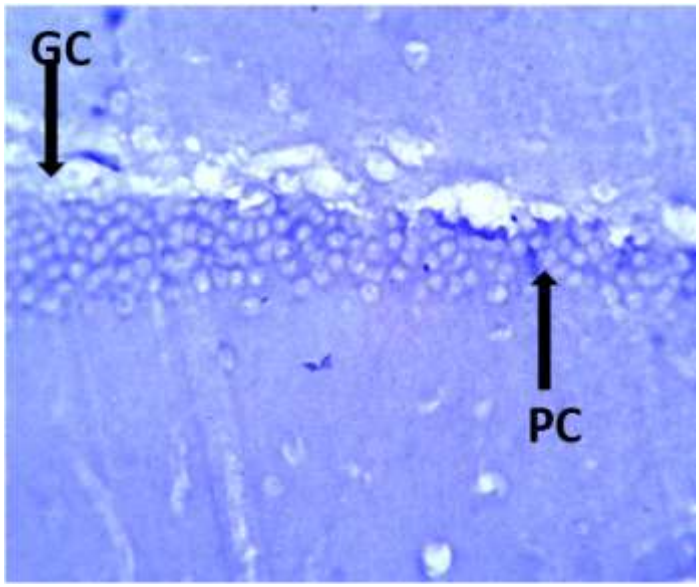

Figure 5a: Sections of dentate gyrus region of control group of Wistar rat stain with cresyl fast violet (x250). GC (Granular Cell); PC (Pyramidal Cell)

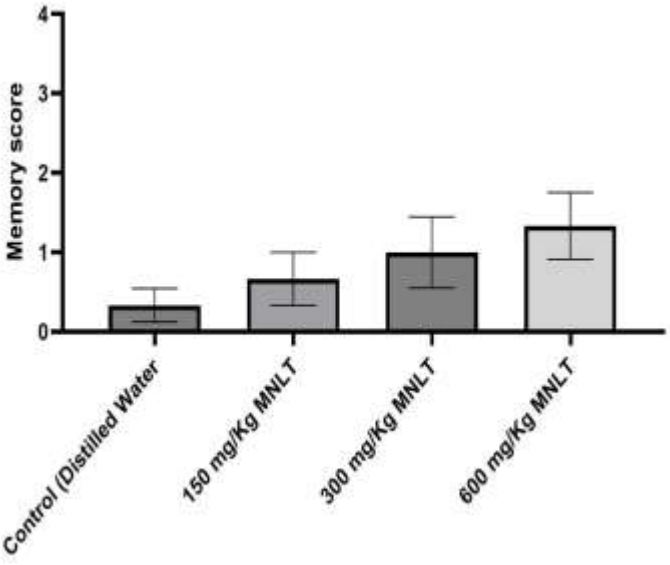

Figure 4: Effect of MLNT on memory score in SDIA of the Wistar rats. n= 5; mean± SEM, one-way ANOVA *Tukey's post hoc* (MLNT= Methanol Leaf Extract of *Nicotiana tabacum*, SDIA= Step-Down Inhibitory Apparatus).

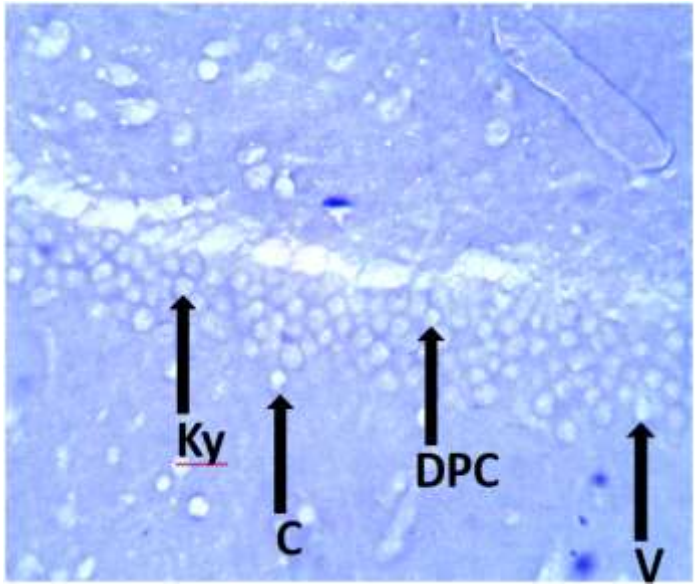

Figure 5b: Sections of dentate gyrus region of Wistar rat administered with 150 mg/kg body weight of MLNT stain with cresyl fast violet (x250). Ky (Karyolysis); C (Chromatolysis); V(Vacuolation) and DPC (Degenerating Pyramidal Cell)

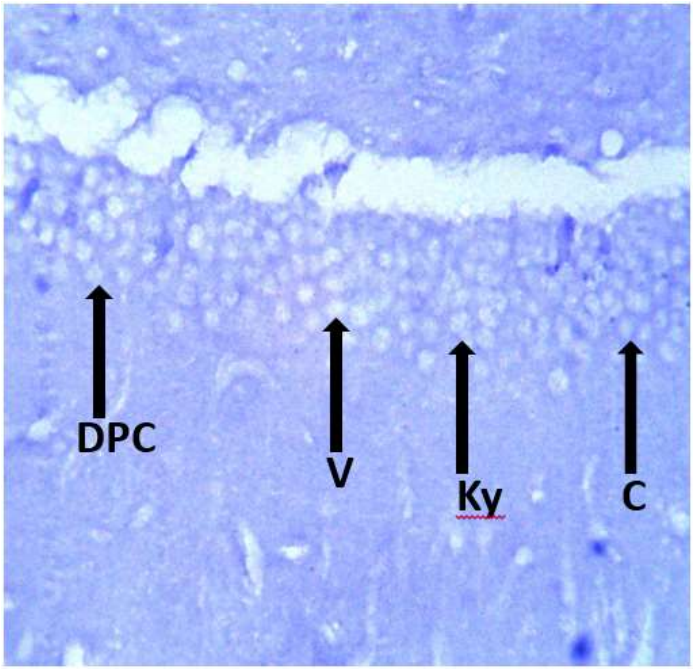

Figure 5c: Sections of dentate gyrus region of Wistar rat administered with 300 mg/kg body weight of MLNT stain with cresyl fast violet (x250). DPC (Degenerating Pyramidal cell); C (Chromatolysis); V (Vacuolation) and Ky (Karyolysis).

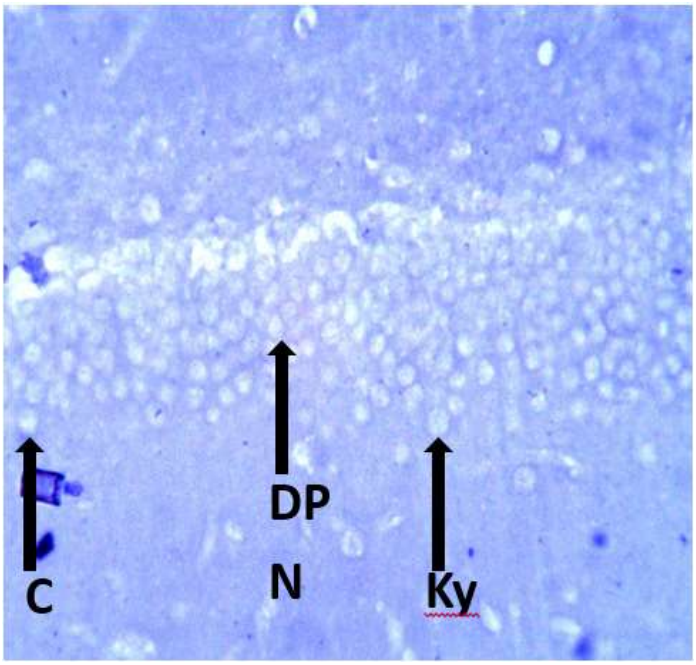

Figure 5d: Sections of dentate gyrus region of Wistar rat administered with 600 mg/kg body weight of MLNT stain with cresyl fast violet (250x). DPN (Degenerating Pyramidal Neuron); C (Chromatolysis); Ky (Karyolysis)

RESULTS

Neurobehavioral study:

There was no significant difference in latency in seconds in the acquisition phase in all treated groups when compared with the control (Figure 1). There is also no significant difference in latency in seconds in the retention phase in all treated groups when compared with the control (Figure 2).

Furthermore there was no significant difference in the memory score in all treated groups when compared to the control (Figure 3) and there is also no significant difference in the learning score in all treated groups when compared to the control (Figure 4).

Histochemical studies:

Sections of the dentate gyrus of Wistar rat of the control group showed normal histoarchitectural features with well-preserved pyramidal cell and granular cell (Figure 5a). Section of Wistar rat administered with 150 mg/kg body weight of MLNT showed a neurodengenerative changes such as karyolysis, degenerating pyramidal cell, vacuolation and chromatolysis when compared with the control (Figure 5b). Section of Wistar rat administered with 300 mg/kg body weight of MLNT showed a neurodengenerative changes such as degenerating pyramidal cell, vacuolation, karyolysis and chromatolysis when compared with the control (Figure 5c). Section of Wistar rat administered with 600 mg/kg MLNT showed a neurodengenerative changes such as degenerating pyramidal cell, karyolysis and chromatolysis when compared with the control (Figure 5d).

DISCUSSION

Nicotiana tabacum (tobacco) plant has been reported to have several medicinal properties but chronic consumption may lead to various forms of neurodegenerative pathologies as a result of its high alkaloid contents, nicotine (Shekins *et al*., 2016). As a correlative test for neuropathological changes within the brain, we assessed long-term memory by using elevated plus maze and step-down inhibitory apparatus in Wistar rats. During acquisition and retention phase in EPM, no significant difference was seen among the groups. This is in agreement with the finding of Itoh *et* al. (1990) who reported that, animal in the EPM escape from the open arm to the enclosed arm because of fear and anxiety as a result of their fear of open arm and elevated places. Also, Muhammad *et al*., (2021) reported there is no significant difference in shisha smoke inhalation mices exposed to EPM when compared with the control. SDIA incorporates critical element of contextual discrimination as an episode for the assessment of episodic like-memory (Atuche and Roozendaal, 2015) in which Wistar rats were tested by different mnemonics processes such as extinction, learning, safety learning or reconsolidation that might all require memory consolidation (Cammarota *et al*., 2004; Alberini, 2011). Findings from this study show no significant difference in both learning and memory score in MLNT-treated groups when compared to the control.

Histoarchitecture of dentate gyrus is important because of the particular vulnerability of the neurons to the toxic event (Seidman, 2011). Observed histochemical assessment revealed neuronal degeneration characterized by chromatolysis, degenerating pyramidal cell, vacuolation and karyolysis. These neurodegenerative changes are due to the neurotoxin effect of the extract in the brain under consideration. Finding from this study are in agreement with Adeniyi and Musa 2011; Adeniyi and Ogundele 2014; Wahab *et al*., 2023; Wahab *et al*., 2024.

Neuronal degeneration has been reported to result in cell death (Faust *et al*., 2009) which might due to extrinsic or intrinsic insults (Natale *et al*., 2004). Rough endoplasmic reticulum and free ribosomes appear under a light microscope as basophilic granular areas called Nissl bodies with cresyl fast violet stain. Histochemical assessment showed a reduced staining intensity in MLNT treated groups which are an indicator of a reduced protein synthesis by the rough endoplasmic reticulum in the cells.

Degenerating pyramidal neuron and chromatolysis are histopathologically characterized by the neuronal atrophy (Mena *et al*., 2004). Neuronal atrophy, a descriptive term given to wide variety of irreversible neuronal injuries resulting in slow cell death which occur in many degenerative disorder (Seilhean *etal*., 2004; Susan, 2007). Feature of which are neuronal cytoplasmic shrinkage, disappearance of Nissl bodies and intense eosinophilia (Mena *et al*., 2004; Seilhean *et al.,* 2004).

Conclusion: The outcomes of the study revealed that MLNT could acts as a potential neurotoxicant through the histoarchitectural distortion of the dentate gyrus in the brain of Wistar rats which might affect the neurocongitive change at later stage.

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