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ORIGINAL ARTICLE

Moringa Regimen Corrects Nicotine-induced Deficits in Behaviour, Altered Energy Metabolism and Neurotransmitter Processing in Rat Brain

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Abstract:

Background: Nicotine is the addictive component of tobacco smoking. It has been reported to have a negative neuromodulatory role in the CNS. *Moringa oleifera* is a medicinal plant with reported antioxidant, anticonvulsant, anti-inflammatory and neuro-protective properties. *Aim and Objectives*: This study was purposed to investigate the neuronal adaptation potentials of *Moringa Oleifera* (MO) on nicotine-induced behavioural decline and perturbed bio-energetics. *Material and Methods*: Twenty-four adult male Wistar rats were used. The treatment regimen was as follows; control group received distilled water, MO group received 200 mg/kg of MO, Nicotine Group received 1.38 mg/kg body weight of nicotine, and Nicotine + MO group received combined treatment of 200 mg/kg body weight of MO after 1.38 mg/kg body weight of nicotine for 28 days. The animals were subjected to Morris water maze for spatial memory, Y-maze for working memory and elevated-plus maze tests for anxiety levels after which they were sacrificed for spectrophotometric analysis of global protein expression, neural bioenergetics (lactate dehydrogenase and glucose-6-phosphate dehydrogenase), and Acetylcholinesterase (AChE) levels. Results: Nicotine infusion caused a reduction in the escape latency period, increased the percentage incorrect alternation, and elevated the anxiety levels of rats. These observations were indicative of decreased synaptic activity in the brain. Together with, nicotine induced chromatolytic changes in cells of the frontal cortex and hippocampus. Co-administration with MO prevented nicotine-associated memory decline, perturbed glucose bioenergetics, induced chromatolysis and histomorphological distortion in the frontal cortex and hippocampus. *Conclusion*: Our data demonstrate that MO administration enhances experience-dependent neuroplasticity and cognitive behaviour function in laboratory animals, modulates energy metabolism and reduced oxidant stress possibly through enhanced production of key antioxidant enzymes against the damaging effects of nicotine. It provided evidence that MO can be further developed as a means to protect the brain from oxidative stress-induced injury.

Keywords: Nicotine, *Moringa oleifera*, Frontal Cortex, Hippocampus, Memory

Introduction:

Smoking is hazardous to health. It exposes an individual to carcinogens, cytotoxins such as carbon monoxide and cadmium as well as a neuromodulatory molecule, nicotine, which is the major chemical component responsible for addiction in tobacco products [1]. It has been

argued that the risk of nicotine addiction depends on the dose delivered and the route of delivery [2]. The cessation of smoking in addicts, results in withdrawal symptoms caused by nicotine dependence. Experimental data have shown that nicotine has neuromodulatory effect on both cognitive and behavioural functions, some of which are positive [3-4] while others are negative [5- 7]. In fact, there are data stating that nicotine has no significant effect on the central nervous system [8]. One notable controversy on nicotine in literature is the effect that it has on reactive oxygen and nitrogen species [7, 9]. 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 [7]. Another study also found that nicotine compromises the intrinsic antioxidant enzyme activity and increases lipid peroxidation [10]. Keeping in mind the role that nicotine plays in creating dependence and addiction in tobacco smokers, it has been used in the form of nicotine replacement therapy to help manage withdrawal symptoms in individuals that are sober from smoking. Due to the addictive nature of nicotine-containing products, nicotine has become a substance of abuse. The capacity of substances of abuse to interact with and modulate the biochemical activities in the areas of the brain associated with learning may underwrite to the robust addictive characteristics of these drugs. Moreover, drugs of abuse may perhaps have an expressly strong impact on the declarative memory system which is responsible for recollection of facts and events [11]. Brain areas involved in declarative memory includes the prefrontal cortex and hippocampus [11, 12]. These areas are also involved in addiction [13, 14].

Several experimental studies have shown the potential of plant extracts in protection against neurotoxicity through activation or boosting intrinsic antioxidant molecules such as catalase, superoxide dismutase, and glutathione reductase enzymes, which diminish the cellular concentration of free radicals and subsequent apoptosis signaling [7, 15]. The variety of neuroprotective mechanisms of natural plant extracts may allow the discovery of more efficient therapies in the management of neurotoxicity and neurodegeneration. One such medicinal plant with neuroprotective properties is *Moringa oleifera* (MO). Growing in many tropical areas and commonly known as "drumstick", MO is the most distributed species in the genus *Moringa* [16]. Different parts of MO plant have been shown to exert many beneficial activities, including anti-inflammatory, antioxidant and neuroprotective effects [7, 17, 18]. Phytochemicals present in the leaves of MO include tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones, alkaloids, niazimicin, moringin and reducing sugars [19, 20]. The essential oil of MO has been found to contain flavonoids quercetin and luteolin [21]. The neuroprotective potentials of MO can be attributed to its ability to decrease oxidative stress and the enhancement of cholinergic function, as research has shown it to reduce Malondialdehyde (MDA) and Acetylcholinesterase (AChE) levels, and cause a corresponding increase in Superoxide Dismutase (SOD) and Catalase (CAT) [22]. It has also been said that MO acts as a free radical scavenger in the central nervous system, thereby boosting the intrinsic antioxidant system [23]. This study aimed to characterize the neuroprotective roles of MO in nicotine-mediated behavioural and histopathological deficits in the prefrontal cortex and hippocampus of Wistar rats.

Material and Methods:

Laboratory Animals and Care:

Twenty-four adult male Wistar rats were used for this study. Ethical approval was sought and obtained from the ethical committee of the College of Health Sciences, University of Ilorin. Wistar rats were housed in a wire-gauzed cage at the animal holding facility of the Faculty of Basic Medical Sciences, University of Ilorin. The animals were allowed to acclimatize for two weeks prior to the commencement of the study.

Preparation of Treatment Solutions:

MO leaves were procured from a local vendor in Ilorin, Nigeria and authenticated by the Department of Plant Biology, Faculty of Sciences, University of Ilorin. The leaves were dried, weighed, pulverized and soaked in distilled water at 100°c for 10 minutes. After cooling overnight, the extract was filtered over Whatmann No. 1 filter paper. The resultant filtrate was dried in a water bath at 40°C for 96 hours to get the concentrate which was then diluted to stock. The extract was administered via the oral route. Proper volume was ensured through the use of a calibrated syringe fitted with an oral cannula. 95% nicotine was obtained from the British Drug House Chemical Ltd., Poole, England.

Treatment of Animals:

The animals were randomly divided into four groups A-D of six animals each. Group A received 1 ml of distilled water orally, Group B was administered with 200 mg/kg aqueous extract of MO orally, Group C was administered 1.38 mg/kg nicotine bitartrate intraperitoneally (IP), while Group D was concomitantly administered 200 mg/kg MO orally and 1.38 mg/kg nicotine bitartrate (IP); all the groups were treated for 28 consecutive days. The dose of nicotine and MO were selected according to the methods of Lv *et al*. [24] and Karthivashan *et al.* [25] respectively.

Neurobehavioural Study:

The animals were subjected to three neuro-behavioural paradigms: Morris water maze, Y-maze and elevated plus maze to assay for spatial memory, working memory and anxiety in the experimental animals.

Morris Water Maze:

This test was carried out to assay for the spatial learning and memory of the rats. A pool of water measuring about 100 cm in diameter and 30 cm in depth was used. An escape platform about an inch deep from the surface of the water was placed in one of the quadrants outside of which were visual cues. The animals were trained 24 h prior the actual test. During the training, each rat was placed in each of the other three quadrants for a maximum of 60 sec to find the escape platform at an interval of 15 min between quadrants until the escape latency period reduced to less than 15 sec. During the test, the pool was coloured and the animals were placed in each of the three quadrants different from the escape platform quadrant at an interval of 15 min between quadrants. The time it took to find the escape platform was recorded as the escape latency period.

Y-Maze:

This test was used to examine for the working memory of the rats. The animals were placed in a Y-maze which arms measured 75 cm in length and 15 cm in breath with an angle of 120° in between arms. The animals were allowed to explore the maze for duration of 5 min. The manner of alternation was recorded. The percentage correct

alternation of each rat was estimated as a ratio of the correct alternation to the total alternation multiplied by 100.

Elevated Plus Maze:

This test was conducted to evaluate the rats' anxiety level. The animals were introduced into an elevated plus apparatus that stood 45 cm tall with two open arms and two closed arms for a b exploration time of five minutes. The open arm duration was estimated as total time the rats spent in the open arm of the elevated plus maze for the duration of the test.

Tissue Processing:

Twenty four hours after the last treatment, rats for histochemical studies were euthanized by intramuscular injection of 25 mg/kg of ketamine and subjected to transcardial perfusion during which a flush of 50 ml of normal saline was followed by 500 ml of 4% Paraformaldehyde (PFA). The brain tissues were thereafter excised and post-fixed in 4% PFA for 48 hours and then processed for histochemistry. Rats processed for enzymatic studies were sacrificed by cervical dislocation (to eliminate interference of anesthetic agent with biochemical redox); the brains were then excised, rinsed in 0.25 M sucrose 3 times for 5 minutes each and stored in 30% sucrose at 4°C. PFA-fixed tissue sections were stained using Cresyl Fast Violet (CFV) staining techniques.

Colourimetric Assay for Enzyme Analysis:

Enzymatic assay for Glucose-6 Phosphatase Dehydrogenase (G6PDH), Lactate Dehydrogenase (LDH), Acetylcholinesterase (AChE) and total protein was quantified in the brain tissues of rats using the spectrophotometric technique at the central research lab, Kwara state. The assay kit of the aforementioned proteins was procured from Abcam, USA. Equal weighing brain tissues were homogenized in 0.25 sucrose at 40º C. The tissue homogenate was centrifuged for 15 min at 5000 rpm, after which the supernatant containing tissue lysate was aspirated. The activities of G6PDH, LDH, AChE and total protein were assayed for according to the manufacturer's instruction in the assay kit pack.

Photomicrography and Statistical Analysis:

The photomicrograph of the hippocampus was obtained using an Amscope microscope camera attached to an electrical light microscope. The data obtained from the neurobehavioural and enzymatic assay were subjected to statistical analysis using Graph-pad Prism (version 6). The values were plotted in ANOVA with Tukey's multiple comparison tests. Data obtained were presented as Mean ± Standard Error of Mean (SEM) with the determination of the level of significance of p-value less than 0.05, 0.01 and 0.005. The results obtained were represented in bar charts with error bars to show the mean and standard error of mean respectively.

Results:

Effect of Nicotine and MO on Spatial and Working Memory:

In the present study, both short and long-term memory of experimental animals were quantified using the Y-maze (working memory) and Morris water maze (spatial memory) respectively. Morris water maze is a stress-induced neurobehavioural paradigm as animals are expected to find the escape platform in the pool to avoid drowning. The time it takes to find this escape platform is referred to as the escape latency period. In the present study, the escape latency period of animals treated with nicotine only was significantly higher

than the control and MO treated animals ($p < 0.005$ for both). This finding suggests a perturbation in the long-term memory of experimental animal as induced by nicotine. Notably, there is no significant difference in the escape latency period of animals that were concomitantly treated with nicotine and MO when compared to the control and MO only group, suggesting the role that MO plays in preventing nicotine memory depleting action. Comparing the nicotine only animals and the co-treated with nicotine and MO animals, it can be observed that the escape latency period in the nicotine plus MO treated rats was significantly lower than that of the nicotine only group ($p > 0.05$). A similar trend was observed in the paradigm used to explore the short-term memory of the Wistar rats. The Y-maze test works with the tendency of a rat to explore each of the three arms of the maze without returning to a recently explored arm, hence the term working memory. The percentage of correct alternation is the index used to quantify the performance of the animals in this test. In the present study, animals treated with nicotine only had a significantly lower percentage correct alternation when compared to the control and MO treated rats ($p < 0.01$). These findings suggest that nicotine-induced a reduction in the working memory of experimental animals. Co-treatment of nicotine and MO resulted into a significantly lower correct alternation relative to the control ($p<0.05$). It is, however, important to note that nicotine plus MO group had a higher percentage correct alternation compared to the nicotine only ($p>0.05$) suggesting the role that MO plays in preventing a depletion in the memory index of the experimental animal.

MO Counteracts the Anxiogenic Effect of Nicotine:

To quantify the effects of MO and nicotine on anxiety, it was subjected the rats to elevated plus maze. This maze tests the tendency of the animal to explore either of the open arm or the closed arm of the maze. Exploring the open arm more than the closed arm connotes that the animal has low anxiety levels, whereas exploring the closed arm more than the open arm suggests that the animal has anxiety. The two parameters that were scored in this test were the open arm entry and the open arm duration; compared to the control and MO treated animals, nicotine only had a significantly lower open arm entry ($p<0.005$). Interestingly, animals co-treated with both nicotine and MO also had a significantly higher open arm entry than the nicotine only animals ($p<0.005$). Similarly, nicotine-treated animals presented with a significantly lower open arm duration relative to control ($p<0.01$), MO ($p<0.01$) and nicotine plus MO ($p>0.05$). These findings suggest that nicotine is anxiogenic when given to the animals alone. This anxiogenic effect of nicotine was counteracted by the actions of MO in the animals that were concomitantly treated with both nicotine and MO.

Counterbalancing Role of MO on Nicotine-induced Glucose Bioenergetics Perturbation:

G6PDH and LDH are two enzymes that play a key role in the glucose bioenergetic pathway. In the present study, nicotine significantly depleted the level of G6PDH in nicotine-treated animals when compared to the MO treated rats ($p<0.05$). Relative to the control and nicotine plus MO, the G6PDH level was lower in the nicotine only group ($p>0.05$). Conversely, nicotine induced a

significant increase in LDH activity relative to the control and MO groups ($p>0.005$). The action of MO suppressed and normalized LDH activity in animals that were treated with both nicotine and MO as it was observed that the LDH level in these rats was significantly lower than those of the nicotine-treated rats ($p<0.005$).

Effects of Nicotine on Total Protein and Acetylcholinesterase (AChE) Turnovers in the Brain:

In the present study, it was assayed for the total protein in the brain of the experimental animals and it was observed that the action of MO and nicotine both independently and concomitantly caused a significant increase in total protein ($p<0.05$). AChE is an enzyme responsible for the degradation of acetylcholine at synaptic junctions thereby regulating signal transduction and nerve impulses. Nicotine depleted the level of AChE in the animals that were treated with nicotine only relative to the control ($p<0.05$ in both). MO had no effect on AChE activity as it was observed that there was no significant difference in the AChE level between the control and MO treated rats. Similarly, co-administration of nicotine and MO did not result into any significant difference in the AChE level when compared with nicotine only group. But when compared with the control and MO group, AChE level was significantly reduced in the nicotine plus MO ($p<0.05$).

MO Prevented Nicotine-induced Chromatolysis:

Thin sections of tissues were qualitatively analyzed histochemically. The Nissl substance in the neurons of the frontal cortex and hippocampus were characterized using Cresyl fast violet stain. Histomorphologically, at the high powered magnification of the external pyramidal layer of the frontal cortices of the control and MO groups, the pyramidal neurons are clearly appreciable with large cell bodies and long axons jutting out of the body. The Nissl substance of the soma is also intensively stained. In the nicotine-treated rats, the pyramidal neurons in the external pyramidal layer of the frontal cortex appeared in clusters suggesting pyknotic changes. These pyramidal neurons also presented with mild chromatolytic changes (central chromatolysis) as the soma of these cells were not properly stained in the animals that were concomitantly treated with nicotine and MO, the pyramidal cells of the external pyramidal layers appeared characteristically similar to those of the control and MO treated rats. The soma's Nissl bodies were intensively stained, and the apical and basal dendrites are conspicuous. The dentate gyrus of the four experimental groups presented with densely packed granule cells in the granular layer with proper delineation from the outer molecular layer and the internal plexiform layer. The cellular density of the granule cells in the granular layer of the dentate gyrus of the hippocampus in the nicotine only group appeared lower than the other experimental groups. Notably, the Nissl substance of the granule cells of the granular layer across the four experimental groups with no signs of chromatolysis. The *Cornuammonis* region three (CA3) of the hippocampus of the Control and MO treated animals presented with large pyramidal cells in the pyramidal layer that appeared intensively stained. The apical and basal dendrite of these cells are conspicuously adjoining the soma, the pyramidal cells in the pyramidal layer of the CA3 region of the hippocampus of rats treated with nicotine only appeared chromatolytic in nature as the soma were

not intensively stained. These chromatolytic changes were subverted by the actions of MO in the group that was co-treated with both nicotine and MO. These findings suggest that nicotine compromises the integrity of the Nissl substance in the hippocampus and frontal cortex of experimental animals thereby leading to chromatolysis, while MO prevented this kind of neuropathological changes by sustaining the Nissl substance's intactness (Fig. 3).

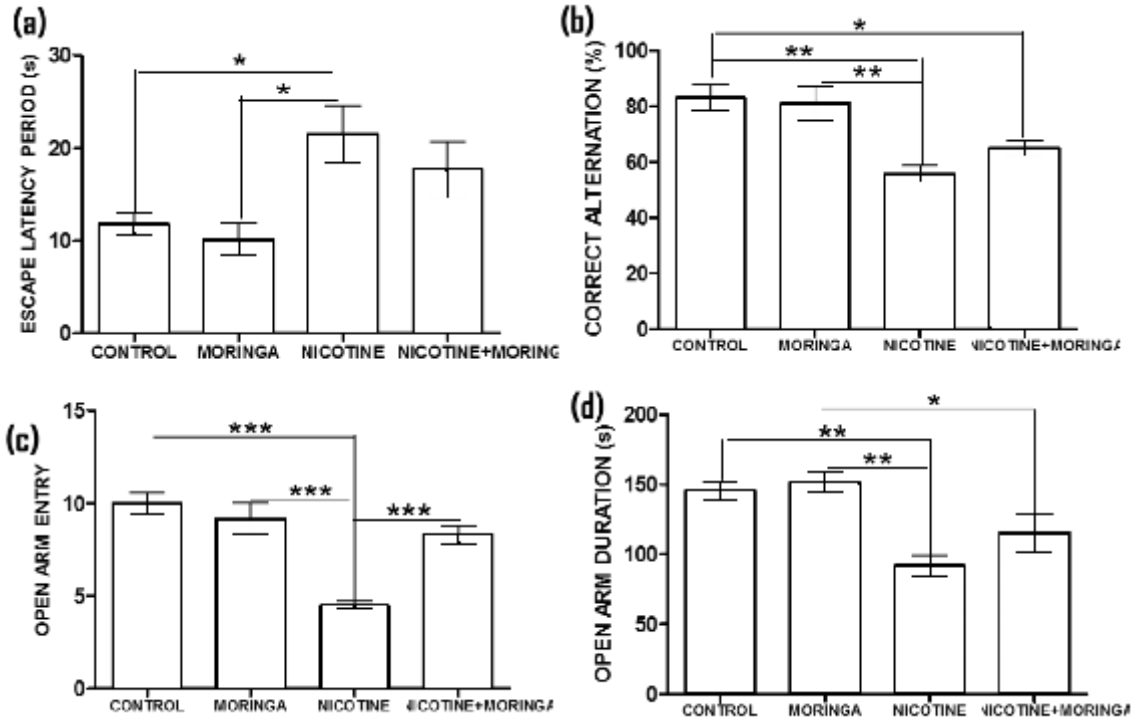

Fig. 1a-d: Performance Outcome of Experimental Animals in the Neurobehavioural Test

Fig. 1a shows the Escape Latency Period of Rats in the Morris Water Maze.

Nicotine treated animals had a significantly higher escape latency period relative to the control and in *Moringa* treated animals. There is no statistical significance between animals that were treated with nicotine only and those that were treated with both nicotine and *Moringa*.

Fig. 1b shows the Percentage of Correct Alternation in the Y-maze

Relative to the control and *Moringa* treated rats, the percentage correct alternation of nicotine-treated rats significantly reduced. Compared to the control, animals that were treated with both nicotine and *Moringa* also had a significantly correct low percentage correct alternation.

Fig. 1c and d show Activities of Animals in the Elevated plus Maze

Nicotine treated animals had a significantly lower open arm entry relative to *Moringa* control and nicotine plus *Moringa*. Similarly, the open arm duration of the nicotine-treated animals was significantly lower compared to the control and *Moringa* treated animals, while the nicotine *+Moringa* treated animals was significantly lower compared to the *Moringa* treated animals. *, ** and *** are p values less than 0.05, 0.01 and 0.005 respectively

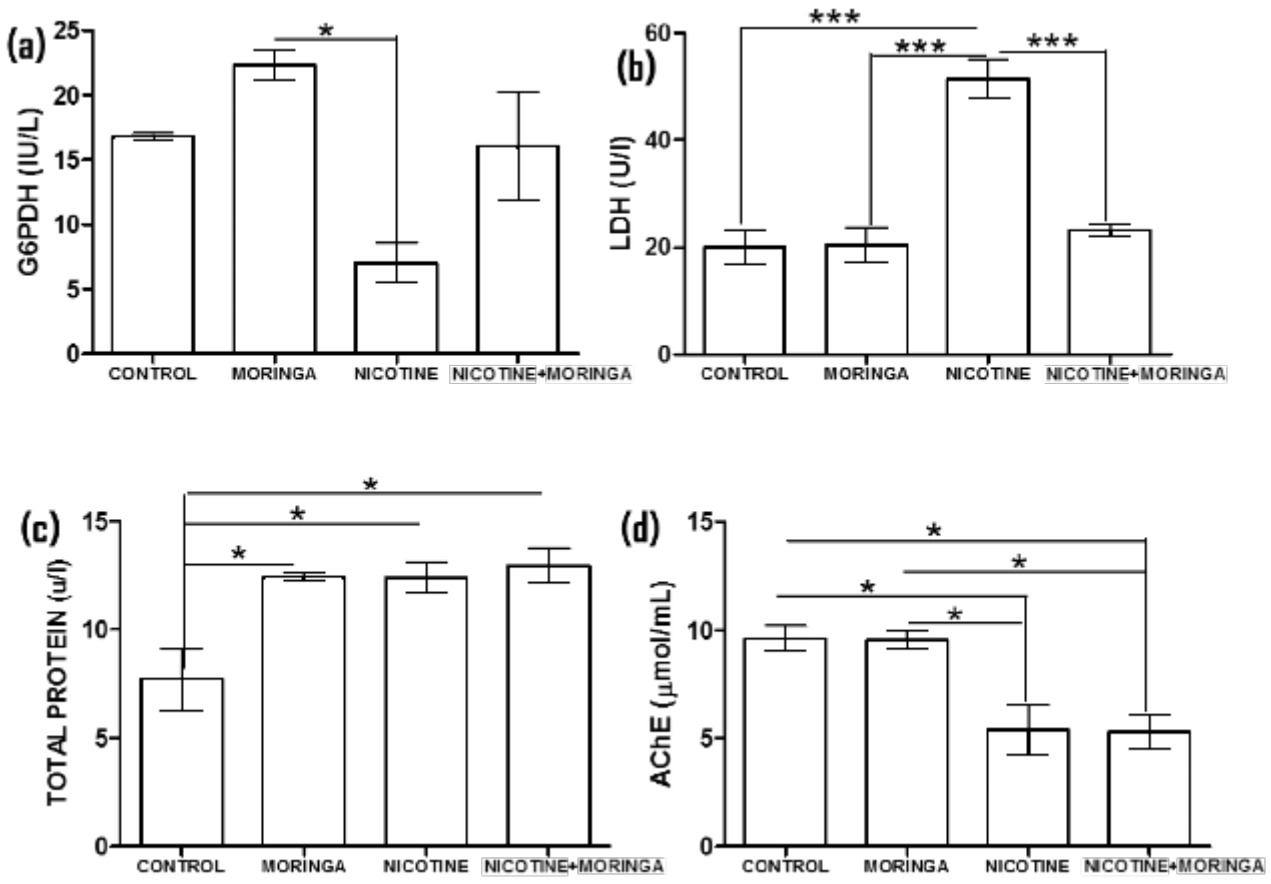

Fig. 2a-d: Brain Level of G6PDH (2a), LDH (2b), protein level (2c) and AChE (2d).
G6PDH levels are significantly reduced in nicotine-treated animals relative to *Moringa* treated rats. LDH activity significantly increases in nicotine-treated rats, relative to other experimental groups. The total protein in the tissue lysate was significantly increased in the *Moringa*, nicotine, and nicotine plus *Moringa* relative to the control. Acetylcholinesterase activity is significantly reduced in the rats that were treated with nicotine and nicotine plus *Moringa*, relative to the control and *Moringa* treated rat group. *, ** and *** are p values less than 0.05, 0.01 and 0.005 respectively.

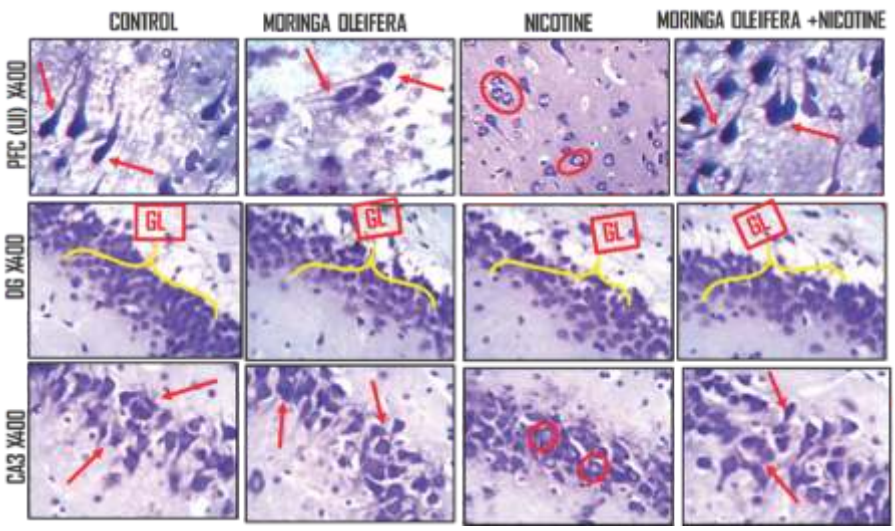

Fig. 3: Representative Photomicrographs of the Prefrontal Cortex (External Pyramidal Layer) and Hippocampus (Dentate Gyrus and CA3) of Wistar Rats at a High Power Magnification.

The external pyramidal layer of the PFC presents with pyramidal neurons possessing apical and basal dendrites and intensively stained Nissl substance in the control and *Moringa* treated animals (red arrow). Nicotine treated animals presented with large pyramidal neurons with mild chromatolytic changes (red circles). These chromatolytic neurons appear in clusters as early signs of pyknosis. There are no chromatolytic changes in animals treated with both *Moringa* and nicotine. The photomicrographs of the hippocampus show the Granular Layer (GL) of the Dentate Gyrus (DG) and pyramidal layer of the *Cornuammonis* three (CA3) region. The granule cells of the control and MO treated rats appeared densely packed with intensively stained Nissl substance, while those of the nicotine-treated animals are less dense but characteristically stained like those of the control and *Moringa*-treated rats. The granular layer of the dentate gyrus of animals treated with both nicotine and *Moringa* appear characteristically similar to those of the control and *Moringa*-treated rats. The pyramidal cells (red arrows) in the CA3 region of the control, *Moringa* and the *Moringa* + nicotine rats appeared normal with large soma with apical and basal dendrites jutting away from the body. Their Nissl substances were intensively stained, unlike, those of the nicotine-treated rats that appeared with mild chromatolytic changes (red circle).

Discussion:

The present study sought to characterize the neuroprotective role of MO in nicotine-induced neurobehavioural perturbation, glucose bioenergetics pathway and histomorphological changes. Nicotine has been recorded to have both positive and negative neuromodulatory role in the central nervous system [3-7]. In the present study, nicotine caused a reduction in long and short-term memory which was inferred from the significantly reduced escape latency period in the Morris water maze and increased percentage correct alternation in the Y-maze. It has been argued that nicotine enhances memory which negates the current findings in this study [26]. The animals in this study were subjected to neurobehavioural paradigms twenty-four hours after the last

administration of nicotine, a period during which nicotine would have been fully metabolized and completely degraded in the system. The behavioural outcome in the present study can be attributed to the fact that nicotine level had diminished. Co-treatment of animals with MO prevented the reduction in the long and short-term memory of the experimental animal. This suggests that MO confers a degree of protection on the pathway involved in long and short-term potentiating against nicotine memory declining activities. Studies have suggested that chronic oral treatment with MO alters electrical activity in the brain and the production of monoamines, including norepinephrine, dopamine, and serotonin, involved in memory processing thereby ameliorating cognitive functions [27].

Glucose metabolism provides the energy needed for biochemical and physiological processes in the central nervous system through the generation of ATP and neurotransmitters [28]. It was investigated that the brain level of important enzymes implicated in the glucose metabolism pathway to understand that nicotine and MO play in modulating the activities of neuronal and non-neuronal cells in the central nervous system. Quantification of G6PDH level in the brain of the experimental animals was carried out as a measure of energy generation in the metabolic pathway that supplies reduced energy to neurons by maintaining the level of coenzyme Nicotinamide Adenine Dinucleotide Phosphate (NADP). This metabolic pathway, pentose phosphate pathway is the key to maintaining the functional and structural integrity of neuronal and non-neuronal cells in the CNS and hence a disturbance of key enzymes involved in this pathway results into the compromised functioning of neurons. In the present study, the neural level of G6PDH was significantly reduced in the group that received nicotine alone when compared to the control, MO and nicotine plus MO group. A previous study has shown that reduction in the neural level of G6PDH leads to decreased energy and ribose production through the pentose phosphate pathway [29]. One possible neuro-protective mechanism of MO is through the prevention of perturbation of cortical and hippocampal glucose bioenergetics. This is shown by the normal level of G6PDH expressed by animals concomitantly treated with nicotine and MO. It was also observed that animals treated with MO only had a comparatively higher G6PDH compared to the control group. It has been shown that MO potentiates the synthesis of antioxidants such as SOD, GPx, and catalase in the CNS. Therefore, this relatively higher G6PDH in the MO treated rat is required to meet the energy demand in a bid to synthesize proteins involved in endogenous neuromodulatory pathways to sustain neuronal integrity and prevent them from oxidative damage. It can, therefore, be said that MO confers its neuroprotective capacity by upregulating energy production while it boosts the antioxidant system. It was previously shown that MO increases the level of SOD, CAT and GPx in the frontal cortex and cerebellum of Wistar rats [7, 30]. Quantification of LDH levels in brain lysates further supports our finding in the glucose bioenergetics pathway. During glycolysis, when there is a reduced supply of cellular oxygen (oxygen debt) required to convert pyruvate to acetate, lactate dehydrogenase catalyzes the conversion of pyruvate to lactate instead. In the present study, the actions of nicotine significantly increased the production of lactate dehydrogenase suggesting that nicotine might be inducing chemical hypoxia which will then result into reduced energy generation in the mitochondria with increased release of reactive oxygen species

[31]. MO was able to sustain the level of LDH in the rats that were treated with both nicotine and MO even though MO only treated rats presented with a neural LDH level similar to what is observed in the Control Group. This finding suggests that the presence of MO is able to sustain the line of supply of oxygen to the cellular and subcellular level, thereby maintaining energy production in the mitochondria. Colourimetric assay of AChE in the present study revealed that nicotine reduced the level of these enzymes in both groups that were treated with nicotine MO had no impact on the activity of AChE. It was observed that AChE level of animals treated with MO was similar to that of the control. This finding does not agree with a previous study that MO treatment at varying doses alters neurotransmitter activities in the CNS [27]. It was also quantified total protein level in the brain lysates of experimental animals in a bid to find out the rate of protein synthesis in the experimental animal. Animals treated with MO only, nicotine only and both MO and nicotine had a significantly higher total protein count relative to the control group, but there was no significant difference in the level of total protein among the three aforementioned groups. This suggests that both nicotine and MO potentiates neuromodulatory activity in the CNS by continuous protein synthesis. For example, MO induces the production of antioxidant enzymes, thereby boosting the antioxidant system. MO has also been reported to induce the production of monoamines in the CNS [27]. While nicotine exacerbates lactate dehydrogenase production and also increased expression of nicotinamide Acetylcholine Receptors (nAChRs) in the CNS. nAChRs are a class of ligand-gated ion channels assembled from several subunit through which nicotine exert its actions [32].

Qualitative analysis in the present study was carried out using the Cresyl fast violet technique which is specific for Nissl substance in neurons. Histomorphological presentation of the hippocampus and medial prefrontal cortex of rats treated with MO only appeared characteristically similar to those of the control. Structural disposition of neural cells and evaluation of Nissl substance confirmed the deleterious role that nicotine plays within the frontal cortex. Neurons in the external pyramidal layer of the prefrontal cortex and the CA3 region of the hippocampus of nicotine-treated rat showed signs of pyknosis which were characterized by the central chromatolysis, neuronal clustering, and degradation of nuclear materials. These degenerative changes can be attributed to the excessive generation of ROS sequel to exacerbated neural LDH and perturbed glucose bioenergetics. Disproportionate ROS generation results in aggravated lipid peroxidation, degradation of nuclear material and ultimately cell death. It was earlier reported that nicotine compromises the intrinsic antioxidant system by exacerbating the generation of reactive species which results into the depleting the level of SOD and CAT thereby leading to excessive lipid peroxidation [7, 30]. MO sustained the functional and structural integrity of pyramidal cells in the hippocampus and prefrontal cortex. The neuroprotection manifested by MO in the present study is due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids [33]. The quercetin in MO is of particular interest. It contains phenolic hydroxyl groups with antioxidant action with reported therapeutic uses [34]. In fact, studies have shown that quercetin strongly inhibits the production of both reactive oxygen species [35]. Luteolin present in MO

extract also has a strong anti-inflammatory, antioxidant activity by scavenging free radicals and exhibits a protective capability on DNA [36-37].

Conclusion:

Cumulatively, MO has proven to be neuro-protective in the present study against memory decline, perturbed glucose bioenergetics and chromatolysis induced by nicotine. A major highlight in the neuroprotective role of MO is the boosting of the antioxidant system by increasing energy production, thereby ultimately sustaining the integrity of neuronal and nonneuronal cells in the CNS. The current result suggests that aqueous extract of MO leaf possesses neuroprotective and nootropic properties against the negative neuro-modulatory effect of nicotine. The underlying mechanism behind the neuroprotective potential of MO lies in the antioxidant capacity of MO. Further mechanistic research using bioactive phyto-constituents of MO will help to further show other pathways through which MO exerts its effects.

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