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Afr. J. Biomed. Res. Vol. 24 (May, 2021); 257- 263

Research Article

Protective Effect of Alkaloid-rich Extract of Brimstone Tree (*Morinda lucida*) on Neurotoxicity in the Fruit-fly (*Drosophila melanogaster*) Model

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ABSTRACT

Brimstone plant is one of the medicinal plants found in Nigeria used in fore-lore medicine with little scientific information on its alkaloid constituents. This study was aimed at investigating the effect of alkaloid-rich compounds from the plant in manganese-induced (MgCl2) neurotoxicity in the fruit fly. In addition, alkaloid compounds will be characterized using gas chromatograph coupled with flame ionization detector (GC-FID). Alkaloid-rich extract was prepared by solvent extraction method, fruit flies were pre-treated with the extract (0.5 – 1.0mg/ml) in a fortified diet before induction with $MgCl_2$. The survival rate and negative geotaxis were observed. Thereafter, the activity of acetylcholinesterase (AChE) enzyme, antioxidantive potentials in in-vivo reactive oxygen species (ROS) thiobarbituric acid reactive species (TBARS), total thiol content, nitric oxide (NO*), hydroxyl oxide (OH*) scavenging ability, ferric reducing antioxidant property (FRAP) and 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTs*) radical scavenging ability were carried out on the flies' homogenate. Results obtained revealed that the alkaloid-rich extract regulated the neuro-protective activity of AchE, reduced the reactive oxygen species level in the induced flies with an increased in antioxidantive potential, higher survival rate and increases in the life span of the flies with 50% reduction in the mortality rate. The GC-MS showed a total of (1.55/100mg) of different plant-derived alkaloids such as dicentrine, atropine, aporphine. These alkaloids-rich compounds were found to have anti-oxidative, anti-nociceptive, anti-inflammatory and anti-cholinergic activities. In conclusion, this study suggests that alkaloids from brimstone plant could be the reason for the observed biological activities for the prevention of neuronal related complications.

Keywords: *Brimstone plant; Alkaloid-rich, Neurotoxicity; Drosophila melongaster, Acetylcholinesterase*

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Received: July 2020; Accepted: December, 2020

INTRODUCTION

Neurodegeneration is the gradual degradation of the function of neurons in the brain which has led to neuronal disorders when uncontrolled could exacerbate into ageing of the brain depending on the type of molecules or proteins involved in each pathology such as in Parkinson's diseases, Alzheimer's diseases, Huntington's diseases (Stephan and Brayne 2008). In the same vein, it could be due to deficiency or increase of some of these biomolecules needed in neurotransmission such as serotonin, choline, dopamine, Gamma aminobutyric acid (GABA), tau proteins, cholinesterases, 5′-nucleotidase, monoamine NTPDase, and adenosine deaminase enzymes (Stephan and Brayne, 2008). World Health Organization (WHO) reported that by 2025, about three-quarter of the estimated 1.2 billion people from 60 years and older would be living with neurodegenerative disorders mostly from developing countries and which would continue to increase if measures are not put in place to reduce and or prevent the incidence. The climax phase of the aforementioned diseases is incurable due to death of neuron cells which eventually causes loss of memory, cognitive decline, and mood changes which are the common characteristic symptoms (Bruggink *et al*., 2011).

Different models such as animals, cell-based system have been used to investigate brain related studies but come with its disadvantages (Baker and Thummel 2007; Jafari,2010). This is why drosophila melanogaster also known as fruit fly has been recommended by the european centre for the validation of alternative methods (Benford *et al*.,2000). Drosophila possesses systems which control nutrient uptake, storage and metabolism and these systems have been reported to be analogous to those of humans (Baker and Thummel 2007; Jafari, 2010). It is highly sensitive to toxic substances, which make it useful in toxicity studies and in the evaluation of activity of pharmacological agents (Mora *et al*., 2014).

Medicine from plants are the most common source of life saving medication for most population in the world today

(Tripathi *et al*., 2009; Nwanna *et al*., 2018). The World Health Organization estimated that 80% of people in Africa rely on herbs from plants for their medicine because these plants are rich source of phytochemicals like polyphenols, flavonoids, terpenoids, alkaloids, saponins, tannins (Cyril –Olutayo *et al*., 2012) to mention but few. These plant chemicals increase antioxidant status, improve memory and cognitive learning (Ramassamy, 2006). Several studies have highlighted the roles of these phytochemicals from fruits, vegetables and medicinal plant-derived polyphenol extracts as regulator of healthy brain functions in amnesia, dementia and insomnia in Nigeria (Nwanna *et al*., 2019; Cyril –Olutayo *et al*., 2012; Spencer, 2009; Spencer, 2008) with little or no information on alkaloid-rich component of these medicinal plants.

Brimstone tree known as *Morinda lucida* is an evergreen medium-sized tree bearing a dense crooked branches (Adeleye *et al*, 2018), the crude polyphenols extract of the different parts of the tree-plant do have medicinal values, rich in an essential nutrient with abundant bioactive compounds with antioxidants, anti-allergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic properties. It is a drought resistant plant thus available throughout the year however, it is mainly found in south-western Nigeria (Adeleye *et al*, 2018).

Plant derived alkaloids contains nitrogen, carbon, hydrogen and oxygen which could affect positively the nerve cells of the brain for proper body functions and behaviour (Pearson,2001).For example indole alkaloids found in fungal, tropane alkaloids like atropine, and scopolamine found in datura plant which controlled concentration could is found useful in central nervous system (CNS) (Pearson, 2001). Iso-quinoline alkaloid such as morphine isolated from *Papaver somniferum*. The mechanism of action of morphin is tightly bind to the μ-opioid receptor that is MOR site in the CNS, resulting in an increase of GABA in the synapses of the brain (Ortiz *et al*., 1999).Although drugs which could serve as an inhibitors or activators of some of the biomolecules in neurodegeneration have been produced like scopolamine, tacrine, donepezil, rivastigimine (Gutierres *et al*., 2012; Marisco *et al*., 2013), either they are not easily accessible and affordable to the low income earner especially those living in Africa. These factors have necessitated the need to source for alternative treatment from natural plant-based alkaloids from brimstone tree leafy parts and assess the effect of the extract diet in some biological parameters such as the enzyme linked to neurodegeneration (acetylcholinesterase), the extract antioxidative potentials in reactive oxygen species (ROS), nitric oxide (NO*), hydroxyl oxide (OH*) scavenging ability, ferric reducing antioxidant property (FRAP) and 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTs*) in manganese-induced (MgCl2) neurotoxicity in the fruit fly. Thereafter gas chromatograph coupled flame ionization detector (GC-FID) was used to quantify and characterize the alkaloid -rich compounds.

MATERIALS AND METHODS

All chemicals used were of analytical grade

Acquisition and Preparation of plant sample: Brimstone leafy part was gotten from the premises of the Federal University of Technology Akure (FUTA) Ondo State, Nigeria. The identification of the sample was carried out at the Department of Forestry and Wood Technology (FUTA) by Mr Omomoh B.E. with voucher number IFE-17692.

Preparation of alkaloid-rich extracts was carried out using the method of (Harborne, 1998).

Stock solution preparation: From the 50g of the 258ulverized leafy sample of which 5.68g of the alkaloid-rich extract was gotten. Thereafter, 1g alkaloid-rich extract was dissolved with dimethyl sulphur oxide (DMSO) in the ratio 1:3, after which 60mls of water was added. Thereafter, the solution was poured into a stock bottle and stored in the refrigerator at -4ºC for subsequent analysis.

Biochemical assays on the alkaloid- rich extract

*2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ABTS*radical scavenging ability:* The ABTS* scavenging ability of the extract was determined according to the method described by (Re *et al*., 1999).The Trolox equivalent antioxidant capacity (TEAC) was subsequently calculated using trolox as standard.

Determination of reducing property (FRAP): The ability of the extract to reduce $FeCl_3$ solution as described by (Oyaizu 1996) was determined, while the absorbance was measured at 700 nm in the JENWAY UV-Visible spectrophotometer. The ferric reducing antioxidant property was calculated as ascorbic acid equivalent.

Determination of nitric oxide (NO) scavenging assay:* The (NO*) capacity of the extract was measured by Griess reaction according to the method of (Sangameswaran *et al*., 2009). Sodium nitroprusside was prepared such as (2.7 mL, 10 mM) in phosphate buffered saline (PBS) was added to 0.3 mL of the extract and incubated at 25°C for 150 min. 0.5 ml of the incubated aliquot was added to 0.5 mL of Griess reagent. The absorbance was measured at 546 nm then calculated as % of inhibition of the nitric oxide evolved relative to the control and test samples.

***Drosophilia melanogaster* Stock Culture:** Wild type *D. melanogaster* (Oregon strain) stock culture was obtained from Department of Biochemistry, University of Ibadan, Nigeria.The flies were maintained on constant temperature and humidity (25 ± 1ºC; 60% relative humidity respectively) under 12 h dark/light cycle condition. The flies were placed on normal diet of corn meal medium containing 1% w/v brewer's yeast and 0.08% v/w nipagin according to the method of Drosophila media recipes (2002). All the experiments were carried out with the same strain of *D. melanogaster*.

Experimental Design: The flies (both gender, 3–5 days old) were divided into 4 groups containing 60 flies each. Group I was placed on normal diet, while group II – IV, were placed on basal diet containing; alkaloid-rich extract of 1% and 0.5% inclusive diet (equivalent weight replacement) as follows:

Group I - Basal Diet
Group - II Basal Diet+ manganese chloride ($Mgcl_2$) 30 mmol per kg
Group - III Basal Diet + Manganese chloride ($Mgcl_2$) + 0.5% MD
Group IV - Basal Diet + Manganese chloride ($Mgcl_2$) + 1% MD

These treatments were carried out for 7 days and the vials containing flies were all maintained at room temperature. All experiments were carried out in triplicate

Survival Study: Flies survival rate study was carried out using various concentration between 0.1 to 2% of pretreatment of the extracts to assess the effect of alkaloid-rich extract inclusive diet on the flies between 0-7 days after exposure to 30 mmol per kg, Mn using the method of Abolaji *et al*., (2014). Flies, of 3–5 days old were divided into four groups containing 60 flies each, which were observed daily for the incidence of mortality. The survival rate were determined by counting the number of dead flies within the five days. The data were subsequently analysed and plotted as cumulative mortality and percentage survival after the treatment period relative to control group 1 (Adedara *et al*, 2016). After which, 1% and 0.5% were chosen as the concentration to be used for the assays after the standardization.

Measurement of Locomotor Performance (Negative geotaxis): The method of (Le Bourg and Lints 1992) was used to determine the locomotor performance (negative geotaxis) of the flies at the end five days treatment, the flies from each group were briefly immobilized in ice and transferred into a clean tube of 11 cm in length 3.5 cm in diameter thereafter the flies were allowed to recover from immobilization within 10 mins the tube was tapped at the bottom, the number of flies that crossed the 6 cm line within a period of 6 s was observed and recorded. The results was expressed as % of flies that escaped beyond a minimum distance of 6 cm in 6 s during three independent experiments.

In-vivo biochemical assays

Preparation of Tissue Homogenate: The method of Abolaji *et al*., (2014) was used to prepare the tissue homogenate. The flies were immobilized in ice and homogenized in 0.1 M phosphate buffer, pH 7.4. Cold refrigerated centrifuge at 10,000 X g was used to homogenised the tissue for 10 mins in Kenxin Model KX3400C (KENXIN Intl. Co., Hong Kong) centrifuge. After which the supernatant was separated from the pellet into labelled eppendorf tubes and stored in the refrigerator at -4 ºC.

Determination of Total Protein: Total Protein content of the homogenates were measured using Coomassie blue method according to (Bradford, 1976) while bovine serum albumin (BSA) was used as the standard.

Reactive Oxygen Species (ROS) level: ROS level in the flies' tissue homogenate was estimated as H_2O_2 equivalent according to by the method of (Hayashi *et al*., 2007) with slight modifications. The absorbance was measured at 505 nm with a spectrophotometer. ROS levels was estimated from an H_2O_2 standard calibration curve and expressed as unit/mg protein.

Determination of the Total Thiol Content: The level of total thiol content in tissue homogenate was done by the method of Ellman and Fiches (1959). The reaction mixture was made up of 270 µL of 0.1 M potassium phosphate buffer (pH 7.4), 20 µL of homogenate, and 10 µL of 10 mM DTNB. This was followed by 30 mins incubation at room temperature, and the absorbance was measured at 412 nm. The total-thiol content was subsequently calculated and expressed as µmol/mg protein.

Lipid Peroxidation and thiobarbibutric Acid reactions: The lipid peroxidation assay was carried out using the modified method of (Ohkawa *et al*., 1979) TBARS (thiobarbituric acid reactive species) produced was measured at 532 nm using spectrophotometer subsequently malondialdehyde (MDA) produced was calculated and reported as % of control.

Acetylcholinesterase (AChE) Activity Assay: Acetylcholinesterase activity was assayed according to the method of (Ellman and Fiches 1959) The AChE activity was thereafter calculated and expressed as mmolAChE/min/mg protein.

Characterization of alkaloid compounds with GC-FID: This was carried using the modified method of (Ngounou *et al*., 2005). 5.0g of the pulverised sample was incarcerated in hexane of 25 mls for 72 hrs. The extract was filtered and the residue was air- dried, later treated with 10% aqueous (NH3) and macerated in $CHCL_3$ for 24 hrs. After the filtration and evaporation, the reduced aqueous phase was made alkaline with aqueous NH_3 and extracted 3times with $CHCl_3$. The $CHCl_3$ fraction was washed with distilled H_2O, which was poured into the round bottom flask of the rotatory evaporator and was separated by drying the solvent off the extract. The concentrated extract was dried by using anhydrous (Na_2SO_4) before applying the gas chromatograph analysis at HP 6890 powered with HP chemstation Rev 0901 [1200] software with split injection of ratio 20:1 using nitrogen as the carrier gas, with inlet temperature of 280º C, DB-5MS capillary column was used while the oven was program such that at initial temperature of 60 º C for 5minutes, first ramping at 10 º C /min for 20 min, second ramping at 15 º C /min for 4min thereafter FID detector of temp of 320 º Cat hydrogen pressure 28psi with compressed air of 38 psi was used. Standard library was used to identify the compounds characterised and quantified.

Data Analysis

The results of replicate readings were pooled and expressed as mean ± SEM values. One-way Analysis of Variance (ANOVA) was used to analysed the results while the significant was accepted at ($p<0.05$). All statistical analysis were carried out using the software Graph pad PRISM version 6. Zar, (1986).

RESULTS

Table 1 shows that the alkaloid-rich extract from the brimstone leafy plant had (0.94 ± 0.27) mmol TEAC/ µM of ABTS radical scavenging ability and (0.36 ± 0.02 mg AAE/g) of ferric reducing antioxidant property (FRAP).

Table 1:
Antioxidant property of alkaloid extracts of *Morinda lucida* (Brimstone tree)

ABTS (mmolTEAC/µM)	FRAP (mgAAE/g)
0.94±0.27	0.36±0.02

Figure 1 shows that the extract exhibited hydroxyl (OH) scavenging ability in dose dependent manner between (0-9mg/ml). While figure 2 shows the effect of the extract on nitric oxide (NO) radical species between (0 - 17mg/ml) concentration the higher the concentration of the sample extracts the lower the NO radicals species significantly.

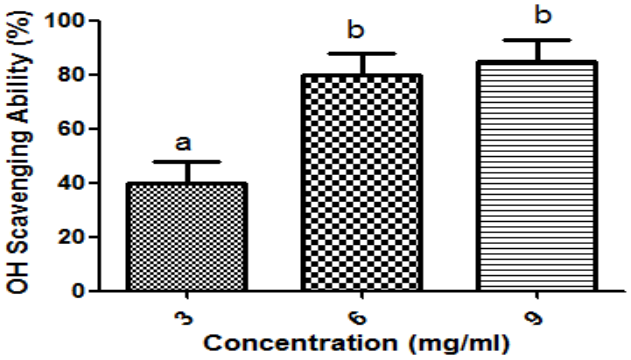

Figure 1:
This figure indicates *in-vitro* antioxidant activity *Morinda lucida* extract in hydroxyl radical scavenging in a dose dependent manner. Mean ±SD with significant (P<0.05)

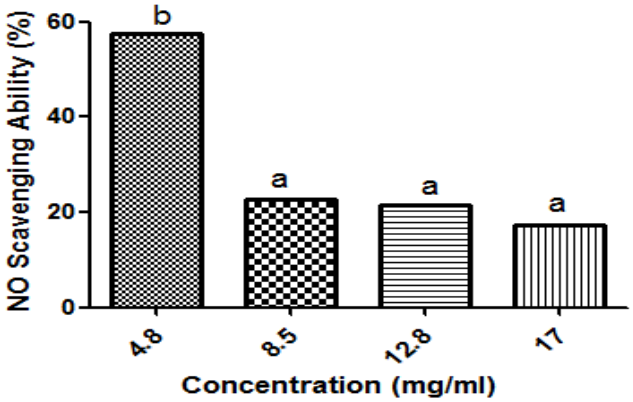

Figure 2:
This indicates that there is a significant decrease (P<0.05) in the NO radicals due to the scavenging ability of the alkaloid-rich extract of *Morinda lucida*.

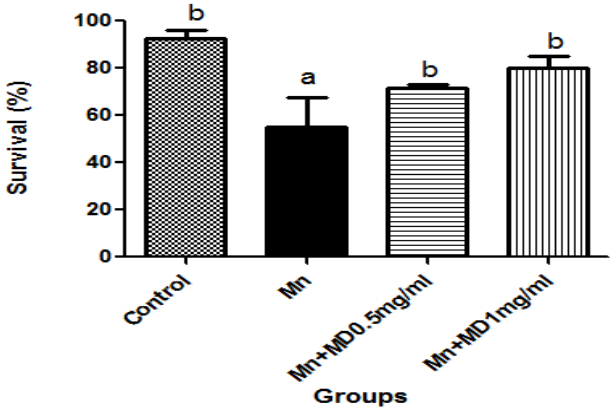

Figure 3
This figure shows that the survival rate of the manganese-induced flies reduced significantly (P<0.05) but increases in treated groups

Figure 3 shows the survival rate in (%) of *D. melanogaster* pretreated with alkaloid-rich extract of *Morinda lucida* and then induced with (30 mmol per kg, Mn). While group II induced with manganese of 30 mmol per kg had 50% survival rate when compared to positive control group I of 100% survival rate meanwhile the pretreatment groups III-IV (0.5mg/ml and 1.0 mg/ml) of the extract had 70%-80 % survival rate respectively.

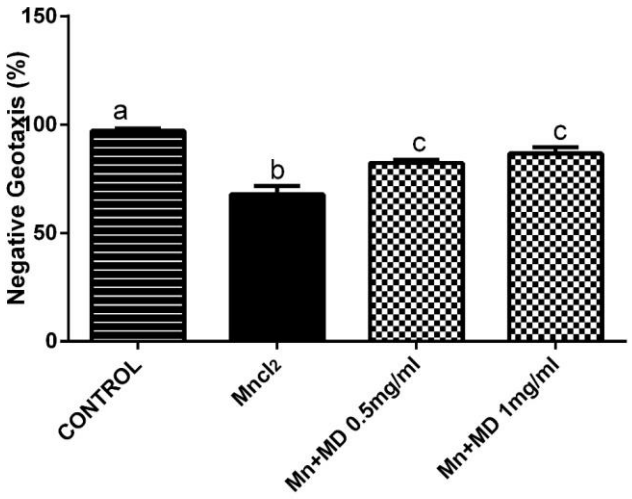

Figure 4:
Negative geotaxis in control and manganese-exposed flies.

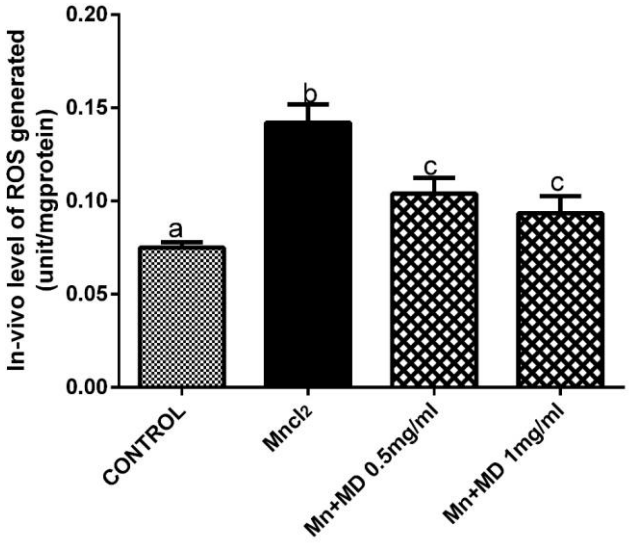

Figure 5:
This figure shows that the level of reactive oxygen species (ROS) generated within the flies. There was significantly (P<0.05) difference in the induced group when compared the control and treated groups

Figure 4 shows the effect of the extract diet of *Morinda lucida* on *D. melanogaster* on locomotion movement (% negative geotaxis) there was an increased in the locomotor of the flies within (80-90%) in the pre-treatment groups (III-IV) when compared to untreated group (II) with (70%) while the control group (I) is 100%. In addition, figure 5 shows the effect of the pretreatment on the endogenous level of reactive oxygen species generated in *D. melanogaster* the results revealed reduction from (0.14 unit/mg protein) in the untreated group

to (0.09-0.10 unit/mg protein) in the treated groups when compare with the control group (0.08 unit/mg protein).

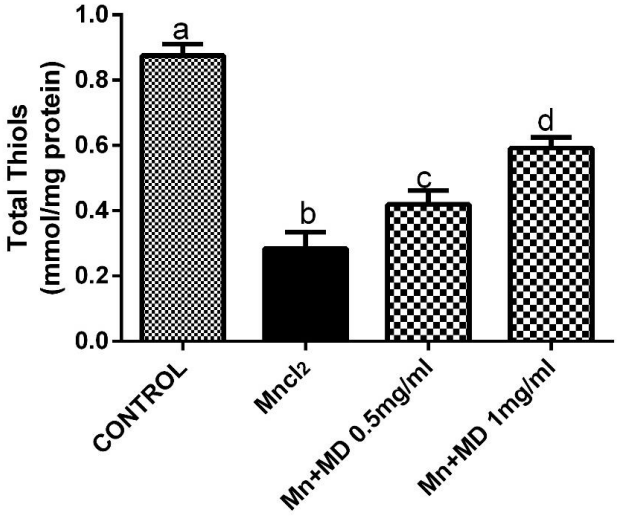

Figure 6
This figure shows that the level of total thiols produced within the flies. There was significantly (P<0.05) difference in all the groups

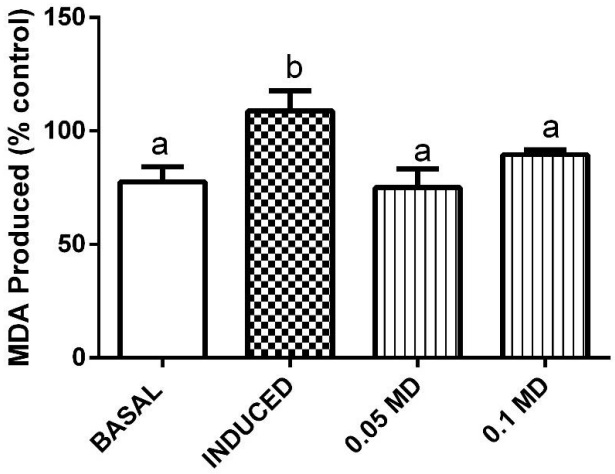

Figure 7:
This figure shows that the level malonaldehyde (MDA) produced within the flies. There was significantly (P<0.05) difference when compared the control to the treated groups

Figure 6 shows the total thiol content in the pretreated flies increased within (0.43-0.59 mmol/mg protein) when compared to the untreated induced group without treatment (0.29 mmol/mgprotein) relative to the control (0.88 mmol/mgprotein). In the same vein, figure 7 shows the % level of malondialdehyde (MDA) produced as a results of lipid peroxidation the extract diet reduced significantly (P>0.05) its production from 105% in the induced group II to 70% (treated groups) with no difference between the control group I and the treated groups II-IV. Furthermore, figure 8 shows the effect of the alkaloid-rich extract on acetylcholinesterase (AchE) enzyme activity within the flies. There was significantly (P<0.05) increased (0.035unit/mgprotein) in the AchE enzyme activity in the induced group II with a reduction in treated groups III and IV (0.25-0.28unit/mg protein) relative to the control group I (0.25unit/mg protein).Gas chromatographic technique coupled with flame ionization detector (GC-FID) was used to identify alkaloid -rich compounds as depicted in figure 9 while figure 10 shows the chromatogram. The result shows a total of (1.55mg/100g) of different forms of alkaloids in the plant sample such as ammodendrine, angustifoline, rhombifoline aporphine atropine, dicentrine as shown in the chromatogram and the table.

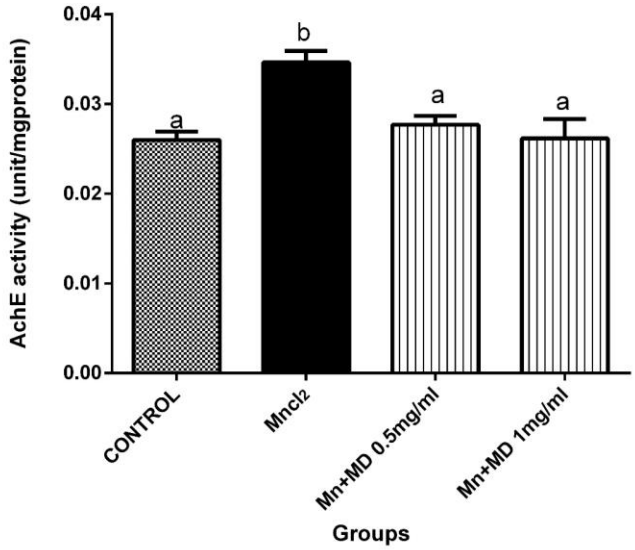

Figure 8:
This figure shows the effect of the alkaloid-rich extract on acetylcholinesterase activity within the flies. There was significantly (P<0.05) difference when compared the control to the treated groups

DISCUSSION

Neurodegenerative diseases would be among the diseases that could inflate death rates by the year 2050 if care is not taken, especially in the sub-Saharan Africa and other developing regions (Stephan, 2008; Hussain *et al*., 2018). Although, several factors such as uncontrolled environmental pollution of metals could predisposed one to the assaults (Abolaji *et al*., 2017) among such metals is Manganese (Mn). This metal is needed in trace quantity for the regulation of many biochemical processes including the development and maintenance of the central nervous system (Longman and Xiaobo, 2018). Although humans is constantly expose to manganese via different sources such as the leaching of this element into the water from occupational setting such as mining, smelting, welding which have appeared to be the major contributor route to its toxicity for those living in these sites. Uncontrolled constant contact to manganese could have a neurological impact characterized by psychological and neurological abnormalities (Abolaji *et al*., 2017). One of the preventive measures is the intake of rich foods in order to live a healthy life in such polluted environment (Abolaji *et al*., 2017). In Nigeria, vegetables of medicinal properties form major constituents of local diets desired for their nutritional benefits, but also for their phytomedical properties as reported in folklore medicine (Nwanna *et al*., 2018).

The present study demonstrated the neuroprotective potential of alkaloid-rich extract of *Morinda lucida* (brimstone tree) against manganese-induced neurotoxicity in *Drosophila melanogaster*. This model used serves as an excellent features with the genetic tools for toxicology experiment that could be utilized to find out the medicinal properties of various plant-based compounds (Ávila *et al*., 2008).).Cellular macromolecules are protected primarily from the assaults of free radical species by an abundant endogenous antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase which are activated by vitamins and nutrients from plant-based products with strong antioxidative potential (Ighodaro and Akinloye, 2018). Alkaloid-rich extract was found to have anti-oxidative property was shown in the ferric reducing antioxidant property using (FRAP) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ABTS radical activity. The results for the *in-vitro* antioxidants properties showed that the alkaloid-rich extract had better antioxidative potential which scavenged nitric oxide (NO*) and also mop up hydroxyl (OH*) radicals species in a dose dependent manner. This clearly confirm that brimstone tree plant could act as a free radical scavenger, inactivating and inhibiting production of superoxide anions while activating endogenous antioxidant enzymes (Kurutas,2016). Although there was no difference significantly with the locomotor performance in the various treatment groups but there was an increased in the survival rate of the groups fortified with the diet extract. This clearly show that over time the lives of those constantly expose to Mn is endanger but a pretreatment in form of the alkaloid rich fortified diet could help to reduce this assault or incidence of neurodegerative diseases because the system was fortified with an increased antioxidative compounds from *morida lucida* which would have assisted to remove the oxidants. The *in-vivo* analysis showed that Manganese (Mn) induction without pretreament had an increased radicals in terms of ROS, MDA and decreased in Thiols levels. Thiol is biomarker used to determine the level of oxidative stress, thus an indication of chemical changes in thiol groups of proteins and peptides (Abolaji *et al*., 2014; Adedara *et al*.,2015; Adedara *et al*.,2016). Higher reactive oxygen species level is directly proportional to lower total thiol content, as seen from this study, the pretreated groups significantly had an increased thiols level which was better with the group III. This showed that the alkaloid-rich compounds in this plant could prevent the degradation of essential biomolecules and therefore protect the entire system. Invariably, reactive oxygen species (ROS) and lipid peroxides in form of MDA measure were increased in induced group but the pretreament with the alkaloid-rich extract was able to alleviate and mop up oxidative species generated during metabolism in the flies This means the lives of those constantly expose to Mn is endanger but a pretreatment in form of the alkaloid rich fortified could help to reduce this assault or incidence because the system was fortified with increased antioxidative compounds which have assisted to remove the oxidants. This positive effect might have contributed to an increased in the survival rate of the flies. Furthermore, the effect of the alkaloid-rich extract on acetylcholinesterase enzyme activity within the flies was investigated, as observed, Mn-induced oxidative stress could have led to the activation or increased in AchE enzyme activity but the plant diet was able to counter react this negative effect and it was controlled. The use of alternative measure from plant- based products which is readily available and cheap especially for those inhabitants in metals polluted environs could be used to manage and or treat induced neurodegenerative disorders. Acetylcholine (ACh) molecule is a neuro-essential transmitter (Nwanna *et al*., 2019), that could be reduced in concentration and function relative to an increased acetylcholinesterase (AchE) action in neurotoxicity induced degeneration. In addition, cholinergic cell uses ACh after its discharge from the cleft of synaptic cell therefore uncontrolled AchE activity could lead to loss of neurotramission. Though there are several synthetic drugs which have been used as an inhibitor of acetylcholinesterase but comes with its side effect (Nwanna *et al.*, 2019). As seen from this study there is a link between oxidative stress and enzymes involved in neurodegeneration (Nwanna *et al.*, 2019), with means that an antioxidative potential system could have a neuroprotective system. In the same vein, the overall observed activities was actually due to the rich alkaloid compounds in *morida lucida* tree plant, from the GC-FID characterization and the quantification such as the aporpine, ammodendrine etc. Each of these compounds have various biological properties such as antioxidative, signalling inhibitors to mention but few. The high antioxidant activity from the study could be due to the compounds α-pyridine rings, the more the ring the higher the antioxidative power.

Alkaloid-rich compounds from the *Morida lucida* tree plant with anticholingernic, antinociceptive properties might have additively or synergically contributed to the positive attributes seen in the study. In conclusion, this is the first reported study on this plant using fruit fly model, have revealed that alkaloid-rich compounds in *Morinda lucida* (Brimstone) tree plant could serve as a rich source of antioxidant with neuroprotective on impaired activity of brain AchE thus making the plant a potent therapeutic agent as prodrug. However, there is need to further understand the possible underlying biochemical mechanisms in order to improve possibly the diagnosis and treatment of Mn-induced neurotoxicity

Acknowledgement

The author acknowledges the technical support of Inumisan Priscilla

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