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Extraction, isolation and evaluation of anti-toxic principles from *Moringa oleifera* (MOF_6) and *Myristica fragrans* (*Trimyristin*) upregulated Acetylcholinesterase concentrations in Sodium arsenite-induced neurotoxicity in rats.

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Abstract

This study evaluated the neuroprotective effects of MOF_6 (isolated from *Moringa oleifera* leaves) and *Trimyristin* (isolated from *Myristica fragrans* seeds) on Acetylcholinesterase concentrations in cerebral cortices of rats with Sodium arsenite-induced neurotoxicity. Sixty-five adult male rats (150 g-250 g) were randomly divided into thirteen groups comprising of five rats per group. Groups 1 and 3 received physiological saline and 1 ml/200 g bodyweight of Olive oil respectively for 9 weeks. Group 2 received 20 mg/kg bodyweight of Sodium arsenite (SA) for 6 weeks and left untreated for another 3 weeks. Groups 4-5 received 20 mg/kg bodyweight of SA for 3 weeks followed by treatments with 5.0 and 7.5 mg/kg bodyweight of MOF_6 respectively for 6 weeks. Groups 6-7 received 20 mg/kg bodyweight of SA for 3 weeks followed by treatments with 15 and 30 mg/kg bodyweight of *Trimyristin* respectively for 6 weeks. Groups 8-11 received 5.0 and 7.5 mg/kg bodyweight of MOF_6; 15 and 30 mg/kg bodyweight of *Trimyristin* respectively for 9 weeks. Groups 12-13 received 7.5 mg/kg bodyweight of MOF_6 and 30 mg/kg bodyweight of *Trimyristin* respectively for 6 weeks followed by co-administration of each extract dose with 20 mg/kg bodyweight of SA for another 3 weeks. Histological examination of cerebral cortices and biochemical analyses of Acetylcholinesterase concentrations were carried out in all rats. Computed data were analyzed using Microsoft Excel 2016 with statistical significance at $p \leq 0.05$. Histo-pathological evaluations revealed normal histo-architecture of cerebral cortices of all rats. Results showed statistically significant ($p \leq 0.05$) increases in Acetylcholinesterase concentrations in rats of Groups 1-10 and 12 compared with Group 2 (2.78±1.76 μmole/min/g). 7.5 mg/kg bodyweight of MOF_6 showed the best therapeutic and neuro-regenerative potential against SA-induced neurotoxicity.

Conclusions: Our findings implied that MOF_6 and *Trimyristin* reversed downregulation of Acetylcholinesterase concentrations in SA-induced neurotoxicity in rats; and possess neuro-protective and neuro-regenerative potentials.

Keywords: *Sodium arsenite; Neurotoxicity; Acetylcholinesterase; Moringa oleifera; Myristica fragrans*

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Introduction

Arsenic compounds are readily available in nature, and are dissipated into the environment through agricultural and industrial processes and also in a number of medical applications (1). Inorganic arsenic is the predominant form found in meats, poultry, dairy products and cereal, while organic arsenic (such as arsenobetaine) predominates in seafood, fruit, and vegetables (2,3). The highest concentrations of arsenic have been found in seafood (2.4–16.7 mg/kg in marine fish, 3.5 mg/kg in mussels, and more than 100 mg/kg in certain crustaceans), followed by meats, cereals, vegetables, fruit, and dairy products. The main source of arsenic exposure in the world population is drinking water with a high inorganic arsenic concentration (4, 5). Arsenic usually is found in water in the form of arsenite and arsenate, the proportions of each depending on conditions. Likewise, inhalation of arsenic compounds has been reported to be another risk factor in carcinogenesis aside ingestion of arsenic contaminated drinking-water at high concentrations over prolonged periods of time (6).

Sodium arsenite is rapidly absorbed into the gastrointestinal tract and it also reacts with other substances like metals to boost its effect resulting in induction of oxidative stress and impaired functions of the brain, liver, testis, lung, spleen and kidney. Hence, Sodium arsenite-induced toxicity is associated with health hazards such as cancer, dermal diseases, cardiovascular defects and gastrointestinal problems. Exposure to Sodium arsenite results in increased generation of free radicals and induction of oxidative stress leading to chronic demyelination, morphological changes in axons of peripheral nerves, alteration in the formation and spread of action potentials in peripheral nerves and obstruction in transmission of nerve impulses (7). Sodium arsenite causes defects in the cerebral cortex due to its ability to bind to acetylcholinesterase and also inactivates (downregulates) it through the thiol group which then enables it to cross the blood-brain barrier (1).

Acetylcholinesterase (AChE) has a biochemical function which is to halt transmission of impulse at cholinergic synapses through immediate hydrolysis of the neurotransmitter, acetylcholine (1). Abnormal alterations in AChE levels have been associated with neurodegenerative disorders such as Alzheimer's disease. Antioxidants help in obstruction and elimination of free radicals, and protects the body systems against infections and degenerative diseases. *Moringa oleifera* (MO) leaves and *Myristica fragrans* (MF) seeds are traditionally used to treat many diseases such as diabetes, hypertension, and cancer. In a previous study, we isolated MOF_6 from MO and evaluated its antioxidant potentials in Cuprizone-induced demyelination in rats (8); and reported that MOF_6 possessed antioxidant potentials (8). Similarly, MF seeds have been reported to possess antioxidant potentials (9, 10). In this study, MOF_6 and *Trimyristin* were purified and isolated from MO leaves and MF seeds respectively, in-order to further determine their possible neuro-

protective and neuro-regenerative potentials on acetylcholinesterase levels of the cerebral cortex in Sodium arsenite-induced neurotoxicity in adult male wistar rats.

In addition, can prior consumption of MO leaves and MF seeds prevent against the onset of SA-induced neurotoxicity when the organism is later exposed to arsenic contamination? Hence, in this study, the higher doses of MOF_6 and *Trimyristin* were administered to rats for 6 weeks followed by co-administrations of extract doses and Sodium arsenite for 3 weeks in-order to evaluate the neuro-protective potentials of MOF_6 and *Trimyristin*.

Materials and Methods

Ethical Approval

Ethical approval for this study was sought and received from the University of Ilorin Ethical Review Committee. The ethical approval number is UERC/ASN/2018/1161. This research study was conducted in accordance with the internationally accepted principles for laboratory animal use and care as provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985).

Collection, Preparation, Purification and Fractionation of *Moringa oleifera* Leaves

The plant name '*Moringa oleifera*' (MO) was checked with http://www.theplantlist.org and accessed via http://www.theplantlist.org/tpl1.1/record/tro-21400003. MO leaves were obtained locally from forest reserves in Ilorin and samples were authenticated at the Forest Research Institute of Nigeria, Ibadan and assigned Voucher Number 110648. MO leaves were air-dried at room temperature (25 ^{0}C–30 ^{0}C) for two weeks in the Department of Chemistry, University of Ilorin, Nigeria; in-order to prevent thermal degradation of the phytochemical constituents of the plant material. The dried plant materials were pulverized and 2 kg of the dried plant material extracted with 70 % ethanol for 72 hours. The ethanol extract of MO leaves was fractionated in a silica gel open column, using n-hexane, dichloromethane, ethyl acetate and ethanol in an increasing order of polarity (N-hexane; N-hexane:Dichloromethane [3:1, 3:2, 1:1, 1:2, 1:3]; Dichloromethane; Dichloromethane: Ethylacetate [3:1, 3:2, 1:1, 1:2, 1:3]; Ethylacetate; Ethylacetate:Methanol [3:1, 3:2, 1:1, 1:2, 1:3] and Methanol, to afford fifty-five eluents of 200 ml each. The eluents were pooled based on the colour of the eluents and the solvents that elute them to give a total of seven combined fractions (MOF_1 – MOF_7). The fraction MOF_6 which had the highest yield and whose antioxidant potentials in Cuprizone-induced neurotoxicity we previously established (8) was used in the present study to evaluate the neuro-protective and neuro-regenerative potentials of *Moringa oleifera* leaves in Sodium arsenite-induced neurotoxicity in rats.

Collection, Preparation, Purification and Fractionation of *Myristica fragrans* Seeds

The plant name '*Myristica fragrans*' (MF) was checked with http://www.theplantlist.org and accessed via http://www.theplantlist.org/tpl1.1/record/kew-

2500629. MF seeds were obtained locally from forest reserves in Ilorin and samples were authenticated at the Forest Research Institute of Nigeria, Ibadan. Extraction of *Trimyristin* from MF seeds was carried out at the Research Laboratory of the Department of Anatomy, University of Ilorin, Nigeria using the modified method described by (11). 30 g (±0.05) of powdered MF seeds was weighed onto a weighing paper and transferred to the microscale round-bottomed flask, using the plastic funnel to assist in the transfer. 90 ml of tert-butyl methyl ether and three boiling chips were added to the flask and, using the black plastic connector, the distillation column/air condenser was connected to the flask. The position of the flask was carefully adjusted so that the mixture boiled very gently. The finely divided seeds caused the mixture to bump (boil violently). Most of the liquid were allowed to filter by gravity, then, when it became slower, a pipet bulb was attached and squeezed gently to apply pressure to complete the filtration. After most of the solvent had evaporated, a gummy yellowish solid remained as *Trimyristin*.

Chemicals and Reagents

Sodium arsenite (SA) was a product of Sigma–Aldrich Japan Co. (Tokyo, Japan). Normal Saline and Olive Oil were obtained from MOMROTA pharmaceutical company in Ilorin, Kwara State, Nigeria.

Animal Care and Feeding

Seventy adult male Wistar rats (average weight of 200 g) were acclimatized in the animal house of the Faculty of Basic Medical Sciences of University of Ilorin. The rats were randomly divided into 14 Groups of 5 rats each and fed with standard rat diet bought from Ogo-Oluwa Livestock Feed Enterprise, Ilorin, Kwara State, Nigeria. Normal Saline and Olive Oil were obtained from MOMROTA pharmaceutical company in Ilorin, Kwara State, Nigeria. All rats were provided with water ad libitum. MOF_6 and Sodium arsenite (SA) (Sigma–Aldrich Japan Co.) were dissolved in Normal Saline, while *Trimyristin* was dissolved in Olive Oil.

Experimental Procedures and Drugs Administration

Groups 1 and 3 received 1 ml/kg bodyweight of Normal Saline and Olive Oil respectively for nine weeks. Group 2 received 20 mg/kg bodyweight Sodium arsenite (SA) for 6 weeks and left untreated for another 3 weeks. Groups 4 - 7 received 20 mg/kg bodyweight SA for 3 weeks followed by treatment for 6 weeks with 5 mg/kg bodyweight MOF_6, 7.5 mg/kg bodyweight MOF_6, 15 mg/kg bodyweight *Trimyristin* and 30 mg/kg bodyweight *Trimyristin* respectively. Groups 8 and 9 received 5 mg/kg bodyweight MOF_6 and 7.5 mg/kg bodyweight MOF_6 respectively for six weeks; while Groups 10 and 11 received 15 mg/kg bodyweight *Trimyristin* and 30 mg/kg bodyweight *Trimyristin* respectively for nine weeks. Groups 12 and 13 received 7.5 mg/kg bodyweight MOF_6 and 30 mg/kg bodyweight *Trimyristin* respectively for 6 weeks, followed by co-administrations of 20 mg/kg bodyweight SA with 7.5 mg/kg bodyweight MOF_6 and 30 mg/kg bodyweight *Trimyristin* respectively for 3 weeks. The model/guidelines on which the experimental procedure used in this study was based, is in accordance with the internationally

accepted principles for laboratory animal use and care as provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985).

All doses of drugs and plant extracts were orally administered to rats. The experimental procedures used in this study were modified from a previous study on Sodium arsenite-induced toxicity in rats (1) and our previous study on neuro-protective effects of MOF_6 (8). The doses of MOF_6 used in this study were determined from our previous study on antioxidant potentials of MOF_6 in Cuprizone-induced neurotoxicity in rats (8), while the doses of *Trimyristin* were determined based on which therapeutic doses were effective against Sodium arsenite-induced toxicity in rats during the pilot phase of the present study as modified from a previous study (11).

Relative Weights of the Cerebrum in Rats of Control and Experimental Groups

The final bodyweight (g) of all rats were computed at the end of experimental procedure. Subsequently, the skull of each rat was opened and the cerebrum was removed and weighed (g). The Relative Weight of the Cerebrum of each rat was determined using the formula: Weight of Cerebrum (g)/Bodyweight (g) of rat X 100.

Assay procedure for Acetylcholinesterase (AChE) concentrations

At the end of experimental procedures, all rats were sacrificed by inhumane method as described in our previous study on neuro-protective effects of MOF_6 (8). The skull of each rat was opened and the cerebrum was removed, grounded and homogenized with sucrose solution, where the amount of sucrose used was based on the tissue size (1 ml = 0.62 g), after which they were placed in cold ice packs. AChE activity was estimated using the modified George Ellman's method of 1961 described by (12). Briefly, 0.4 ml aliquot of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1 M, pH8) and 100 µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm in a LKB spectrophotometer. When absorbance reached a stable value, it was recorded as the basal reading. 20 µl of substrate of AChE was added and change in absorbance was recorded for a period of 10 mins at intervals of 2 minutes. Change in the absorbance per minute is thus determined.

Calculations: AChE activity was calculated using the following formula;

$R = 5.74 \times 10^{-4} \times A/CO$ Where,

R= Rate in moles of substrate hydrolyzed/ minute/ gm tissue

A= Change in absorbance / min

CO = Original concentration of the tissue (mg/ml).

Statistical Analysis

Numerical data obtained from the weight measurements and quantitative histochemical study of AChE were analyzed statistically by the application of t-test, using Microsoft Excel 2016 to calculate the Mean and Standard Deviation (SD). Values of $p \leq 0.05$ were considered to be statistically significant.

Results

Incidence of Mortality

All rats of Group 13, which received 30mg/kg bodyweight of *Trimyristin* for 6 weeks followed by 20mg/kg bodyweight of Sodium arsenite co-administration with 30mg/kg bodyweight of *Trimyristin* for 3 weeks died between the 8th and 9th week of experimental procedure.

Behavioural Analyses

No behavioural anomalies were observed in rats of Control and Experimental Groups.

Relative Weight of Cerebrum in Rats of Control and Experimental Groups

There were no statistical significant ($p \geq 0.05$) differences in weights of the cerebrum and relative weights of cerebrum between the rats of Control Group 1 and Experimental Groups 2 – 12 (Table1).

Histopathological Evaluations of the Cerebral Cortex in Rats of Control Group 1 and Experimental Groups 2 - 12.

Histopathological examinations showed normal cytoarchitectures of cerebral cortices of rats of Control Group 1 and Experimental Groups 2 - 12. The neurons appeared to be distributed in a background meshwork of neutrophils, with numerous capillaries; and there were no significant inflammation or degenerative changes.

Evaluations of Acetylcholinesterase (AChE) concentrations in Rats of Groups 1 – 12.

Analyses showed statistically significant decreased AChE levels in rats of Group 2 compared to Groups 1 and 3 – 12 (Table 2).

Discussion

There were no behavioural anomalies in rats of Control Group 1 and Experimental Groups 2-13. This implied that the administrations of Sodium arsenite, MOF_6 and *Trimyristin* had no negative effects on body organs such as the cerebellum, cerebrum and internal ear, which are involved in the control of behavioral functions, equilibrium and balance. There were statistically significant increases in the Final Weight compared to the Initial Weight of rats in Groups 1 - 13, but there were no statistically significant ($p \geq 0.05$) differences in the weight of the cerebrum and relative weights of cerebrum between Group 1 and Groups 2 - 13. This implied that administration of Sodium arsenite, MOF_6 and *Trimyristin* had no adverse effects on the gross morphology of the cerebral cortices of rats of Control and Experimental Groups 1 - 13.

Histopathological examinations showed normal histo-architectures of the cerebral cortices of rats of Control Group 1 and Experimental Groups 2 - 13. This suggests that administrations of different doses of Sodium arsenite, MOF_6 and *Trimyristin* had no adverse effects on the histo-architecture of the cerebral cortex in adult male Wistar rats. Our findings which showed no evident histopathological effects of 20mg/kg bodyweight of Sodium arsenite on the cerebral cortex of rats is in contrast with the findings of (13), which reported histopathological effects of Sodium arsenite on the brain in rats.

One of the most important molecular players in the cholinergic neurotransmitter system is Acetylcholinesterase (AChE) (1, 14), as it is responsible for terminating nerve

impulses at cholinergic synapses by splitting the neurotransmitter acetylcholine (ACh) into choline and acetate. Hence, the implication of AChE's role is to prevent excess accumulation of acetylcholine at synaptic terminals. However, excess AChE at synaptic junctions will hamper proper neurotransmission because it will mediate excess breakdown of acetylcholine (1, 14). In the present study, AChE level was significantly reduced and downregulated in Experimental Group 2 (Sodium arsenite only-treated group) (Table 2). This suggests that Sodium arsenite caused reduced clearance of Acetylcholine thereby resulting into its accumulation. This kind of accumulation can cause continuous excitation of postsynaptic neurons and ultimately culminating to excito-toxicity and cell death. Our findings are in agreement with (1), which reported that Sodium arsenite-induced neurotoxicity resulted in downregulation of AChE levels in rats.

Post-treatment of rats with doses of MOF_6 and *Trimyristin* following exposure to 20 mg/kg bodyweight of Sodium arsenite upregulated and restored the activities of AChE to baseline control level (Table 2). This suggests that MOF_6 and *Trimyristin* conferred a degree of neuroprotection against Sodium arsenite-induced excitotoxicity and neurotoxicity.

However, the marked reduction of AChE in the Experimental Group 11 that were treated with 30 mg/kg bodyweight of *Trimyristin* for 9 weeks (Table 2) suggests the excito-toxic role that chronic or high dose of *Trimyristin* treatment could cause. The possible excito-toxicity and cell death effects of 30 mg/kg bodyweight of *Trimyristin* could have been responsible for the death of all rats of Group 13, which received 30 mg/kg bodyweight of *Trimyristin* for 6 weeks followed by 20 mg/kg bodyweight of Sodium arsenite co-administration with 30 mg/kg bodyweight of *Trimyristin* for 3 weeks.

In addition, can prior consumption of MO leaves and MF seeds prevent against the onset of SA-induced neurotoxicity when the organism is later exposed to arsenic contamination? The death of all rats of Group 13, which received 30 mg/kg bodyweight of *Trimyristin* for 6 weeks followed by 20 mg/kg bodyweight of Sodium arsenite co-administration with 30 mg/kg bodyweight of *Trimyristin* for 3 weeks suggests clearly that *Trimyristin* at higher dose of 30 mg/kg bodyweight has possible excito-toxicity and cell death effects in rats. Furthermore, 30 mg/kg bodyweight of *Trimyristin* does not have neuro-protective potentials against Sodium arsenite-induced neurotoxicity in rats. Notably and conversely, there was upregulation of AChE in rats of Group 12, which received 7.5 mg/kg bodyweight of MOF_6 for 6 weeks followed by 20 mg/kg bodyweight of Sodium arsenite co-administration with 7.5 mg/kg bodyweight of MOF_6 for 3 weeks (Table 2). This suggests that 7.5mg/kg bodyweight of MOF_6 further possesses neuro-protective potentials against Sodium arsenite-induced neurotoxicity in rats. Our observed neuro-protective effects of MOF_6 (isolated from *Moringa oleifera*) are in agreement with the opinions of (8, 15), which reported antioxidant and neuro-protective effects of *Moringa oleifera* against the

neurotoxic effects of Cuprizone and Sodium arsenite on rat cerebellum.

Conclusions

In conclusion, our findings suggest that post-treatment of rats with doses of MOF_6 and *Trimyristin* following exposure to Sodium arsenite upregulated and restored the activities of AChE to normal levels. This suggests that MOF_6 and *Trimyristin* conferred a degree of neuroprotection against Sodium arsenite-induced excitotoxicity and neurotoxicity; and are recommended for further evaluation as potential drug candidates for the treatment of neurodegenerative diseases such as Alzheimer's disease.

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Table 1: Relative Weights of the cerebrum in rats of Control and Experimental Groups.

Groups	Bodyweight of Rats (g)	Weight of the Cerebrum (g)	Relative Weight of the Cerebrum (g)
1	275.31±37.27	1.25±0.06	0.45
2	216.80±6.47	1.15±0.04	0.53
3	268.74±3.65	1.3±0.02	0.48
4	247.60±7.11	1.2±0.03	0.48
5	254.68±2.63	1.26±0.02	0.49
6	207.93±3.71	1.18±0.02	0.56
7	221.67±8.59	1.265±0.13	0.57
8	202.24±2.73	1.21±0.04	0.55
9	250.54±2.96	1.33±0.05	0.53
10	269.43±7.84	1.325±0.15	0.49
11	234.28±9.44	1.225±0.03	0.52
12	255.53±2.18	1.06±0.05	0.46

Table 2: Acetylcholinesterase (AChE) concentrations (μmole/min/g) in rats of Control and Experimental Groups.

Groups	Doses of drugs and extracts	AChE activity (μmole/min/g) of tissue/mg protein) $\times 10^{-4}$
Group 1	1 ml/200 g bodyweight of Normal Saline for 9 weeks	4.39±2.18
Group 2	20 mg/kg bodyweight of Sodium arsenite for 6 weeks and left untreated for 3 weeks	2.78±1.76[a*]
Group 3	1 ml/200 g bodyweight of Olive oil for 9 weeks	4.68±2.88[a-]
Group 4	20 mg/kg bodyweight Sodium arsenite 3 weeks followed by treatment with 5 mg/kg bodyweight MOF_6 for 6 weeks	8.23±1.18[a*]
Group 5	20 mg/kg bodyweight Sodium arsenite 3 weeks followed by treatment with 7.5 mg/kg bodyweight of MOF_6 6 weeks	4.354±1.486[a-]
Group 6	20 mg/kg bodyweight Sodium arsenite for 3 weeks followed by treatment with 15 mg/kg bodyweight of *Trimyristin* for 6 weeks	5.026±1.158[a-]
Group 7	20 mg/kg bodyweight Sodium arsenite for 3 weeks followed by treatment with 30 mg/kg bodyweight of *Trimyristin* for 6 weeks	4.66±2.302[a-]
Group 8	5 mg/kg bodyweight of MOF_6 only for 6 weeks	6.47±1.80[a-]
Group 9	7.5 mg/kg bodyweight of MOF_6 only for 6 weeks	5.28±0.951[a-]
Group 10	15 mg/kg bodyweight of *Trimyristin* only for 9 weeks	6.68±1.26[a*]
Group 11	30 mg/kg bodyweight *Trimyristin* only for 9 weeks	3.345±2.19[a-]
Group 12	7.5 mg/kg bodyweight of MOF_6 for 6 weeks followed by 20 mg/kg bodyweight of Sodium arsenite co-administration with 7.5mg/kg bodyweight of MOF_6 for 3 weeks	4.424±1.430[a-]

a = T-test comparison between rats of Group 1 and Groups 2 - 13.

Where [*-] represents statistical significance at $p \leq 0.05$ and [-] represents statistical non-significance at $p \geq 0.05$.

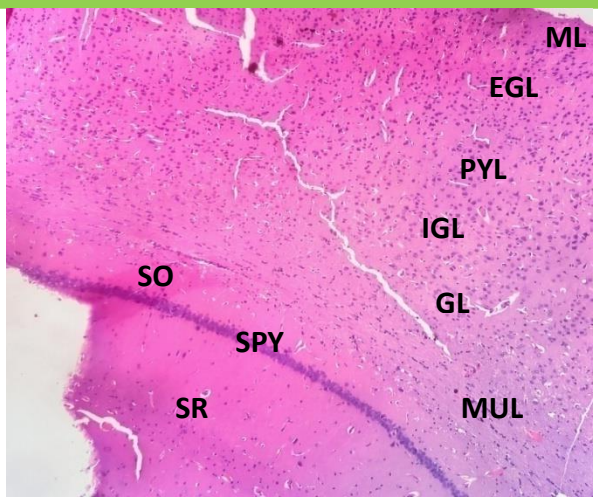

Figure 1: Transverse section of the cerebral cortex of rats of Group 1 which received Normal Saline for 9 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, GL-ganglionic layer, MUL- multiform layer SR- stratum radiatum, SPY- Stratum pyramidal layer of hippocampus and SO- stratum oriens of hippocampus. Neurons are distributed in a background meshwork of neutrophils with no feature of significant inflammation or degenerative changes.

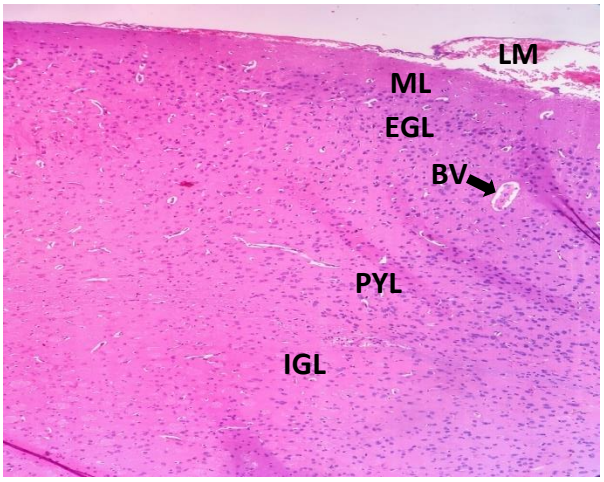

Figure 2: Transverse section of the cerebral cortex of rats of Group 2 which received 20 mg/kg bodyweight of Sodium arsenite for 6 weeks and left untreated for 3 weeks. Haematoxylin and Eosin X 100. LM- leptomeninges, ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer and BV- blood vessel. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to Control Group 1.

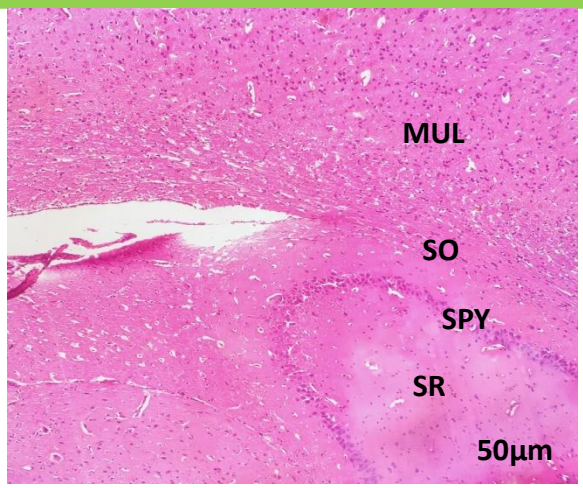

Figure 3: Transverse section of the cerebral cortex of rats of Group 3 which received 1 ml/200 g bodyweight of Olive oil for 9 weeks. Haematoxylin and Eosin X 100. MUL- multiform layer, SO- stratum oriens of hippocampus, SPY- stratum pyramidalis layer of hippocampus and SR- stratum radiatum of hippocampus. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to rats of Control Group 1.

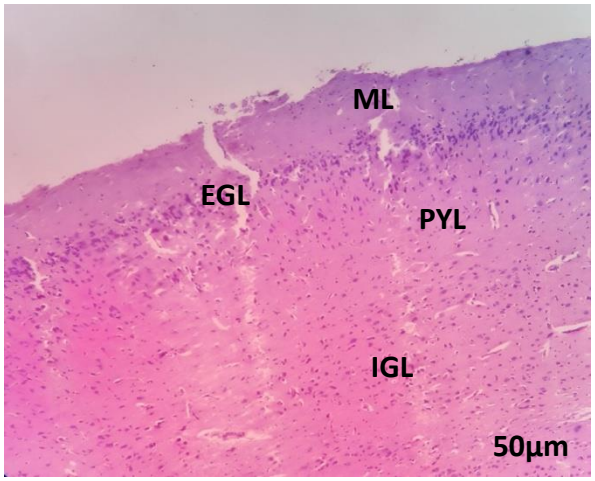

Figure 4: Transverse section of the cerebral cortex of rats of Group 4 which received 20 mg/kg bodyweight Sodium arsenite for 3 weeks followed by treatment with 5 mg/kg bodyweight MOF_6 for 6 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer and IGL- internal Granular layer. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to rats of Control Group 1.

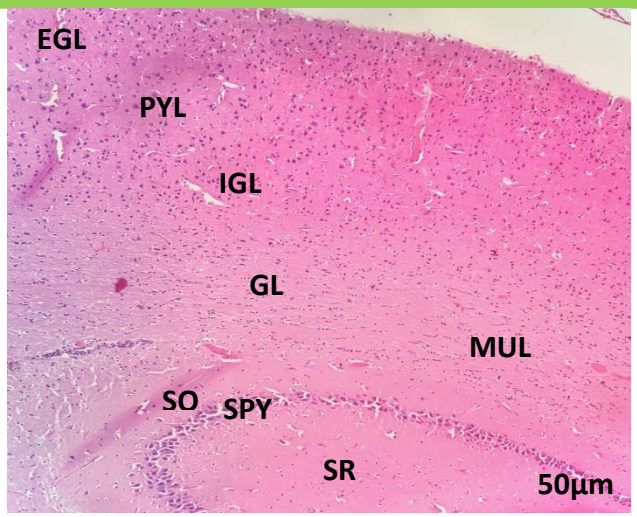

Figure 5: Transverse section of the cerebral cortex of rats of Group 5 which received 20 mg/kg bodyweight Sodium arsenite for 3 weeks followed by treatment with 7.5 mg/kg bodyweight of MOF_6 for 6 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, GL-ganglionic layer, MUL- multiform layer, SO- stratum oriens of hippocampus, SPY- stratum pyramidalis layer of hippocampus and SR- stratum radiatum of hippocampus. Histo-pathological evaluations showed normal histo-architecture of the cerebral cortex.

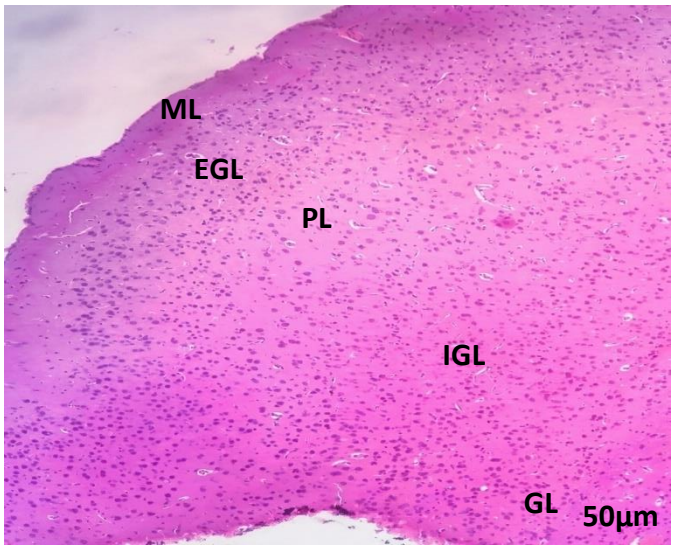

Figure 6: Transverse section of the cerebral cortex of rats of Group 6 which received 20 mg/kg bodyweight Sodium arsenite for 3 weeks followed by treatment with 15 mg/kg bodyweight of *Trimyristin* for 6 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer and Gl- ganglionic layer. Histo-pathological evaluations showed normal histo-architecture of the cerebral cortex.

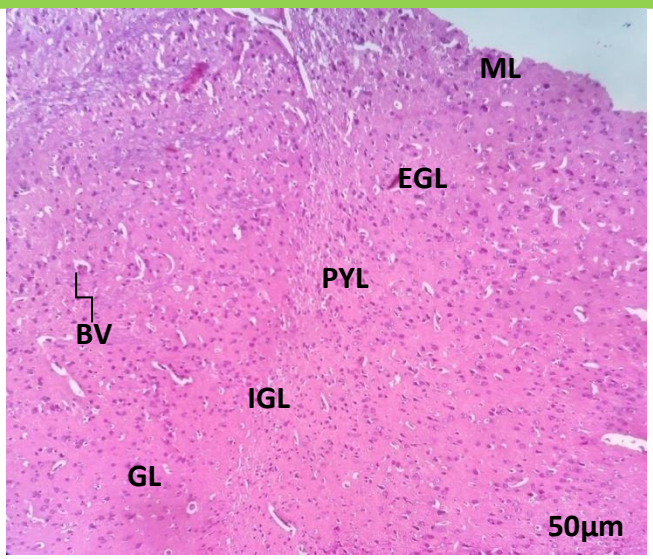

Figure 7: Transverse section of the cerebral cortex in rats of Group 7 which received 20 mg/kg bodyweight Sodium arsenite for 3 weeks followed by treatment with 30 mg/kg bodyweight of *Trimyristin* for 6 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, Gl- ganglionic layer and BV- blood vessel. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to rats of Control Group 1.

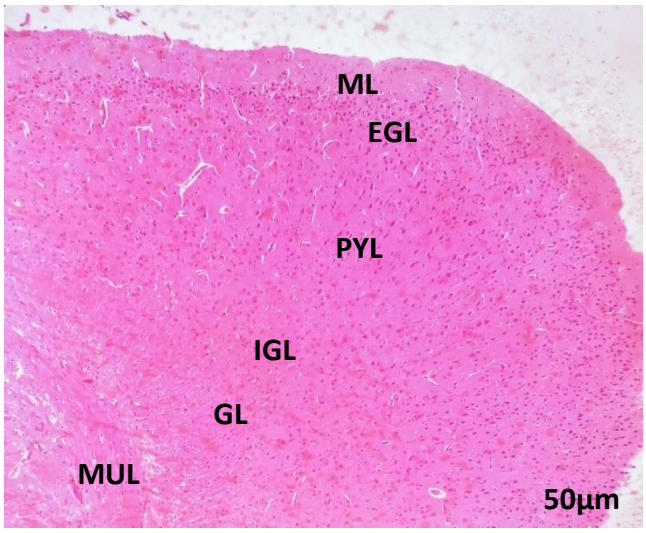

Figure 8: Transverse section of the cerebral cortex of rats of Group 8 which received 5 mg/kg bodyweight of MOF_6 only for 9 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, Gl- ganglionic layer and MUL- multiform layer. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to rats of Control Group 1.

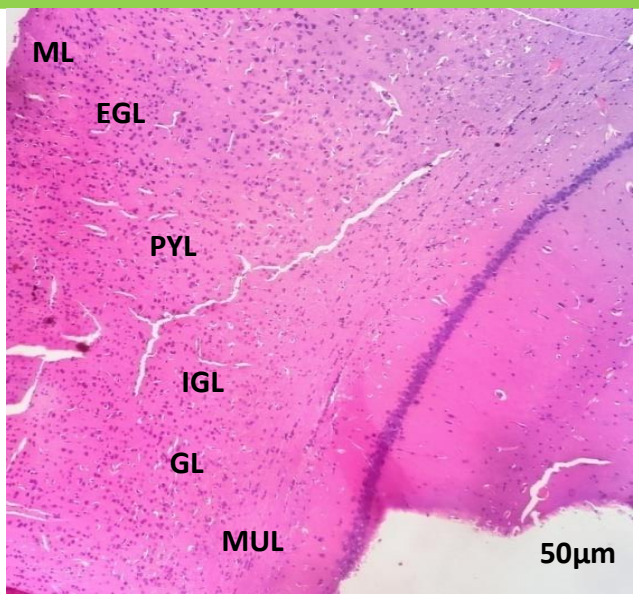

Figure 9: Transverse section of the cerebral cortex of rats of Group 9 which received 7.5 mg/kg bodyweight of MOF_6 only for 9 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, Gl- ganglionic layer and MUL- multiform layer. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to rats of Control Group 1.

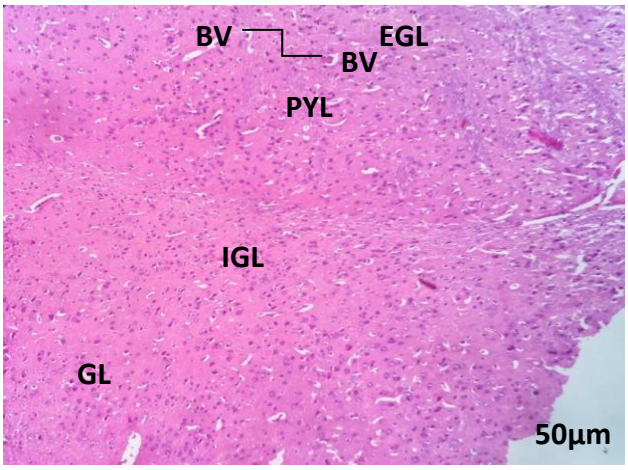

Figure 10: Transverse section of the cerebral cortex of rats of Group 10 which received 15 mg/kg bodyweight of *Trimyristin* only for 9 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, Gl- ganglionic layer and MUL- multiform layer. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to rats of Control Group 1.

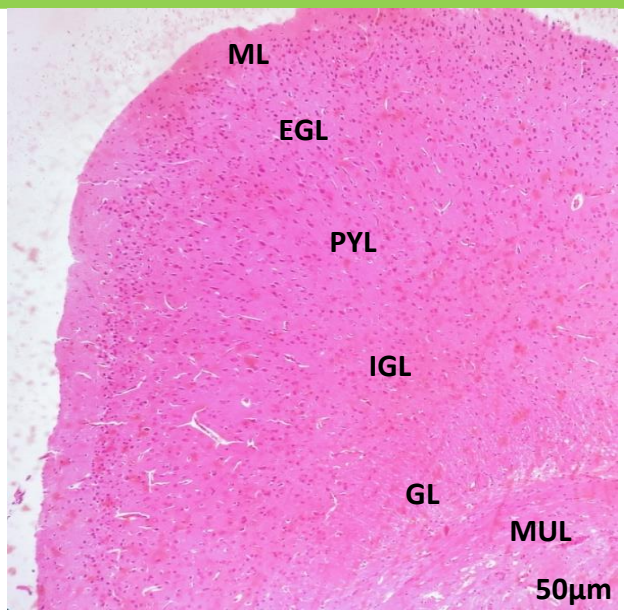

Figure 11: Transverse section of the cerebral cortex of rats of Group 11 which received 30 mg/kg bodyweight *Trimyristin* only for 9 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, Gl- ganglionic layer and MUL- multiform layer. Neurons are distributed in a background mesh work of neutrophils with no feature of significant inflammation or degenerative changes compared to Control Group 1.

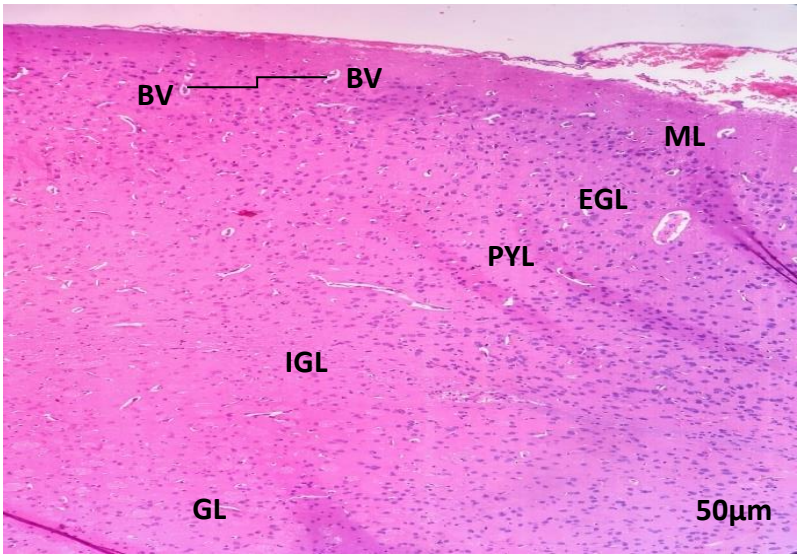

Figure 12: Transverse section of the cerebral cortex of rats of Group 13 which received 7.5 mg/kg bodyweight of MOF_6 for 6 weeks followed by 20 mg/kg bodyweight of Sodium arsenite co-administration with 7.5 mg/kg bodyweight of MOF_6 for 3 weeks. Haematoxylin and Eosin X 100. ML- molecular layer, EGL- external granular layer, PYL- pyramidal layer, IGL- internal Granular layer, Gl- ganglionic layer and BV- blood vessel. Histo-pathological evaluations showed normal histo-architecture of the cerebral cortex.