MO11 and MS06 ameliorated cadmium chloride-induced neuro-inflammation, hyperplasia and apoptosis via NF-kB/ Caspase-3/p53 pathway and down-regulated sVEGFR in rats

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SUMMARY

Cadmium is a neurotoxin, carcinogen and a suspected agent in aetiology of Parkinson's disease and Alzheimer's disease (AD). Furthermore, upregulations of Caspase-3 and p53 were reported in brains of AD patients. This study evaluated the neuroprotective potentials of MO11 (isolated from *Moringa oleifera* leaves) and MS06 (isolated from *Musa sapientum* suckers) in Cadmium Chloride (CdCl)-induced neurotoxicity in the cerebrum of rats.

Twenty-eight adult male wistar rats (average weight of 155 g) were randomly divided into 7 groups (n = 4). Group 1 received physiological saline. Groups 2-4 and 7 received single 1.5 mg/Kg bodyweight of CdCl (i.p.) (Day 1). Groups 3-4 and 7 were post-treated with 15 mg/Kg bodyweight of MO11, 15 mg/Kg bodyweight of MO11 + 7 mg/Kg bodyweight of MSF1 and 3.35 mg/Kg bodyweight of Doxorubicin respectively (Days 1-17). Groups 5-6 received only MO11 and Vegetable Oil (vehicle) respectively (Days 1-17). Cerebral histopathology (Cresyl Fast Violet method) was evaluated in rats. ELISA evaluations of biomarkers of pro-inflammation (IL-1β, IL-6, IL-8 and NF-kB), anti-inflammation (IL-4 and IL-10), apoptosis (Caspase-3 and p53), proliferation (Ki67) and angiogenesis (sVEGFR) in cerebral homogenates of rats were also conducted.

Histopathological evaluations showed a high number of chromatolytic cells in Group 2, compared with Groups 1 and 3-7. Post-

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treatments of CdCl-induced neurotoxicity with MO11 and MS06 resulted in decreased levels of IL-1β, IL-6, IL-8, NF-kB, Caspase-3, Ki67, p53 and sVEGFR, but increased levels of IL-4 and IL-10 in Groups 3-4, compared with Group 2. Therefore, MO11 and MS06 possess neuroprotective, neuroregenerative, anti-AD, anti-inflammatory and anticancer potentials.

Key words: Cadmium – Neurotoxicity – Alzheimer's disease – *Moringa oleifera* – *Musa sapientum* – Neuroprotection – Neuroregeneration

INTRODUCTION

Cadmium (Cd), according to the World Health Organization, is one of the 10 chemicals of concern for human health (Andjelkovic et al., 2019). Cd was classified as a human carcinogen by the National Toxicology program (Wang and Du, 2013) and the International Agency for Research on Cancer (Huff et al., 2007). Cd-induced toxicity resulted in systemic dysfunctions such as neurotoxicity (Wang and Du, 2013; Batool et al., 2019), skin alopecia and ulceration (Lansdown et al., 2001), inflammation and hepatotoxicity (Bernhoft, 2013; Wang and Du, 2013; Andjelkovic et al., 2019).

Human Cd-exposure is associated with dysfunctions of the nervous system resulting in symptoms such as impaired learning capacity, headache and vertigo, decreased cognitive functions, olfactory dysfunction, poor vasomotor functioning, parkinsonian-like symptoms, peripheral neuropathy and poor equilibrium and balance co-ordination (Wang and Du, 2013). Cd-exposure has also been suspected as an etiological factor in the development of Parkinson's disease (PD) and Alzheimer's disease (AD) (Wang and Du, 2013). Increased concentrations in total Cd-exposure was associated with dyslexia or learning difficulties, decreased visual motor capacity and mental retardation in children (Wang and Du, 2013). It is, therefore, scientifically relevant to develop drug candidates from plants or other sources which can prevent or eliminate resulting dysfunctions of the nervous system due to Cd-induced neurotoxicity.

Moringa oleifera (MO) and *Musa sapientum* (MS) are ethno-medicinal plants that are well grown in Nigeria (Akinlolu et al., 2021). Furthermore, MOF6, which was fractionated from MO leaves using column chromatography methods, showed significant antioxidant and neuro-protective potentials against Cuprizone-induced cerebellar damage in rats (Omotoso et al., 2018), as well as neuro-protective potentials against dysregulated Acetylcholinesterase concentrations in Sodium arsenite-induced neurotoxicity in rats (Akinlolu et al., 2020a). MOF6 equally showed hepato-protective, anti-proliferation and anti-drug resistance potentials in 7,12-Dimethylbenz[a]anthracene-induced hepato-toxicity in rats (Akinlolu et al., 2021). Similarly, MSF1, which was fractionated from MS sucker using column chromatography methods possesses hepatoprotective, antiproliferation and antidrug resistance potentials in 7,12-Dimethylbenz[a]anthracene-induced hepato-toxicity in rats (Akinlolu et al., 2021).

Cd-induced neuro-toxicity has been suggested to result from increased oxidative stress, dysregulation and dysfunction of neurotransmitters, estrogen-like effect, interactions with heavy metals such as zinc and cobalt and epigenetic effects (Wang and Du, 2013; Batool et al., 2019). The mechanism underlying Cd-induced neurotoxicity remains poorly understood and unresolved till date. What mechanism underlies Cd-induced neuroinflammation? What is the relationship between Cd-induced neuroinflammation and neuronal cell death? Cd is an established carcinogen (Wang and Du, 2013; Batool et al., 2019), and mutagenesis is associated with increased angiogenesis (Batchelor et al., 2009). What type of relationship exists amongst Cd-induced apoptosis, hyperplasia, angiogenesis and mutagenesis? Finally, what are the effects of post-treatments with MO and MS on the possible mechanisms underlying Cd-induced neurotoxicity, neuroinflammation, apoptosis, hyperplasia, angiogenesis and mutagenesis?

Cd generally exists as a divalent cation, complexed with other elements, such as Cadmium Chloride (CdCl) (Bernhoft, 2013; Andjelkovic et al., 2019). Cd-induced neurotoxicity possibly results from increased oxidative stress, and neuronal cell death (apoptosis) are cell-specific, with cerebral cortical neurons as main targets (Wang and Du,

2013). In this study, the most active antioxidant and antimicrobial cytotoxic compounds were isolated from MO leaves (MO11) and MS suckers (MS06) respectively. Therefore, in order to further understand the mechanisms underlying Cd-induced neurotoxicity and in order to determine the neuroprotective potentials of MO and MS, this study evaluated the effects of MO11 and MS06 on CdCl-induced neuroinflammation, apoptosis, hyperplasia, angiogenesis, mutagenesis and neurodegeneration in the cerebral cortices of adult male Wistar rats.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was sought and received from the Ethical Review Committee of the University of Ilorin, Nigeria. Appropriate measures were observed to ensure minimal pain or discomfort of rats used in this study. The ethical approval number is UERC/ASN/2018/1161. Furthermore, 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), the Directive 2010/63/Eu of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes and the Guidelines of the U.S. Public Health Service and NIH regarding the care and use of animals for experimentation (NIH publication #85-23, revised in 985).

Collection, authentication and deposition of *Moringa oleifera* (MO) leaves and *Musa sapientum* (MS) suckers

Freshly cut MO leaves and MS suckers were obtained locally from forest reserves in Ilorin and samples identified and authenticated by a Pharmaceutical Botanist of the Department of Botany, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. Samples of MO leaves and MS suckers were deposited at the herbarium of the Department of Botany, Faculty of Life Sciences, University of Ilorin, and assigned Herbarium Identification Numbers UILH/001/1249 and UILH/002/1182 respectively.

Extraction and partitioning of fractions of MO leaves and MS suckers

MO leaves were air-dried, grinded, weighed and stored in an air tight container until further analysis. 4.0 Kg weight of the MO leaves were powdered, extracted with distilled ethanol and concentrated on a rotary evaporator. Similarly, MS suckers were air-dried, grinded, weighed and stored in an air tight container until further analysis. 5.2 kg weight of the MS suckers were powdered, extracted with distilled ethanol and concentrated on a rotary evaporator. The crude extract (210.2 g) of MO leaves was successively partitioned into n-hexane (NH), dichloromethane (DCM), ethyl acetate (EA) and methanol (MeOH) soluble fractions in an increasing order of polarity to afford 12 fractions (MO1 – MO12). Similarly, the crude extract (159.32 g) of MS suckers was successively partitioned into n-hexane (NH), dichloromethane (DCM), ethyl acetate (EA) and methanol (MeOH) soluble fractions in an increasing order of polarity to afford 8 fractions (MS01 – MS08). Phytochemical screening of MS showed the presence of saponins, saponin glycosides, tannins, alkaloids and indole alkaloids.

Column Chromatography

Column chromatography of the MO and MS fractions was carried out on silica gel (70 – 230 and 240 – 300 mesh size, Merck, Germany), Merck alumina (70 – 230 mesh ASTM). Thin Layer Chromatography (TLC) was carried out on pre-coated silica gel 60 F_{254} aluminium foil (Merck, Germany) for the establishment of the purity of isolates. Spots on TLC were examined with an ultraviolet lamp operating at a wavelength of 366 nm for fluorescence and at 254 nm for fluorescence quenching spots.

Evaluations of antioxidant and antimicrobial activities of MO and MS fractions

Antioxidant activities of plants' extracts and fractions were evaluated using modified 2,2-diphenyl-1-picrylhydrazyl method as previously described by Chaves et al. (2020). In addition, antimicrobial activities of plants' extracts and fractions were evaluated by testing the cyto-toxic potentials of each fraction against the growths of *Escherichia*

coli and *Salmonella tiphimurium* as previously described by Elisha et al. (2017).

Purification of MO fractions

The antioxidant and antimicrobial activities of the obtained 12 MO fractions (MO1 – MO12) were determined. MO8 and MO11 fractions, which had the best antioxidant and antimicrobial potentials, were selected and further purified on a silica gel open column, using NH, DCM, EA and MeOH in an increasing order of polarity until the most active antioxidant and antimicrobial drug candidates ($MO11_{8,3}$ and $MO11_{8,4}$) were obtained. Phytochemical screenings of $MO11_{8,3}$ and $MO11_{8,4}$ showed the presence of flavonoids, saponin, tannins, alkaloids, glycosides and steroids. The resulting grams of $MO11_{8,3}$ and $MO11_{8,4}$ were mixed together as 1.43 g of MO11, which was used for the evaluations of neuro-protective potentials of MO against CdCl-induced neurotoxicity.

Purification of MS fractions

The antioxidant and antimicrobial activities of the obtained 8 MS fractions (MS01 – MS08) were determined. MS06, which had the best antioxidant and antimicrobial potential, was selected and further purified on a silica gel open column, using NH, DCM, EA and MeOH in an increasing order of polarity to afford seven fractions ($MS06_{1-7}$). $MS06_5$ showed the best antioxidant and antimicrobial potential out of the seven fractions. The resulting 1.24 g of $MS06_5$ was used for the evaluations of neuro-protective potentials of MS against CdCl-induced neurotoxicity.

Chemicals and Reagents

Cadmium Chloride (CdCl) was a product of Sigma-Aldrich Japan Co. (Tokyo, Japan), and was purchased from Bristol Scientific Company, Lagos State, Nigeria. Normal Saline, Doxorubicin and Vegetable Oil were purchased from Olabisi Onabanjo University Teaching Hospital pharmacy, Sagamu, Ogun State, Nigeria. Sucrose crystal produced by Qualikems and Methylated spirit produced by Samstella Industry Nigeria Limited, Abule- Oba, Ogun State, Nigeria were purchased from local suppliers.

Animal care and feeding

28 adult male Wistar rats (average weight of 155 g and 2 months of age) were purchased from a colony breed at Badagry in Lagos state, Nigeria. The rats were randomly divided into 7 groups with 4 rats per group. The rats were acclimatized for a week at the animal house of the Faculty of Pharmacy of Olabisi Onabanjo University, Sagamu campus, Ogun State, Nigeria before the beginning of experimental procedures. The rats were kept under standard conditions and allowed free access to food and drinking water ad libitum. The experimental procedure lasted for 18 Days. The bodyweights of the rats were measured and recorded on daily bases using electronic compact scale (SF-400C weighs in gram) weighing scale; a product of Valid Enterprise, Kalbadevi, Mumbai, India.

Grouping of rats and extracts/drugs administration

MO11 and MS11 were dissolved in Vegetable Oil (vehicle). Rats of Control Group 1 (Baseline Control) received physiological saline only for 17 Days (Days 1-17). Each rat of Experimental Groups 2–4 and 7 received single intra-peritoneal administration of 1.5 mg/Kg bodyweight CdCl on Day 1. Rats of Group 2 (Negative Control) were left untreated throughout experimental procedure for 17 Days (Days 1-17). Thereafter, rats of Group 3 were post-treated with 15 mg/Kg bodyweight of MO11 for 17 Days (Days 1-17). Rats of Group 4 were post-treated with combined mixture of 15 mg/Kg bodyweight of MO11 and 7 mg/Kg bodyweight of MS06 for 17 Days (Days 1-17). Rats of Group 5 received only 15 mg/Kg bodyweight of MO11 for 17 Days (Days 1-17). Rats of Group 6 received only 1 ml/Kg bodyweight of Vegetable Oil (vehicle) for 17 Days (Days 1-17). Rats of Group 7 were post-treated with 3.35 mg/Kg bodyweight of Doxorubicin (standard anticancer drug – Positive Control) for 17 Days (Days 1-17).

Histopathological evaluations of the cerebral cortex

Twenty-four hours after the last day of administration of drugs and extracts on Day 17 and following animal sacrifice, the cranium of each rat was exposed and the cerebrum removed. Each cerebrum was divided into two hemispheres. One cerebral hemisphere was fixed in 10% formalin and

processed for light microscopy using conventional histological procedures. Slices were stained with Cresyl fast violet and examined under the microscope for histopathological changes as earlier described (Omotoso et al., 2018). Photomicrographs of the slides were prepared.

Evaluations of concentrations of IL-1β, IL-4, IL-6, IL-8, IL-10, NF-kB, Caspase-3, Ki67, p53 and sVEGFR in homogenates of cerebral hemispheres of rats using enzyme linked immunosorbent assay (ELISA)

The second cerebral hemisphere from each rat was isolated and then subjected to thorough homogenization using porcelain mortar and pestle in ice-cold 0.25 M sucrose, in the proportion of 1 g to 4 ml of 0.25 M sucrose solution. The tissue homogenates were filled up to 5 ml with additional sucrose, and collected in a 5 ml serum bottle. Homogenates were thereafter centrifuged at 3000 revolution per minute (rpm) for 15 minutes using a centrifuge (Model 90-1). The supernatant was collected with Pasteur pipettes and placed in a freezer at -4°C, and thereafter assayed for concentrations of IL-1β, IL-4, IL-6, IL-8, IL-10, NF-kB, Caspase-3, Ki67, p53 and sVEGFR in the cerebral cortices of all rats of Control Group 1 and Experimental Groups 2 – 7 using ELISA technique as previously described (Akinlolu et al., 2021).

Statistical analyses

Statistical analyses were conducted using the 2019 Statistical Package for the Social Science software Version 23.0. Computed data of concentrations of each biomarker was expressed as arithmetic means ± standard error of mean, and were subjected to statistical analyses using One-way Analysis of Variance to test for significant difference amongst Groups 1-7. Degree of freedom (*df*): (between groups and within groups) and *F*-values were computed. Significant difference was confirmed at 95% confidence interval with associated *P* -value of less than 0.05 ($P \leq 0.05$). In addition, Scheffe Post-hoc analysis was used for separation of Mean values amongst Groups 1-7. The statistical comparison of the concentration of each biomarker between two groups was considered significant only at $P \leq 0.05$.

RESULTS

Histopathological evaluations of the pre-frontal cortices of rats

Histopathological evaluations showed normal histoarchitectures of the prefrontal cortices of rats of Control Group 1 and Experimental Groups 5 and 6 (Figs. 1A, 1B and 5A-6B), with normal staining intensity of Nissl substance, cellular density and delineation. Normal staining neurons (black arrows) present with intensively stained soma both peripherally and centrally. Chromatolytic neurons (red arrows) present with reduced staining intensity in the central or peripheral aspect of the soma of neuron. In contrast to normal stained neurons of rats of Control Group 1 and Experimental Groups 5 and 6 (Figs. 5A-6B), histopathological analyses of prefrontal cortices of rats of Experimental Group 2 showed Nissl substance with reduced staining intensity and high number of chromatolytic cells (Figs. 2A and 2B). In addition, histopathological analyses of prefrontal cortices of rats of Experimental Groups 3, 4 and 7 showed normal staining intensity of Nissl substance and cellular delineation but reduced cellular density and few chromatolytic cells (Figs. 3A-4B and 7A-7B).

Concentrations of IL-1β, IL-4, IL-6, IL-8, IL-10 and NF-kB in cerebral cortices of rats: CdCl-only treated Group 2 versus Normal Saline-only Control Group 1

Results showed statistically significant higher ($P \leq 0.05$) levels of IL-1β (df = 6,14, F = 52.34, $P < 0.001$), IL-6 (df = 6,16, F = 59.22, $P < 0.001$), IL-8 (df = 6,16, F = 127.64, $P < 0.001$) and NF-KB (df = 6,19, F = 36.41, $P < 0.001$) in rats of Group 2, when compared with Control Group 1 (Table 1, Figs. 8, 9, 10 and 11). In addition, results showed statistically non-significant lower ($P \geq 0.05$) levels of IL-4 (df = 6,16, F = 272.42, P = 0.74), but significant lower ($P \leq 0.05$) levels of IL-10 (df = 6,16, F = 272.42, $P < 0.001$) in rats of Group 2, when compared with Control Group 1 (Table 1, Figs. 12 and 13).

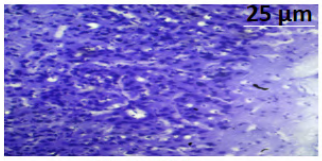


Figure 1A

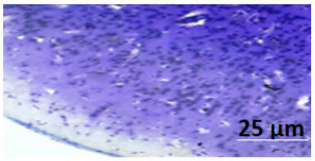


Figure 2A

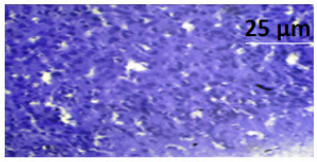


Figure 3A

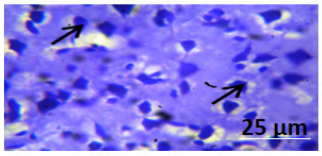
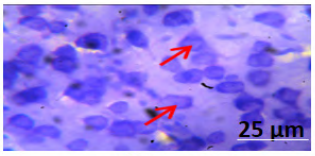


Figure 1B



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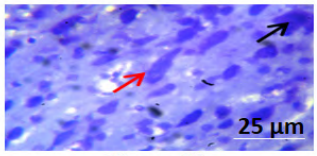


Figure 3B

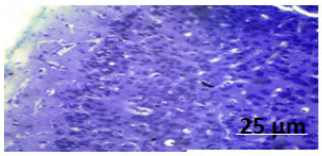


Figure 4A

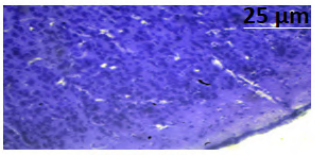


Figure 5A

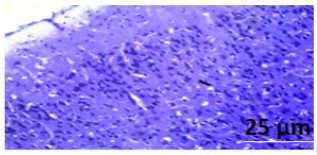


Figure 6A

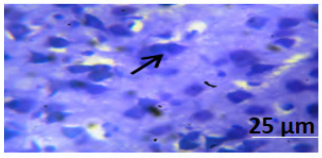


Figure 4B

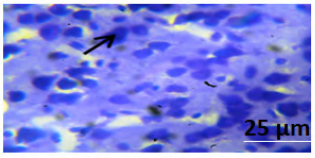


Figure 5B

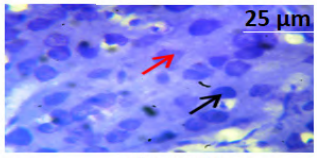


Figure 6B

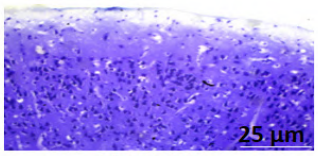


Figure 7A

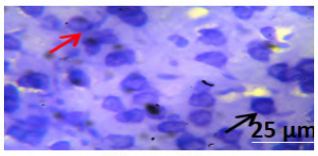


Figure 7B

Fig. 1.- Section of the dorsolateral prefrontal cortex of rats of control Group 1 which received only normal saline. This figure shows (**A**) Cresyl fast Violet x100; (**B**) Cresyl fast Violet x400. Black arrows represent normal neurons. Histopathological evaluation shows normal staining intensity of Nissl substance, cellular density and delineation. Scale bars = 25 µm. **Fig. 2.**- Section of the dorsolateral prefrontal cortex of rats of experimental Group 2 which received intraperitoneal (i.p.) injection of 1.5m/kg bodyweight of CdCl only. This figure shows (**A**) Cresyl fast Violet x100; (**B**) Cresyl fastViolet x400. Red arrows represent chromatolytic neurons. Histopathological evaluation shows Nissl substance with reduced staining intensity, but high number of chromatolytic cells. Scale bars = 25 µm. **Fig. 3.**- Dorsolateral prefrontal cortex of rats of experimental Group 3 which received 1.5 mg/kg bodyweight of CdCl (i.p.) and post-treatment with 15 mg/kg bodyweight of MO11. This figure shows (**A**) Cresyl fast Violet x100; (**B**) Cresyl fast Violet x400. Black arrow represents a normal neuron while red arrow represents a chromatolytic neuron. Histopathological evaluation shows normal staining intensity of Nissl substance but reduced cellular density and few chromatolytic cells. Scale bars = 25 µm. **Fig. 4.**- Section of the dorsolateral prefrontal cortex of rats of experimental Group 4 which received 1.5 mg/kg bodyweight of CdCl (i.p.) and post-treatment with 15 mg/kg bodyweight of MO11 + 7 mg/kg bodyweight of MS06. This figure shows (**A**) Cresyl fast Violet x100; (**B**) Cresyl fast Violet x400. Black arrow represents a normal neuron. Histopathological evaluation shows normal staining intensity of Nissl substance and cellular delineation but reduced cellular density and few chromatolytic cells. Scale bars = 25 µm. **Fig. 5.**- Section of the dorsolateral prefrontal cortex of rats of experimental Group 5 which received 15 mg/kg bodyweight of MO11 only. This figure shows (**A**) Cresyl fast Violet x100; (**B**) Cresyl fast Violet x400. Black arrow represents a normal neuron. Histopathological evaluation shows normal staining intensity of Nissl substance, cellular density and delineation. Scale bars = 25 µm. **Fig. 6.**- Section of the dorsolateral prefrontal cortex of rats of experimental Group 6 which received 1 ml/kg bodyweight of Vegetable Oil only. This figure shows (**A**) Cresyl fast Violet x100; (**B**) Cresyl fast Violet x400. Black arrow represents a normal neuron while red arrow represents a chromatolytic neuron. Histopathological evaluation shows normal staining intensity of Nissl substance, cellular density and delineation. Scale bars = 25 µm. **Fig. 7.**- Section of the dorsolateral prefrontal cortex of rats of experimental Group 7 which received 1.5 mg/kg bodyweight of CdCl (i.p.) and post-treatment with 3.35 mg/kg bodyweight of Doxorubicin. This figure shows (**A**) Cresyl fast Violet x100; (**B**) Cresyl fast Violet x400. Black arrow represents a normal neuron while red arrow represents a chromatolytic neuron. Histopathological evaluation shows normal staining intensity of Nissl substance, but reduced cellular density and few chromatolytic cells. Scale bars = 25 µm.

Table 1. Concentrations (ng/ml) of IL-1β, IL-4, IL-6, IL-8, IL-10 and NF-kB in cerebral cortices of rats.

Drug/Extract administered	IL-1β	IL-4	IL-6	IL-8	IL-10	NF-kB
Normal Saline only	80.83±5.75^{c}	31.54±4.11^{c}	99.26±3.99^{b}	63.79±6.22^{b}	35.75±3.44^{ab}	38.29±1.66^{c}
CdCl only	481.25±41.37^{a}	19.31±0.58^{c}	125.18±2.33^{a}	84.82±2.16^{a}	17.68±0.35^{d}	56.82±1.78^{a}
CdCl-exposure + MO11 post-treated group	312.75±8.13^{b}	24.56±0.84^{c}	97.52±0.99^{b}	65.67±1.03^{b}	6.48±0.88^{e}	39.12±1.5^{b}
CdCl-exposure + MO11 + MS06 post-treated group	302.38±4.84^{b}	32.88±1.07^{c}	100.25±2.51^{b}	29.43±1.58^{d}	28.91±1.97^{b}	39.32±1.71^{b}
MO11 only	293.67±21.36^{b}	154.57±13.09^{a}	97.72±1.48^{b}	58.73±3.05^{b}	58.88±3.11^{a}	38.5±1.31^{c}
Vegetable Oil only	249.75±40.62^{b}	109.03±11.27^{b}	97.68±1.43^{b}	44.18±1.49^{c}	25.34±2.01^{c}	37.5±1.13^{c}
CdCl-exposure + Doxorubicin post-treated group	291.33±27.54^{b}	15.65±2.35^{c}	101.71±1.78^{b}	62.3±0.43^{b}	2.4±0.21^{e}	44.98±4.54^{ab}

Results of One-way ANOVA from Days 1-17. Mean ± SEM across the columns between groups are significantly different with a>ab>b>c>d>e. (n = 4 per group).

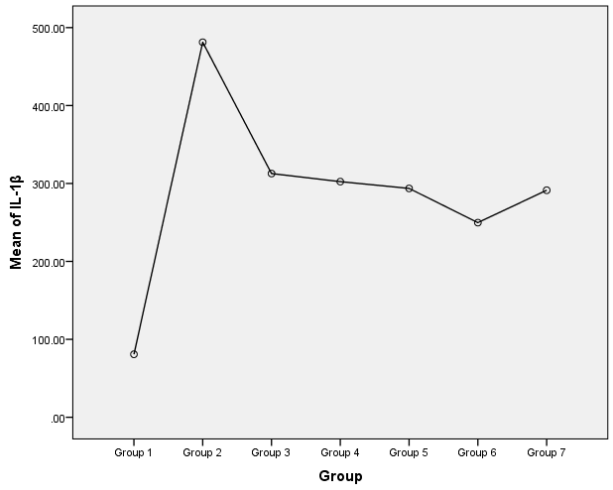


Figure 8.

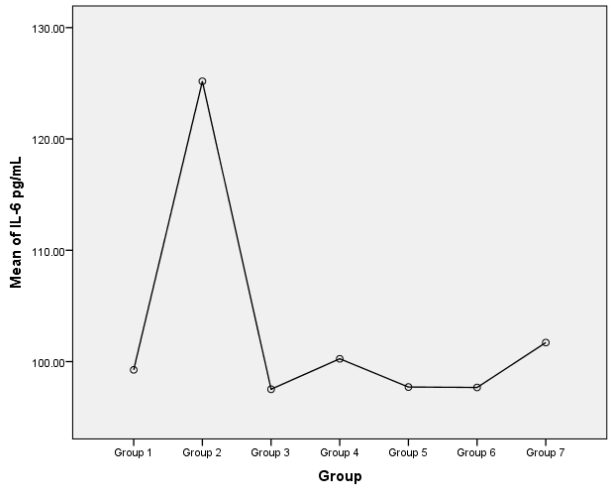


Figure 9.

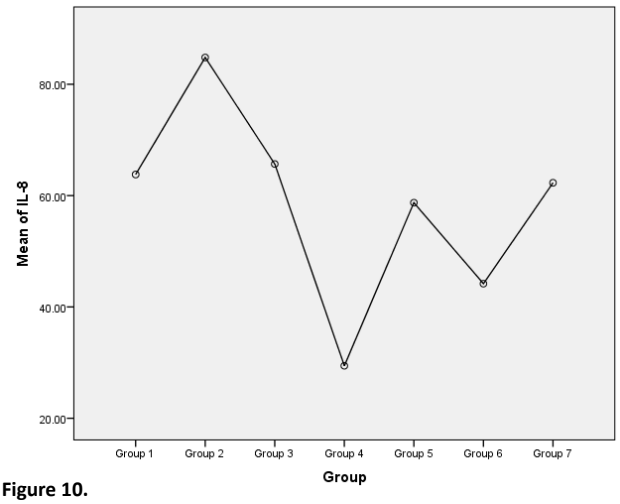


Figure 10.

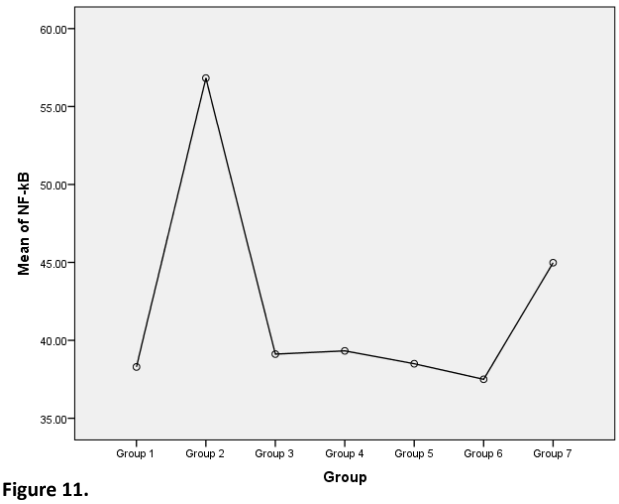


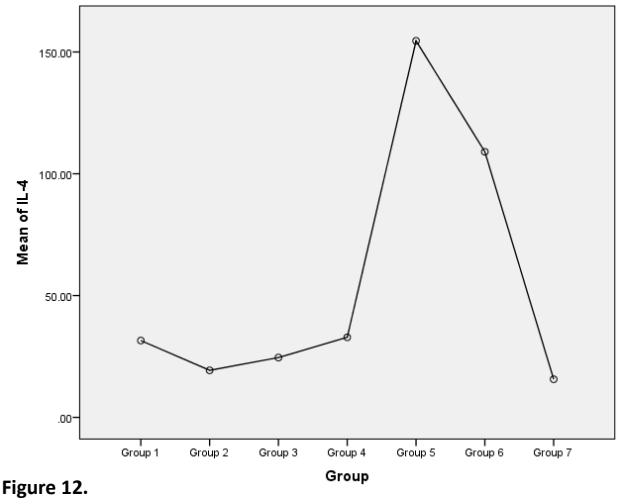
Figure 11.

Fig. 8.- Concentration (ng/ml) of IL-1β in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

Fig. 9.- Concentration (ng/ml) of IL-6 in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

Fig. 10.- Concentration (ng/ml) of IL-8 in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

Fig. 11.- Concentration (ng/ml) of NF-kB in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.



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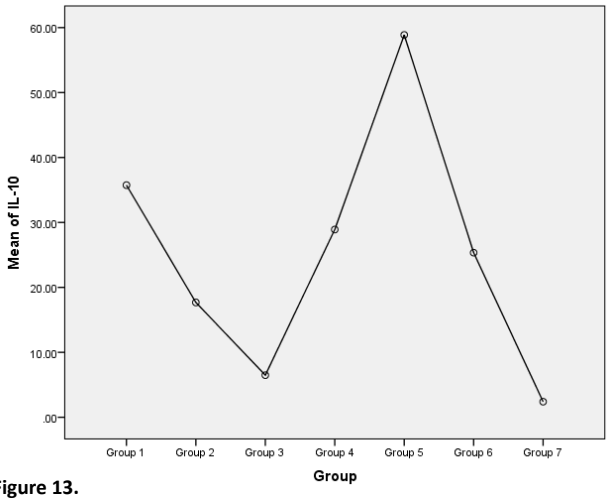

Figure 13.

Fig. 12.- Concentration (ng/ml) of IL-4 in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

Fig. 13.- Concentration (ng/ml) of IL-10 in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

Concentrations of Caspase-3, Ki67, p53 and sVEGFR in cerebral cortices of rats: CdCl-only treated Group 2 versus Normal Saline-only Control Group 1

Results showed statistically significant higher ($P \leq 0.05$) levels of Caspase-3 (df = 6,18, F = 21.11, $P<0.001$), Ki67 (df = 6,11, F = 8.95, P = 0.01) and sVEGFR (df = 6,20, F = 386.68, $P<0.001$), but statistically non-significant higher ($P \geq 0.05$) levels of p53 (df = 6,13, F = 8.54, P = 0.74) in rats of Group 2, when compared with Control Group 1 (Table 2 and Figs. 14-16).

Concentrations of IL-1β, IL-4, IL-6, IL-8, IL-10 and NF-kB in cerebral cortices of rats: CdCl-only treated Group 2 versus CdCl-exposure + MO11 post-treated Group 3 and CdCl-exposure + MO11 + MS06 post-treated Group 4

Results showed statistically significant higher ($P \leq 0.05$) levels of IL-1β (df = 6,14, F = 52.34, $P<0.001$) and (df = 6,14, F = 52.34, $P<0.001$), IL-6 (df = 6,16, F = 59.22, $P<0.001$) and (df = 6,16, F = 59.22, $P<0.001$), IL-8 (df = 6,16, F = 127.64, $P<0.001$) and (df = 6,16, F = 127.64, $P<0.001$) and NF-KB (df = 6,19, F = 36.41, $P<0.001$) and (df = 6,19, F = 36.41, $P<0.001$) in rats of Group 2, when

Table 2. Concentrations (ng/ml) of Caspase-3, Ki67, p53 and sVEGFR in cerebral cortices of rats.

Drug/Extract administered	Caspase-3	Ki67	p53	sVEGFR
Normal Saline only	121.56±23.71^{c}	1.94±1.05^{b}	4.58±0.37^{b}	114.21±7.53^{b}
CdCl only	799.22±9.86^{a}	5.13±0.61^{a}	18.31±10.80^{ab}	440.04±11.47^{a}
CdCl-exposure + MO11 post-treated group	396.25±281.18^{c}	2.88±0.07^{ab}	3.36±0.79^{b}	105.46±7.56^{b}
CdCl-exposure + MO11 + MS06 post-treated group	662.5±12.76^{ab}	2.51±0.10^{b}	6.42±2.36^{b}	104±11.48^{b}
MO11 only	643.28±35.52^{ab}	1.46±0.67^{b}	23.61±0.82^{ab}	87.33±10.10^{c}
Vegetable Oil only	452.19±14.93^{b}	4.0±0.51^{ab}	14.94±1.28^{ab}	102.13±14.54^{b}
CdCl-exposure + Doxorubicin post-treated group	657.29±44.26^{ab}	2.23±0.67^{b}	27.94±3.91^{a}	302.75±29.06^{ab}

Results of One-way ANOVA from Days 1-17. Mean ± SEM across the columns between groups are significantly different with a>ab>b>c>d>e. (n = 4 per group).

compared with Groups 3 and 4 respectively (Table 1 and Figs. 8-11). In addition, results showed statistically non-significant lower ($P \geq 0.05$) levels of IL-4 in rats of Group 2, when compared with Group 3 ($df = 6,16$, $F = 204.89$, $P = 0.99$) and Group 4 ($df = 6,16$, $F = 204.89$, $P = 0.59$) (Table 1 and Fig. 12). Furthermore, results showed statistically significant lower ($P \leq 0.05$) levels of IL-10 in rats of Group 2, when compared Group 3 ($df = 6,16$, $F = 272.42$, $P = 0.003$) and Group 4 ($df = 6,16$, $F = 272.42$, $P = 0.02$) (Table 1 and Fig. 13).

Concentrations of IL-1β, IL-4, IL-6, IL-8, IL-10 and NF-kB in cerebral cortices of rats: CdCl-only treated Group 2 versus CdCl-exposure + Doxorubicin post-treated Group 7

Results showed statistically significant higher ($P \leq 0.05$) levels of IL-1β ($df = 6,14$, $F = 52.34$, $P < 0.001$), IL-6 ($df = 6,16$, $F = 59.22$, $P < 0.001$), IL-8 ($df = 6,16$, $F = 127.64$, $P < 0.001$), IL-10 ($df = 6,16$, $F = 272.42$, $P < 0.001$) and NF-KB ($df = 6,19$, $F = 36.41$, $P < 0.001$), but non-significant higher ($P \geq 0.05$) levels of IL-4 ($df = 6,16$, $F = 272.42$, $P = 0.99$), in rats of Group 2, when compared with Group 7 (Table 1 and Figs. 8-13).

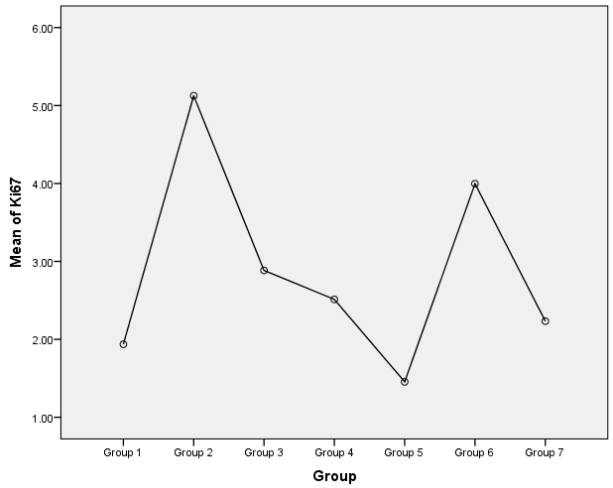

Figure 14.

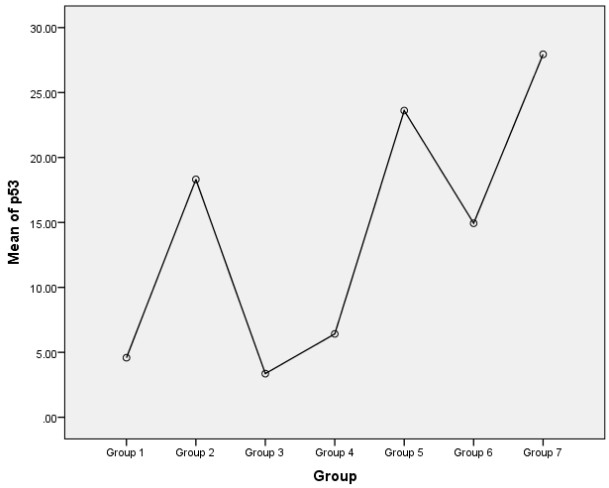

Figure 15.

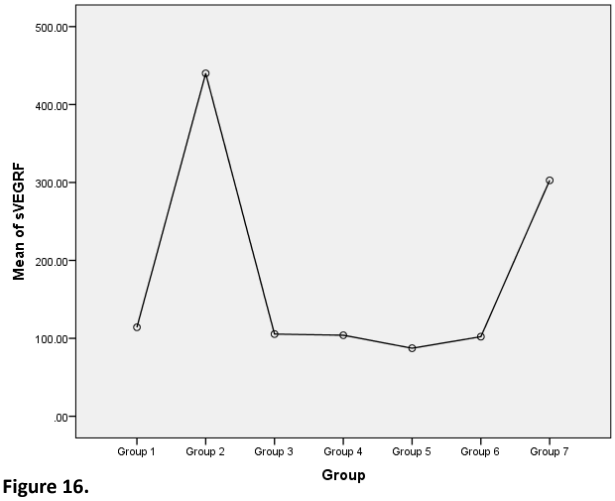

Figure 16.

Fig. 14.- Concentration (ng/ml) of Ki67 in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

Fig. 15.- Concentration (ng/ml) of p53 in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

Fig. 16.- Concentration sVEGFR in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post-treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post- treated group.

Concentrations of Caspase-3, Ki67, p53 and sVEGFR in cerebral cortices of rats: CdCl-only treated Group 2 versus CdCl-exposure + MO11 post-treated Group 3 and CdCl-exposure + MO11 + MS06 post-treated Group 4

Results showed statistically significant higher ($P \leq 0.05$) levels of Caspase-3 ($df = 6,18$, $F = 21.11$, $P = 0.04$) and non-significant higher ($P \geq 0.05$) levels of Caspase-3 ($df = 6,18$, $F = 21.11$, $P = 0.75$) in rats of Group 2, when compared with Groups 3 and 4 respectively (Table 2 and Fig. 17). In addition, results showed statistically non-significant higher ($P \geq 0.05$) levels of Ki67 ($df = 6,11$, $F = 8.95$, $P = 0.17$) and ($df = 6,11$, $F = 8.95$, $P = 0.08$); and p53 ($df = 6,13$, $F = 8.54$, $P = 0.23$) and ($df = 6,13$, $F = 8.54$, $P = 0.46$) in rats of Group 2, when compared with Groups 3 and 4 respectively (Table 2 and Figs. 14-15). Furthermore, results showed statistically significant higher ($P \leq 0.05$) levels of sVEGFR in rats of Group 2, when compared with Group 3 ($df = 6,20$, $F = 386.68$, $P < 0.001$) and Group 4 ($df = 6,20$, $F = 386.68$, $P < 0.001$) (Table 2 and Fig. 16).

Concentrations of Caspase-3, Ki67, p53 and sVEGFR in cerebral cortices of rats: CdCl-only treated Group 2 versus CdCl-exposure + Doxorubicin post-treated Group 7

Results showed statistically non-significant higher ($P \geq 0.05$) levels of Caspase-3 ($df = 6,18$, $F = 21.11$, $P = 0.72$), in rats of Group 2, when compared with Group 7 (Table 2 and Fig. 17). Furthermore, results showed statistically significant higher ($P \leq 0.05$) levels of Ki67 ($df = 6,11$, $F = 8.95$, $P = 0.03$) and sVEGFR ($df = 6,20$, $F = 386.68$, $P < 0.001$), but statistically significant lower ($P \leq 0.05$) levels of p53 ($df = 6,13$, $F = 8.54$, $P = 0.47$) in rats of Group 2, when compared with Group 7 (Table 2 and Figs. 14-16).

DISCUSSION

Chromatolysis of neurons involves the dispersal and redistribution of rough endoplasmic reticulum and polyribosomes (Nissl substance) from the central to the periphery of the perikaryon for

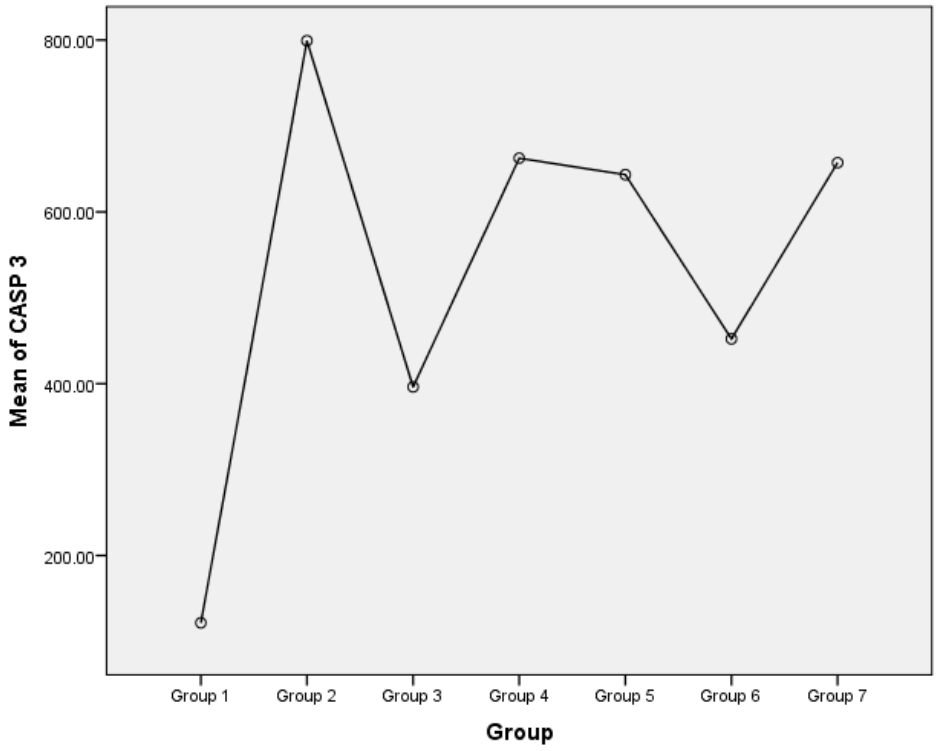

Fig. 17.- Concentration (ng/ml) of Caspase-3 in cerebral cortices of rats. Group 1: Normal Saline-only treated group. Group 2: CdCl-only treated group. Group 3: CdCl-exposure + MO11 post- treated group. Group 4: CdCl-exposure + MO11 + MS06 post-treated group. Group 5: MO11-only treated group. Group 6: Vegetable Oil-only treated group. Group 7: CdCl-exposure + Doxorubicin post-treated group.

increased protein synthesis in response to disruption of axonal transport, axotomy, metabolic nerve injuries, trauma or response to regenerative stimuli (Hanz and Fainzilber, 2006; Bhuiyan et al., 2018). The observed increase in number of chromatolytic cells in the prefrontal cortices of rats of CdCl-only treated Group 2 (Figs. 2A and 2B) confirms CdCl-induced neurotoxicity, chromatolysis and neurodegeneration. In addition, the decreased number of chromatolytic cells in the prefrontal cortices of rats post-treated with MO11 (Group 3), MO11 + MS06 (Group 4) and Doxorubicin (Group 7) (Figs. 3A – 4B and 7A – 7B) following CdCl-exposure indicates that MO11, MS06 and Doxorubicin possess neuroprotective potentials and were able to gradually reverse CdCl-induced chromatolysis and neurodegeneration within 17 days.

Is CdCl-induced chromatolysis associated with neuro-inflammation? Hanz and Fainzilber (2006) opined that axotomy of peripheral nerve resulted in increased expressions of ciliary neurotrophic factor and interleukins (LIF and IL-6) and activation of the JAK-STAT3 signalling pathway. Results showed significant upregulation of IL-6 in rats of CdCl-only treated Group 2, when compared with Normal saline-only treated Control Group 1, CdCl-exposure + MO11 post-treated Group 3, CdCl-exposure + MO11 + MS06 post-treated Group 4 and CdCl-exposure + Doxorubicin post-treated Group 7 (Table 1 and Fig. 9). Chromatolysis is associated with axotomy, hence these observations possibly indicate the involvement of IL-6 in CdCl-induced chromatolysis. It is equally possible that the observed anti-chromatolytic potentials of MO11, MS06 and Doxorubicin are associated with the regulation of IL-6 expression.

What mechanism underlies CdCl-induced neuroinflammation? Neuroinflammation involves the actuation of microglia cells leading to the release of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, TNFα and NF-kB (Shih et al., 2015; Akinlolu et al., 2020b). NF-kB upregulation results in the release and increased levels of other pro-inflammatory cytokines; hence NF-kB is opined to be the central regulator of neuroinflammation (Shih et al., 2015). Furthermore, the central nervous system environment is anti-inflammatory with high levels of anti-inflammatory cytokines such as IL-4 and IL-10. IL-4 and IL-10 inhibit the release of NF-kB and pro-inflammatory cytokines, and are involved in the resolution of neuroinflammation (Shih et al., 2015). Previous studies reported Cd-induction of inflammation via upregulations of IL-1β, IL-6 and IL-8 in rats, but downregulations of IL-4 in mice (Ebrahimi et al., 2020).

Similarly, results of this study showed significant upregulations of IL-1β, IL-6, IL-8 and NF-kB, but downregulations of IL-4 and IL-10 in rats of CdCl-only treated Group 2, when compared with Normal saline-only treated Control Group 1 (Table 1 and Figs. 8-13). These observations confirm CdCl-induction of neuro-inflammation, and equally indicate that CdCl-induced neuroinflammation possibly occurs via the NF-kB pathway.

Do MO11, MS06 and Doxorubicin have neuroprotective potentials against CdCl-induced neuroinflammation? Post-treatments of CdCl-induced neuroinflammation in rats with MO11 (Group 3), MO11 + MS06 (Group 4) and Doxorubicin (Group 7) resulted in significant downregulations of pro-inflammatory cytokines (IL-1β, IL-6, IL-8 and NF-kB), but upregulations of anti-inflammatory cytokines (IL-4 and IL-10), when compared with CdCl-only treated Group 2 (Table 1 and Figs. 8-13). These observations indicate that MO11, MS06 and Doxorubicin possess anti-neuro-inflammatory potentials which were possibly mediated via the NF-kB pathway.

What is the relationship between Cd-induced neuro-inflammation and neuronal cell death (apoptosis)? NF-kB is pro-apoptotic and its upregulation is associated with increased release of p53 and c-myc as well as induction of apoptosis (Barkett and Gilmore, 1999). Ryan et al., 2000 noted that loss or blockage of NF-kB activities resulted in loss of p53-induction of apoptosis, confirming the role of NF-kB in p53-induced apoptosis. In addition, significant upregulation of Caspase-3 is associated with activation of both the intrinsic mitochondrial pathway and the extrinsic death-receptor pathway of apoptosis (Akinlolu et al., 2020b). Hence, significant upregulation of Caspase-3 is associated with promotion of apoptosis. In addition, Genchi et al. (2020) reported Cd-induction of significant upregulation

of Caspase-3, while Huff et al. (2007) reported significant upregulation of p53 in Cd-induced toxicity in *in vivo* and *in vitro* experimental studies.

The findings of this study showed significant upregulations of NF-kB, Caspase-3 and p53 in CdCl-only treated Group 2, when compared with Normal Saline-only treated Control Group 1 (Tables 1, 2 , Figs. 11, 15 and 17). These observations indicate CDCl-induction of apoptosis via the NF-kB/Caspase-3/p53 pathway.

Do MO11 and MS06 have neuro-protective potentials against CdCl-induced neuro-apoptosis? Post-treatments of CdCl-induced neuro-apoptosis in rats with MO11 (Group 3) and MO11 + MS06 (Group 4) resulted in downregulations of NF-kB, Caspase-3 and p53 (Tables 1, 2 and Figs. 11, 15 and 17). These observations indicate that MO11 and MS06 resolved CdCl-induced apoptosis via the NF-kB/Caspase-3/p53 pathway. In addition, these observations indicate that MO11 and MS06 are possible anticancer drug compounds of clinical interest, as opined by Barkett and Gilmore (1999), who noted that drug compounds which interfere with the apoptotic role of Rel/NF-kB transcription factors could have therapeutic and clinical anticancer potentials.

Is there any possible association between CdCl-induced neuronal cell death and neuro-degeneration? Previous studies reported upregulations of Caspase-3 and p53 in brain of AD patients (Seo and Park, 2020). The observed upregulations of Caspase-3 and p53 in rats of CdCl-only treated Group 2, when compared with Normal Saline-only treated Control Group 1 (Table 2, Figs. 15 and 17) confirm Cd-induced toxicity as a possible aetiological factor in the development of AD and neurodegenerative diseases.

Can MO11 and MS06 reverse CdCl-induced neuro-degeneration? The observed downregulations of Caspase-3 and p53 in CdCl-exposed rats post-treated with MO11 (Group 3) and MO11 + MS06 (Group 4), when compared with CdCl-only treated Group 2 (Table 2, Figs. 15 and 17) indicate that MO11 and MS06 reversed CdCl-induced neurodegeneration and possess neuroregenerative potentials.

Does Doxorubicin have neuroprotective potentials against CdCl-induced p-53 dependent apoptosis and neurodegeneration? Results showed significant upregulation of p53 in rats of Doxorubicin-treated group 7, when compared with CdCl-only treated Group 2 (Table 2 and Fig. 15). These observations indicate that Doxorubicin did not reverse CdCl-induced p-53 dependent apoptosis and neurodegeneration.

Is there any relationship between CdCl-induced apoptosis, hyperplasia and mutagenesis? Genchi et al. (2020) noted that Cd-induced apoptosis is p-53 dependent. However, Genchi et al. (2020) equally reported that, while some Cd-treated cells undergo apoptosis, the remaining cells acquire apoptotic resistant capability resulting in increased development of pre-neoplastic cells, neoplastic cells and hyperplasia. Similarly, Prajapati et al. (2014) reported Cd-induced increased Ki67-index and hyperplasia in rats. The results of this study showed upregulation of Ki67 in rats of Cdcl-only treated Group 2, when compared with Normal Saline-only treated Control Group 1 (Table 2 and Fig. 14). This observation confirms CdCl-induction of hyperplasia and mutagenesis, and equally confirms that CdCl-induced hyperplasia, mutagenesis and apoptosis are intimately related.

Do MO11 and MS06 and Doxorubicin have neuroprotective potentials against CdCl-induced hyperplasia and mutagenesis? Post-treatments of CdCl-induced neuroapoptosis in rats with MO11 (Group 3) and MO11 + MS06 (Group 4) resulted in downregulations of Ki67 (Table 2 and Fig. 14). These observations indicate that MO11, MS06 and Doxorubicin possess anti-proliferation potentials.

Cd is an established carcinogen (Wang and Du, 2013; Batool et al., 2019), and mutagenesis is associated with increased angiogenesis (Batchelor et al., 2009). What then is the relationship between Cd-induced mutagenesis and increased angiogenesis in the cerebrum? VEGF is an established angiogenic factor, and abnormal VEGF upregulation is associated with increased angiogenesis, cancer, metastasis, ischemia and inflammation (Mahoney et al., 2021). Results showed significant upregulation of VEGFR in rats of CdCl-only treated Group 2, when compared with Normal Saline-only treated rats Control Group 1 (Table 2 and Fig. 16). This observation confirms

CdCl-induced promotion of angiogenesis, and equally confirms a relationship between CdCl-induced mutagenesis and angiogenesis.

Do MO11, MS06 and Doxorubicin have neuro-protective potentials against CdCl-induced increased angiogenesis? Results showed significant upregulation of sVEGFR in rats of CdCl-only treated Group 2, when compared with rats post-treated with MO11 (Group 3), MO11 + MS06 (Group 4) and Doxorubicin (Group 7) following CdCl-exposure (Table 2 and Fig. 16). These observations indicate that MO11, MS06 and Doxorubicin possess neuro-protective and anti-angiogenesis potentials.

CONCLUSION

The findings of this study suggest that post-treatments with MO11 (isolated from *Moringa oleifera* leaves) and MS06 (isolated from *Musa sapientum* suckers) conferred a degree of neuro-protection against CdCl-induced neurotoxicity and resulted in decreased levels of IL-1β, IL-6, IL-8, NF-kB, Caspase-3, Ki67, p53 and sVEGFR, but increased levels of IL-4 and IL-10 in rats. These observations indicate that MO11 and MS06 ameliorated CdCl-induced neurotoxicity, neuroinflammation, apoptosis, hyperplasia, angiogenesis, mutagenesis and neurodegeneration via the NF-kB/Caspase-3/p53 pathway. Hence, MO11 and MS06 possess neuroprotective potentials and are recommended for further evaluations as potential drug candidates for the treatments of neuroinflammatory and neurodegenerative diseases, as well as cancers.

However, post-treatments of CdCl-induced neurotoxicity with standard anticancer drug Doxorubicin resulted in decreased levels of IL-1β, IL-6, IL-8, NF-kB, Caspase-3, Ki67 and sVEGFR, but increased levels of p53, IL-4 and IL-10 in rats. These observations indicate that Doxorubicin ameliorated CdCl-induced neurotoxicity, neuroinflammation, hyperplasia, angiogenesis, but not p53-dependent apoptosis and neurodegeneration. Therefore, MO11 and MS06 possess better neuroprotective potentials against CdCl-induced neurotoxicity, when compared with Doxorubicin.

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