

## NEUROPROTECTIVE POTENTIALS OF EXTRACTS FROM *MORINGA OLEIFERA* AND *MUSA SAPIENTUM* AGAINST CADMIUM CHLORIDE-INDUCED NEUROTOXICITY IN RATS CEREBRI

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### Abstract

Cadmium (Cd)-exposure in humans causes nervous system dysfunctions. In rats, Cd-exposure resulted in increases of nitric oxide and lipid peroxidation levels in the hippocampus. This study evaluated the neuroprotective potential of active drug compounds extracted from *Moringa oleifera* leaves (MO11) and *Musa sapientum* suckers (MS06) in cadmium chloride (CdCl<sub>2</sub>)-induced neurotoxicity in rats. Adult male Wistar rats totalling 24 in number, were grouped randomly into six with four rats per group. Group 1 served as the control. An intraperitoneal single-dose of CdCl<sub>2</sub> was administered to rats of Groups 2 to 4 and 6 on Day 1. MO11-dose, MO11+MS06-doses, and Doxorubicin-dose were respectively administered to rats of Groups 3, 4, and 6 for post-treatment of CdCl<sub>2</sub>-induced neurotoxicity. Rats of Group 5 were administered Olive Oil-dose (vehicle) for 17 days. Tissue concentrations of catalase, superoxide dismutase, cyclo-oxygenase-2 and cytochrome P450 in rats' cerebri were determined using ELISA. Statistical analyses ( $p \leq 0.05$ ) of data were conducted using the Mann-Whitney U Test. Results showed increased catalase levels, similar superoxide dismutase levels, decreased cytochrome P450 levels and decreased cyclo-oxygenase-2 levels in rats of Groups 3, 4, and 6 in comparison with Group 2. The tested extracts impacted some levels of neuroprotection, neuroregeneration, antioxidant and anticancer capacities against neurotoxicity caused by CdCl<sub>2</sub> exposure.

**Keywords:** Cadmium, *Moringa oleifera*, *Musa sapientum*, neuroprotection, neurotoxicity

## Introduction

The World Health Organization classified cadmium (Cd) as one of ten (10) unsafe chemicals for human health (Andjelkovic et al. 2019). The International Agency for Research on Cancer and the National Toxicology Program further classified Cd as a carcinogen (Huff et al. 2007). Exposures to Cd caused functional anomalies, including hepatotoxicity and inflammation (Andjelkovic et al. 2019), and neurotoxicity (Schad et al. 2003, Batool et al. 2019).

Cd-exposure in humans causes nervous system dysfunctions producing symptoms such as developments of Parkinsonian-like symptoms and Alzheimer's disease, decreased cognitive functions, impaired learning capacity, headache, vertigo, poor vasomotor functioning, olfactory dysfunction, poor equilibrium, poor balance co-ordination, and peripheral neuropathy (Schad et al. 2003). In children, increased levels in total Cd-exposure caused decreased visual motor capacity, mental retardation, and dyslexia (Schad et al. 2003). In rats, Cd-exposure resulted in increases of nitric oxide and lipid peroxidation levels in the hippocampus (Lamtai et al. 2018). It will therefore, be of scientific relevance to develop herbal extracts and drug candidates that can prevent or mitigate the functional anomalies of the nervous system caused by exposures to Cd.

*Musa sapientum* (MS) and *Moringa oleifera* (MO), which are readily available plants in Nigeria, have been confirmed in many studies to have possible ethnomedicinal and therapeutic potentials (Akinlolu et al. 2021). Our research team previously fractionated MOF6 from MO leaves using column chromatography techniques (Omotoso et al. 2018). Omotoso et al. 2018 reported that MOF6 conferred significant neuroprotection and antioxidant potential against cuprizone-induced cerebellar damage in rats. Akinlolu et al. 2020 also reported that MOF6 conferred a degree of neuroprotection which ameliorated disruptions acetylcholinesterase levels in neurotoxicity caused by exposures to sodium arsenite in rats. In rats, MOF6 conferred a degree of anti-proliferation, anti-drug resistance capability, and hepatoprotection against hepatotoxicity caused by exposures to 7,12-Dimethylbenz[a]anthracene (Akinlolu et al. 2021). Furthermore, Akinlolu et al. 2021 disclosed that MSF1, which was fractionated from MS suckers using column chromatography techniques, conferred a degree of anti-drug resistance capacity, anti-proliferation, and hepatoprotection against hepatotoxicity caused by exposures to 7,12-Dimethylbenz[a]anthracene in rats.

The clear understanding of how exposures to Cd result in neurotoxicity is yet to be well deduced. However, studies suggested that Cd-induced neurotoxicity probably results from elevated dysfunctions of neurotransmitters and oxidative stress, interactions with heavy metals, i.e., cobalt and zinc, with accompanied epigenetic effects, as well as estrogen-like effect (Schad et al. 2003, Batool et al. 2019). Generally, Cd occurs as a bivalent, positively charged molecule, usually coupled to another element, for example, cadmium chloride (CdCl<sub>2</sub>) (Andjelkovic et al. 2019). Thus, in order to understand how exposures to Cd result in neurotoxicity, and to test neuroprotective capacities of plant' extracts, this study evaluated the effects of MO11 (extracted from *Moringa oleifera* leaves) and MS06 (extracted from *Musa sapientum* suckers) in cadmium chloride (CdCl<sub>2</sub>)-produced neurotoxicity in rats.

## Materials and Methods

### Ethical considerations

The ethical acceptance number for this study as obtained from the University of Ilorin's Ethical Review Committee is UERC/ASN/2018/1161. Thereafter, experimental procedures of this study were conducted in accordance with the internationally accepted principles for laboratory animal use and care.

### Allocation of Herbarium Identification Numbers (HIN)

Leaves of MO and suckers of MS were freshly cut and collected from different plantations in Ilorin, the capital city of Kwara State in the North Central region of Nigeria. The MO leaves and MS suckers were authenticated at the Department of Botany of the University of Ilorin. Thereafter, the MO leaves were allotted HIN: UILH/001/1249, while the MS suckers were allotted HIN: UILH/002/1182.

### ***Antioxidant and cytotoxic capacities of MO and MS extracts***

The amended 2,2-diphenyl-1-picrylhydrazyl technique outlined by Chaves et al. 2020 was employed to evaluate the antioxidant activities of MO and MS extracts. In addition, the antimicrobial and cytotoxic potentials of MO and MS extracts were determined via evaluations of the cytotoxic effects of each extract against the growths of *Salmonella typhimurium* and *Escherichia coli* using the methods earlier outlined by Elisha et al. 2017.

### ***Isolations of MO11 from MO leaves and MS06 from MS suckers***

In this study, following a series of column chromatography and liquid chromatography-mass spectrometry methods accompanied with evaluations of antioxidant and antimicrobial cytotoxicity effects of plants' extracts, MO11 was isolated as the main drug compound from MO leaves while MS06 was isolated as the main drug compound from MS suckers (Akinlolu et al. 2023, Ameen and Akinlolu, 2023).

### **Animals**

Twenty-four (24) adult male Wistar rats, which were two months old and of average weight of 155 g, were procured from a rat breeding center at Badagry in Lagos state of Nigeria. All rats were acculturated for a week, and thereafter grouped randomly into six with four rats per group and kept in standard laboratory conditions. Consequently, the body weights of rats in grams were computed every day.

### **Design of experimental procedures**

Olive Oil was used as the vehicle to dissolve MO11 and MS06. Physiological saline (only) was administered to rats of Control Group 1 for 17 Days (Days 1-17). For the induction of CdCl<sub>2</sub>-toxicity, the dose of 1.5 mg/kg body weight of CdCl<sub>2</sub> which was previously employed for induction of CdCl<sub>2</sub>-induced testicular toxicity in rats was used in this study (Kawaguchi et al. 2005, Akinlolu et al. 2023). Similarly, the doses of MS06 extract, MO11 extract and Doxorubicin used in the present study were the same as doses employed in our previous studies, which compared the cytoprotective capacities of MO11 and MS06 extracts in CdCl<sub>2</sub>-induced toxicity in comparison with Doxorubicin in rats (Akinlolu et al. 2023, Ameen et al. 2023).

1.5 mg/kg body weight of CdCl<sub>2</sub> (single dose) was given via intraperitoneal administration to each rat of Experimental Groups 2-4 and 6 (Sigma-Aldrich, Japan Co.) on Day 1. No further post-treatment was given to rats of Group 2 (Negative Control) all through Days 1-17. Consequently, 15 mg/kg body weight of MO11 was orally administered to each rat of Group 3 for 17 Days (Days 1-17) for the treatment of CdCl<sub>2</sub>-induced toxicity. Similarly, the added doses of 15 mg/kg body weight of MO11 and 7 mg/kg body weight of MS06 was orally administered to each rat of Group 4 for 17 Days (Days 1-17) for the treatment of CdCl<sub>2</sub>-induced toxicity. The rats of Group 5 were not exposed to administration of CdCl<sub>2</sub> throughout the experimental procedure. However, 1 ml/kg body weight of Olive Oil (vehicle) was orally administered to each rat of Group 5 for 17 Days (Days 1-17). Furthermore, single dose of 3.35 mg/kg body weight of the standard anticancer drug (Doxorubicin) was given via intravenous administration to each rat of Group 6 (Positive Control) for the treatment of CdCl<sub>2</sub>-induced toxicity.

At the end of experimental procedures, each rat was sacrificed by cervical dislocation without anesthesia because concentrations of biomarkers to be evaluated in the present study may be endogenously modified by anesthetic agents demanding post-experimental control of confounding factors (Ogunwobi et al. 2012, Omotoso et al. 2018).

## Tissue-biochemical analyses of levels of Catalase (CAT) and Superoxide dismutase (SOD) in rat cerebrum

Tissue-biochemical analyses of levels of CAT and SOD in rat cerebrum were evaluated using standard spectrometric methods of Sinha, 1972, and Misra and Fridovich (1972), respectively, as modified by Akinlolu et al. 2013.

### Tissue-ELISA analyses of Cyclo-oxygenase-2 (COX-2) and P450 in rat cerebrum

Tissue-ELISA analyses of levels of COX-2 and P450 in rat cerebrum using ELISA technique as described by Akinlolu et al. 2023. The ELISA kits for COX-2 and P450 were manufactured by CUSABIO Technology LLC, Houston, USA. Absorbance read at the wavelength of 450 nm using the AgileReader™ ELISA plate reader.

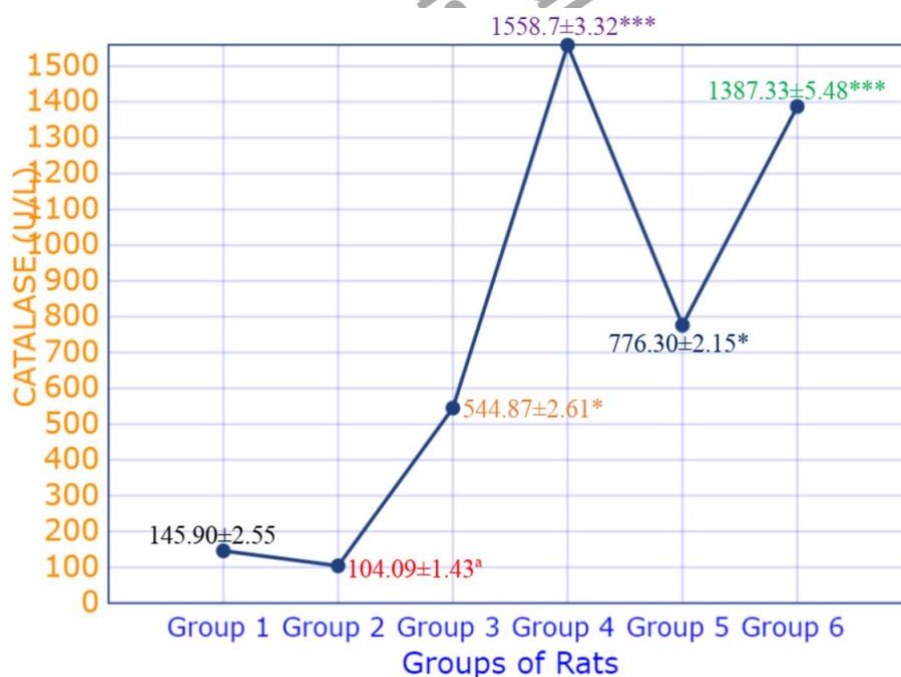
### Analyses of computed data

The concentration of each of CAT, SOD, COX-2, and P450 was presented as arithmetic means  $\pm$  standard deviation. The statistical comparison of the level of each biomarker between two groups was conducted using the Mann-Whitney U test (Wilcoxon-Mann-Whitney Test, 2016). Mann-Whitney U test was chosen because the total number of rats (sample size = 24) employed in this study is not up to 30. 95% confidence interval with  $p \leq 0.05$  was used to determine statistical significant difference.

## Results and Discussion

### 1. Concentrations of CAT in the cerebrum of rats

There were statistically non-significant lower levels of CAT in rats of the CdCl<sub>2</sub>-only treated Group 2, when compared with the normal saline-treated Control Group 1 ( $p = 1.00$ ) (Figure 1). In addition, results showed significantly higher levels of CAT in the CdCl<sub>2</sub>-exposed + MO11 + MS06 post-treated Group 4 ( $p < 0.001$ ), the Olive Oil-only treated Group 5 ( $p = 0.03$ ) and the CdCl<sub>2</sub>-exposed + Doxorubicin post-treated Group 6 ( $p < 0.001$ ), when compared with Group 2. An increase in CAT levels was also observed in the CdCl<sub>2</sub>-exposed + MO11 post-treated Group 3, when compared to Group 2, although statistically non-significant ( $p = 0.21$ ).



**Figure 1.** Concentrations (mean $\pm$ SD) of Catalase (U/L) in cerebrum of rats.

a - significant difference compared with the normal saline-treated control Group 1 at  $p \leq 0.05$ .

\* $p \leq 0.05$  and \*\*\* $p < 0.001$  - significant difference compared with CdCl<sub>2</sub>-only treated Group 2.

In our previous study by Akinlolu et al. 2022, we reported that CdCl<sub>2</sub>-induced neurotoxicity caused an abnormal increase in the population of chromatolytic cells with accompanied neurodegeneration of the prefrontal cortices of rats of Group 2 exposed only to CdCl<sub>2</sub> without further treatment. Contrariwise, there were decreased populations of chromatolytic cells accompanied with evident neuroregeneration in the prefrontal cortices of rats exposed to CdCl<sub>2</sub> but further post-treated with MO11, MO11 + MS06, as well as Doxorubicin (Akinlolu et al. 2022). These findings suggest that MO11, MS06, and Doxorubicin conferred neuroprotection against CdCl<sub>2</sub>-induced neurotoxicity, which resulted in gradual amelioration and reversal of CdCl<sub>2</sub>-induced neurodegeneration and chromatolysis in less than three weeks (Akinlolu et al. 2022).<sup>1</sup>

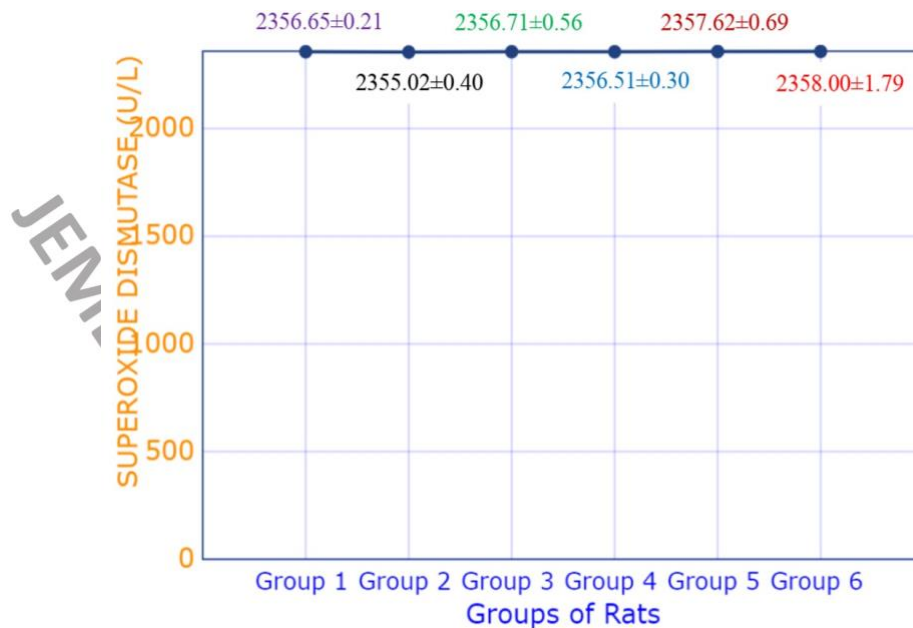
CAT and SOD are antioxidant enzymes, which catalyse the conversion of hydrogen peroxide to water and molecular oxygen, and break down potentially harmful oxygen molecules in cells (Akinlolu et al. 2013, Younus et al. 2018). CAT and SOD, therefore, protect against tissue damage by scavenging free radicals and reversing the effects of oxidative stress. CAT is a peroxisomal marker enzyme and the role of brain CAT in ethanol oxidation as well as in central nervous system disorders due to hereditary peroxisomal diseases such as Zellweger syndrome has been reported (Schad et al. 2003). There was marked cytoplasmic staining of CAT mRNA in a multitude of neurons in the rat brain using tyramine/CARD (catalyzed reporter deposition)-enhanced nonradioactive *in situ* hybridization protocol (Schad et al. 2003). Hence, evaluation of CAT levels in the cerebrum is of interest in neuroregenerative studies.

The decreased CAT level in the CdCl<sub>2</sub>-only treated Group 2, in comparison with the normal saline-only treated Control Group 1 (Figure 1) confirmed CdCl<sub>2</sub>-induction of oxidative stress and reduction of antioxidant enzymes levels. This observation is in agreement with the observations of earlier studies which reported Cd-induced oxidative stress with accompanied increased levels of nitric oxide and lipid peroxidation (Lamtai et al. 2018), but decreased CAT levels (Elkhadragey et al. 2018). However, post-treatments of CdCl<sub>2</sub>-induced cerebral oxidative stress with MO11, MS06 and Doxorubicin showed higher cerebral CAT levels in the CdCl<sub>2</sub>-exposed + MO11 post-treated Group 3, the CdCl<sub>2</sub>-exposed+ MS06 post-treated Group 4, the Olive Oil-treated Group 5, and the Doxorubicin-treated Group 6, when compared with the normal saline-treated Group 1 (Figure 1), confirming the pro-antioxidant potential of the extracts, Olive Oil and Doxorubicin.

Similarly, post-treatments with MO11, MS06, and Doxorubicin resulted in significant elevated levels of CAT in the cerebrum of rats of Groups 3, 4, and 6 respectively, when compared with Group 2 (Figure 1), confirming their antioxidant, neuroprotective and neuroregenerative potentials.

## 2. Concentrations of SOD in the cerebrum of rats

Results showed similar levels of SOD in rats of the CdCl<sub>2</sub>-only treated Group 2, in comparison with the normal saline-treated Control Group 1 ( $p = 0.19$ ), as detailed in Figure 2. No statistically significant changes in SOD levels were found in the CdCl<sub>2</sub>-exposed + MO11 post-treated Group 3 ( $p = 0.17$ ), the CdCl<sub>2</sub>-exposed + MO11 + MS06 post-treated Group 4 ( $p = 0.28$ ), the Olive Oil-only treated Group 5 ( $p = 0.16$ ) and the CdCl<sub>2</sub>-exposed + Doxorubicin post-treated Group 6 ( $p = 0.10$ ), when compared to Group 2.

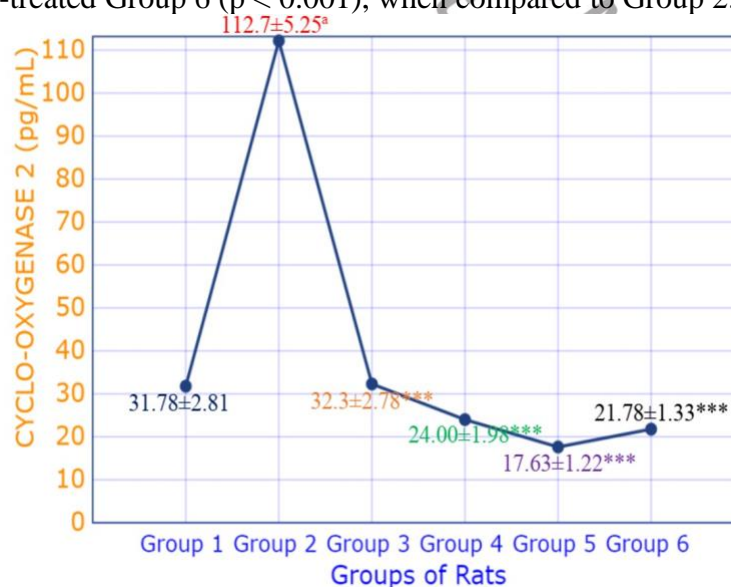


**Figure 2.** Concentrations (mean±SD) of Superoxide dismutase (U/L) in cerebrum of rats. Significant difference -  $p \leq 0.05$ .

Endogenous expressions of SOD in different regions of the brain such as the cortex, the hippocampus, the hypothalamus, the brainstem, and the cerebellum (Ramanathan et al. 2002), and the whole brain (Otitoju et al. 2008 and Rao et al. 2021) were reported in rats. The findings of the present study showed similar SOD concentrations in the cerebrum of rats of Groups 1–6 (Figure 2). These findings suggest that exposure to CdCl<sub>2</sub> followed with post-treatments with MO11, MS06, and Doxorubicin had no significant effects on SOD levels within the 18 days of experimental procedures.

### 3. Concentrations of COX-2 in the cerebrum of rats

Statistical analyses showed significant higher levels of COX-2 in rats of the CdCl<sub>2</sub>-only treated Group 2, in comparison with the normal saline-treated Control Group 1 ( $p < 0.001$ ), as detailed in Figure 3. COX-2 levels were found significantly decreased in the CdCl<sub>2</sub>-exposed + MO11 post-treated Group 3 ( $p < 0.001$ ), the CdCl<sub>2</sub>-exposed + MO11 + MS06 post-treated Group 4 ( $p < 0.001$ ), the Olive Oil-only treated Group 5 ( $p < 0.001$ ) and the CdCl<sub>2</sub>-exposed + Doxorubicin post-treated Group 6 ( $p < 0.001$ ), when compared to Group 2.



**Figure 3.** Concentrations (mean±SD) of Cyclo-oxygenase-2 (pg/mL) in cerebrum of rats. a - significant difference compared with the normal saline-treated control Group 1 at  $P \leq 0.05$ .

\*\*\*P < 0.001 - significant difference compared with CdCl<sub>2</sub>-only treated Group 2.

COX-2 is the main cyclo-oxygenase isoform in the brain. Kawaguchi et al. 2005 reported COX-2 expression in hippocampal CA3, dentate gyrus, and cerebral cortex, emphasizing the relevance of evaluating COX-2-levels in the rat brain. Increased COX-2 activity results in increased oxidative stress and increased release of prostaglandins with accompanied injurious effects (Kawaguchi et al. 2005). Therefore, increased COX-2 levels results in increased oxidative stress, causing induction of inflammation, apoptosis, and carcinogenesis (Ogunwobi et al. 2012, Nørregaard et al. 2015).

The higher level of COX-2 in the CdCl<sub>2</sub>-only treated Group 2, in comparison with the normal saline-only treated Control Group 1 (Figure 3) confirmed CdCl<sub>2</sub>-induction of oxidative stress and promotion of inflammation, neuronal cell death and carcinogenesis. This finding is in agreement with those of Liu et al. 2009 and Junior et al. 2020, which reported Cd-induced increase in COX-2 levels with associated inflammation, apoptosis and carcinogenesis. In contrast, our results showed similar COX-2 levels in Groups 1 and 3, but lower COX-2 levels in Groups 4-6, when compared with the normal saline-treated Group 1 (Figure 3), confirming neuroprotective potential of the extracts, Olive Oil, and Doxorubicin. These findings also suggest that MO11, MS06, and Doxorubicin possess neuroprotective, neuroregenerative, anti-inflammatory, and anticancer potentials, which resulted in significant reduction of COX-2 levels in the cerebrum of rats of Groups 3, 4, and 6 respectively, in comparison with Group 2.

#### 4. Concentrations of P450 in the cerebrum of rats

Statistical analyses showed significant higher levels of P450 in rats of the CdCl<sub>2</sub>-only treated Group 2, in comparison with the normal saline-treated Control Group 1 (p < 0.001), as detailed in Figure 4. P450 levels were found significantly decreased in the CdCl<sub>2</sub>-exposed + MO11 post-treated Group 3 (p < 0.001), the CdCl<sub>2</sub>-exposed + MO11 + MS06 post-treated Group 4 (P < 0.001) and the Olive Oil-only treated Group 5 (p < 0.001) in comparison with Group 2.

P450 levels were also found decreased in rats of the CdCl<sub>2</sub>-exposed + Doxorubicin post-treated Group 6 compared to the CdCl<sub>2</sub>-only treated Group 2, although statistically non-significant (p = 0.34).



**Figure 4.** Concentrations (mean±SD) of Cytochrome P450 (pg/mL) in cerebrum of rats.

a - significant difference compared with the normal saline-treated control Group 1 at p ≤ 0.05.

\*\*\*p < 0.001 - significant difference compared with CdCl<sub>2</sub>-only treated Group 2.

P450s are monooxygenases which oxidize xenobiotics, fatty acids, and steroids promoting the water-solubility and ejection of foreign agents. Hence, P450 regulates several processes such as homeostasis, drugs clearance, drugs detoxification, detoxification of xenobiotics, metabolisms of vitamin D and cells, cholesterol synthesis, and synthesis of hormones (Rodriguez-Antona and Ingelman-Sundberg 2006, Mahmoudi et al. 2018). P450 mediates the activation/inactivation of carcinogens and anticancer drugs; therefore, P450 is of relevance in cancer therapy (Manikandan et al. 2018). The liver is the major site of xenobiotic metabolism and detoxification, and the brain P450 is very low, constituting about 0.5%-2% (Hedlund et al. 2001) or 0.2%-0.5% (Wang et al. 2013) of hepatic P450. Hence, the brain P450 does not appear significantly involved in regulatory roles of pharmacokinetics of the body's hormones and drugs. The brain P450 rather regulates brain cholesterol homeostasis, retinoids elimination, and levels of endogenous GABA receptor agonists (Hedlund et al. 2001, Wojciech et al. 2021). Wojciech et al. 2021 reported expression of different forms of P450 in the frontal cortex, thalamus, hypothalamus, striatum and hippocampus of rat brain. Therefore, the profiling of brain P450 in neurotoxicology becomes very important in understanding the mechanism of action of the neurotoxin and in the design of appropriate chemotherapy.

The higher P450 level in the CdCl<sub>2</sub>-only treated Group 2, compared to the normal saline-only treated Control Group confirmed induction of increased P450 level. This observation is similar to those of Bhattacharyya et al. 2014, which reported that increased P450 levels are associated with increased oxidative stress via oxygen activation. Hence, the observed increased P450 levels could have resulted from CdCl<sub>2</sub>-induced increased oxidative stress and decreased CAT levels in rats of the CdCl<sub>2</sub>-only treated Group 2. Contrari-wise, our results showed similar or lower P450 levels in Groups 3–5, when compared with Group 1. However, results showed higher P450 level in Group 6 compared to Group 1. These findings imply that the extracts and Olive Oil have pro-P450 potential.

The increased P450 levels in the cerebrum of rats in this study is in contrast with previously reported significant decreases in liver P450 levels in Cd-induced hepatotoxicity in hamsters (Sripanidkulchai et al. 2005), and significant decreases in testicular P450 levels in Cd-induced testicular damage in rats (Alkhedaide et al. 2016). These differences could have been due to low brain P450 content versus high liver P450 content. In addition, the reason for the Cd-induced increased brain P450 levels versus Cd-induced liver and testicular decreased P450 levels could have been due to the shielding effect of the protective components of the brain resulting in increased P450 levels to aid clearance of CdCl<sub>2</sub> brain content.

Results showed significant reduction of P450 levels in the cerebrum of rats of Groups 3 and 4 respectively, when compared with Group 2 (Figure 4) confirming the neuroprotective and neuroregenerative potentials of MO11 and MS06. Contrari-wise, results of higher P450 levels in Group 6 compared with Group 2 suggest that Doxorubicin possesses lower neuroprotective and neuroregenerative potential, when compared with MO11 and MS06.

## Conclusion

Overall, the findings of the present study suggest that post-treatments of CdCl<sub>2</sub>-induced neurotoxicity with MO11 (extracted from *Moringa oleifera* leaves) and MS06 (extracted from *Musa sapientum* suckers), and Doxorubicin caused significant elevations of CAT concentrations, but significantly decreased COX-2 levels in rat brains. In addition, post-treatments of CdCl<sub>2</sub>-induced neurotoxicity with MO11 and MS06 caused significantly decreased levels of P450; however post-treatments with Doxorubicin resulted in non-significant decreased levels of P450 in rat brains. These findings suggest that MO11 and MS06 impacted some levels of neuroprotection against CdCl<sub>2</sub>-induced neurotoxicity, oxidative stress, and



promotions of inflammation, apoptosis and carcinogenesis, when compared with Doxorubicin. Thus, MO11 and MS06 are recommended for further evaluations as possible drug agents for the treatments of neurodegenerative diseases and disorders of the central nervous system.

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