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Hepatoprotective and Anticancer Potentials of *Moringa oleifera* and *Musa sapientum* Extracts against Cadmium Chloride Induced Hepatotoxicity in Rats

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Anticancer potential of MO11 (fractionated from *Moringa oleifera* leaves) and MS06 (fractionated from *Musa sapientum* suckers) against cadmium chloride induced hepatotoxicity, demyelination, carcinogenesis, and metastasis is reported. The activity was evaluated for 17 days in 24 adult male Wistar rats randomly divided into six groups (n=4). The baseline control Group 1 received normal saline only for the entire study period. Groups 2, 3, 4 and 6 received single CdCl-dose on Day 1. Group 2 (negative control) received no further treatment, while Groups 3, 4 and 6 were treated with plant extracts MO11, MO11+MS06, and doxorubicin (positive control), respectively, on Days 1-17. Group 5 received olive oil vehicle only for the 17 days. Levels of neurotransmitters (dopamine and glutamate), and biomarkers of myelination (myelin basic protein, MBP), drug metabolism and carcinogenesis (cytochrome p450), apoptosis (caspase-3 and p53), and angiogenesis (soluble vascular endothelial growth factor receptor, sVEGFR) in liver homogenates were determined using enzyme-linked immunosorbent assay. The data were statistically analysed using Mann-Whitney U test with $p \leq 0.05$. The MO11, MO11+MS06, and doxorubicin upregulated dopamine, glutamate, and cytochrome p450, but downregulated MBP, caspase-3, p53 and sVEGFR in Groups 3, 4 and 6, compared with Group 2, implying the hepatoprotective, re-myelination, and anticancer potential of the studied plant fractions.

KEY WORDS: Cadmium, hepatotoxicity, *Moringa oleifera*, *Musa sapientum*, hepatoprotective, re-myelination, anticancer

INTRODUCTION

Moringa oleifera (MO) and *Musa sapientum* (MS) are ethnomedicinal plants in Nigeria [1]. Previous studies indicated that Fraction 6 of *Moringa oleifera* leaves (MOF6) had significant antioxidant and neuroprotective potential against cuprizone induced cerebellar damage in rats [2], as well as neuroprotective potential against

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dysregulated acetylcholinesterase in sodium arsenite-induced neurotoxicity in rats [3]. The MOF6 also showed hepatoprotective, antiproliferation and antidrug resistance potentials in 7,12-dimethylbenz[a]anthracene-induced hepatotoxicity in rats [1]. Similarly, MSF1, fractionated from MS suckers, showed hepatoprotective, antiproliferation and antidrug resistance potentials.

Cancers functionally consist of vascular endothelial cells, macrophages, and cancer stem cells. Hence, cancer cells have the capacities for unlimited growth and metastasis [1, 4]. Metastasis entails interactions amongst interrelated factors such as angiogenesis, dissemination, embolization, hyperplasia, evasion of the immune system and consequent survival in ectopic organs [1, 4]. Localized cancers are treatable, while metastatic cancers are associated with poor prognosis and survival. Cancer cells interact with adjacent cells, hence predictable crosstalk amongst cellular and extracellular constituents such as endothelial cells, immune cells, stroma cells, nerve fibres and their secretory elements impact strongly on cancer prognosis, metastasis and survival [1, 4].

Dopamine-induction results in apoptosis via cytochrome C/caspase-dependent pathway and dopamine-inhibition of tumour growth [5]. Furthermore, neurotransmitters and neuropeptides released by nerve fibres within tumour microenvironment influence cancer prognosis, metastasis and survival via complex neurotransmitter-cancer interactions, and are targets for therapeutic interventions [5]. In addition, sustained myelination is required for proper functioning of nerve fibres [6]. The mechanism underlying metastasis remains poorly understood. Hence, the biology of innervation of tissues is relevant in the search for anticancer drugs from plants or other sources.

Cadmium is an established human and animal carcinogen [7-9]. Cadmium exists as a divalent cation, complexed with other elements, such as cadmium chloride (CdCl) [7-10]. Commercially, Cd is used in home appliances such as television screens, lasers, batteries, paint pigments and cosmetics [9]. Approximately 30% of Cd deposits in the liver and 30% in the kidneys, with the rest distributed throughout the body, resulting in systemic dysfunctions [7-9]. The Cd-induced toxicity results in hepatic inflammation, hepatocyte necrosis, apoptosis, and carcinogenesis [7-10], mild dilation of sinusoids and infiltration of lymphocytes in liver portal spaces [7], reduction in hepatic antioxidant defence system and increased hepatic lipid peroxidation [11]. There are few studies which have investigated the adverse effects of Cd on neurotransmitters-cancer interaction, myelination, apoptosis, angiogenesis, and carcinogenesis.

This study evaluated the hepatoprotective and anticancer potential of MO11 (fractionated from *Moringa oleifera* leaves) and MS06 (fractionated from *Musa sapientum* suckers) against cadmium chloride-induced hepatotoxicity, alterations of neurotransmitter levels, carcinogenesis, angiogenesis, and metastasis using adult male Wistar rats.

EXPERIMENTAL

Collection and authentication of plant materials

Freshly cut leaves of MO leaves and MS suckers were obtained locally from forest reserves in Ilorin. The plant samples were identified, authenticated, deposited, and assigned Herbarium Identification Numbers UILH/001/1249 and UILH/002/1182, respectively, at the herbarium of Department of Botany, University of Ilorin.

Fractionation of *Moringa oleifera* leaves and *Musa sapientum* suckers

The extracts were taken through a series of fractional and column chromatography methods as earlier described [1]. The obtained fractions were evaluated for antioxidant and antimicrobial activities.

Evaluation of antioxidant and antimicrobial activities

Antioxidant activity of plant extracts and fractions was evaluated using modified 2,2-diphenyl-1-picrylhydrazyl method [12], while the antimicrobial activity was evaluated by testing the cytotoxic effect of each fraction against the growth of *Escherichia coli* and *Salmonella typhimurium* as described by Elisha *et al*., 2017 [13].

Column chromatography

Column chromatography of the MO and MS fractions was executed on silica gel (70–230 and 240–300 mesh size, Merck, Germany), Merck alumina (70–230 mesh ASTM). Thin layer chromatography (TLC) was executed on pre-coated silica gel 60 F254 aluminium foil (Merck, Germany) to obtain pure isolates. Spots on TLC were evaluated using an ultraviolet lamp at 366 nm and 254 nm wavelengths for fluorescence and fluorescence quenching spots, respectively.

A series of chromatographic fractionations, and antioxidants and antimicrobial analyses yielded Fraction 11 of MO (MO11) as the most bioactive fraction. Phytochemical screenings of MO11 showed the presence of flavonoids, saponin, tannins, alkaloids, glycosides and steroids. Similarly, Fraction 6 of MS (MS06) had the best antioxidant and antimicrobial potential. Phytochemical screening of MS06 showed the presence of saponins, saponin glycosides, tannins, alkaloids, and indole alkaloids.

Animal care and feeding

The 24 adult male Wistar rats (average weight of 155 g and two months old) were purchased from a colony breeder at Badagry in Lagos State, Nigeria. The rats were randomly divided into six groups of four rats each. The rats were acclimatized for a week at the animal house of Faculty of Pharmacy of Olabisi Onabanjo University, Nigeria, before the beginning of the experimental procedures. The rats were kept under standard conditions and allowed free access to food and drinking water *ad libitum*. The body weights of the rats were computed on daily basis using electronic compact weighing scale (Valid Enterprise, Kalbadevi, Mumbai, India).

Grouping of rats and treatment

The study was conducted for 17 days. The MO11 and MS06 fractions were dissolved in olive oil (vehicle). The baseline control Group 1 received physiological saline only, while experimental Groups 2, 3, 4, and 6 received single intraperitoneal dose of 1.5 mg/kg bodyweight CdCl (Sigma-Aldrich, Japan Co.) on Day 1. Thereafter, Group 2 (negative control) was left untreated throughout the experimental period. Group 3 was treated with 15 mg/kg bodyweight of MO11 while Group 4 was treated with combined mixture of 15 mg/kg bodyweight of MO11 and 7 mg/kg bodyweight of MS06. Group 5 received only 1 ml/kg bodyweight of olive oil for the experimental period, while Group 6 was treated with 3.35 mg/kg bodyweight of doxorubicin (positive control). The rats were sacrificed on Day 18, and their livers excised and stored for further analyses.

Evaluation of levels of biomarkers in homogenates of rat liver

Homogenates of excised liver portions were evaluated for levels of dopamine, glutamate, MBP, cytochromes p450 (CYP450), caspase-3, p53 and soluble

vascular growth factor receptor (sVEGFR) using an ELISA technique as described by Akinlolu *et al*., 2021 [1].

Data analysis

Computed data of concentrations of each biomarker was expressed as arithmetic mean ± standard deviation. Mann-Whitney U test (Wilcoxon-Mann-Whitney Test, 2016) was used for statistical comparison of the concentration of each biomarker between two groups because the total number of rats (n = 24) was less than 30. Significant difference was confirmed at 95% confidence interval with associated p-value of less than 0.05 ($p \leq 0.05$).

Ethical approval

Ethical approval for this study was sought and received from the Ethical Review Committee of the University of Ilorin, Nigeria (UERC/ASN/2018/1161). This research study was conducted in accordance with the internationally accepted principles for laboratory animal use and care as described by Akinlolu *et al*., 2021 [1]. Appropriate measures were observed to ensure minimal pain or discomfort of rats used in this study.

RESULTS AND DISCUSSION

Effects on neurotransmitter levels

Statistically non-significant lower levels ($p \geq 0.05$) of dopamine in Group 2 were noted when compared with Groups 1, 3, 4 and 6 as shown in Table 1 and Figure 1. Similarly, there were statistically non-significant lower levels ($p \geq 0.05$) of glutamate in Group 2 when compared with Groups 1, 3 and 4. Conversely, statistically significant lower levels ($p \leq 0.05$) of glutamate in Group 2 was observed when compared with Group 6.

Dopamine is involved in regulation of activities such as arousal, motor control, motivation, reinforcement, and reward [5, 14]. Lan *et al*., 2017 [5] reported dopamine-induction of apoptosis via cytochrome C/caspase-dependent pathway and dopamine-inhibition of tumour growth. However, dopamine levels are downregulated in tumours [5, 14]. In addition, glutamate is the major excitatory neurotransmitter of the central nervous system, and it is at the crossroad of several metabolic pathways [15, 16]. Metabolism-related genes are mutated in cancers, making cancers glutamate-dependent. Hence, dysregulation of glutamate levels promotes tumour growth [16].

The results obtained in this study confirmed Cd-induced dysregulations of liver levels of dopamine and glutamate. These observations agree with reported Cd-induced down-regulations of dopamine [17] and glutamate [18] in rats. Furthermore, the results of this study suggest that post-treatment with MO11, MO11+MS06, and doxorubicin up-regulated dopamine and glutamate levels and ameliorated the CdCl-induced dys-regulations of neurotransmitter-cancer interactions. Hence, MO11 and MS06 seem to possess anticancer potentials.

Effects on myelination

The MBP is a membrane actin-binding protein and the second most abundant protein of myelin after proteolipid protein. It transmits extracellular signals to tight junctions of myelin and to the cytoskeleton of oligodendrocytes [19]. Astrocytes depletion result in breach of the glial-limiting membrane, Schwann cells invasion for myelin sheath repair, dissociation of MBP from the plasma membrane, de-myelination, and MBP-upregulation in response to axonal degeneration and oxidative stress [6, 19].

Table 1: Levels of monitored biomarkers in liver homogenates of treated rats

Biomarker	Concentration (*p-value)					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Dopamine (pg/ml)	6.06 ± 0.17 (0.12)	4.67 ± 0.22	5.16 ± 0.05 (0.92)	6.64 ± 0.02 (0.02)	6.11 ± 0.19 (0.10)	6.16 ± 0.35 (0.05)
Glutamate (ng/ml)	134.90 ± 2.09 (0.33)	106.12 ± 0.79	123.94 ± 2.85 (0.71)	131.58 ± 3.20 (0.34)	121.91 ± 11.22 (0.89)	153.54 ± 22.05 (0.03)
Myelin basic protein (ng/ml)	3.96 ± 0.04 (<0.01)	5.45±0.06	3.77±0.03 (<0.01)	2.89±0.17 (<0.01)	4.14 ± 0.01 (<0.01)	4.24 ± 0.03 (<0.01)
Cytochrome p450 (ng/ml)	227.60 ± 7.28 (0.29)	203.52 ± 9.20	306.72 ± 2.41 (<0.01)	319.72 ± 1.40 (<0.01)	309.60 ± 0.88 (<0.01)	244.35 ± 4.97 (<0.01)
Caspase-3 (ng/ml)	55.63 ± 5.00 (<0.01)	138.96 ± 1.10	78.13 ± 5.00 (<0.01)	31.46 ± 1.63 (<0.01)	115.00 ± 10.63 (0.31)	126.87 ± 13.75 (0.89)
p53 (ng/ml)	45.44 ± 0.69 (<0.01)	159.75 ± 1.04	38.50 ± 15.42 (<0.01)	47.11 ± 5.14 (<0.01)	86.88 ± 4.79 (0.07)	107.53 ± 5.61 (0.27)
sVEGFR (ng/ml)	58.33 ± 0.83 (<0.01)	161.94 ± 5.62	52.08 ± 0.42 (<0.01)	47.92 ± 0.99 (<0.01)	122.50 ± 0.83 (<0.01)	140.83 ± 7.50 (0.05)

Key: sVEGFR = soluble vascular endothelial growth factor receptor; *All p-values were determined at p≤0.05: Group 2 versus Groups 1, 3, 4, 5 and 6.

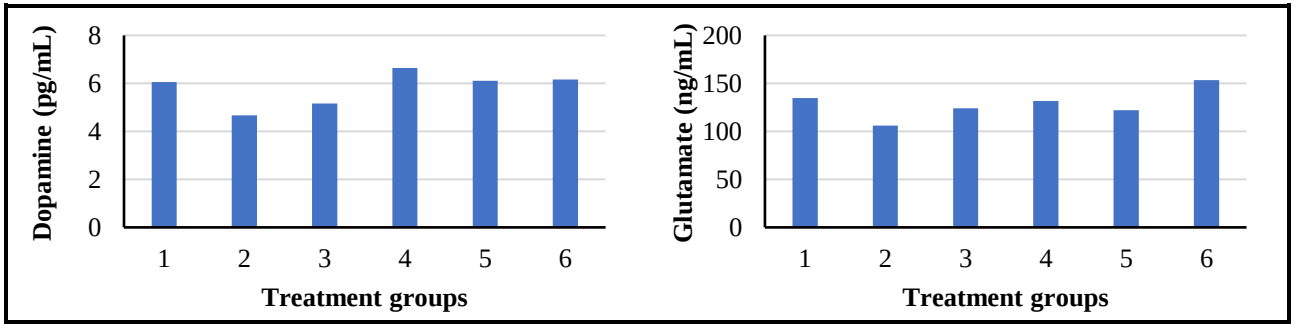

Figure 1: Concentrations of dopamine and glutamate in rat liver homogenates.

Statistically significant higher levels ($p \leq 0.05$) of MBP in Group 2 were observed when compared with Groups 1, 3, 4, and 6 as presented in Table 1 and depicted in Figure 2. The observed MBP levels suggest possible Cd-induction of demyelination of nerve fibres supplying the livers of rats of Group 2. In addition, the findings indicate that post-treatments with MO11, MO11+MS06, and doxorubicin resulted in down-regulation of MBP levels and ameliorations of CdCl-induced demyelination of nerve fibres in the liver.

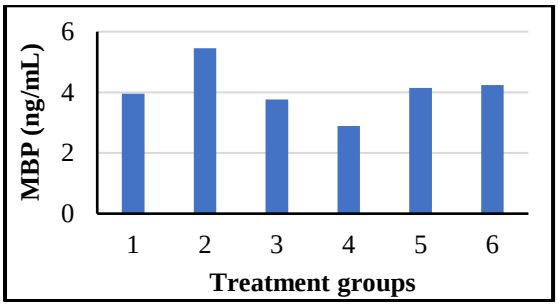

Figure 2: Concentration of myelin basic protein (MBP) in rat liver homogenates.

Effects on biomarkers of drug metabolism, carcinogenesis, apoptosis and angiogenesis

Cytochromes p450 are monooxygenases which oxidize fatty acids, steroids and xenobiotics resulting in expulsion of foreign compounds, clearance and detoxification of drugs and xenobiotics, as well as regulations of vitamin D metabolism and synthesis of cholesterols and hormones [20-22]. CYP450s are also involved in activation/inactivation of carcinogens and anticancer drugs, and play a major role in cancer therapy [22]. In addition, significant up-regulation of caspase-3 activates p53-induction of apoptosis [6]. The VEGF is an established angiogenic factor which is upregulated in increased angiogenesis [23]. Cadmium is an established carcinogen [7-11], while angiogenesis is a significant component of carcinogenesis and related metastasis [1, 4].

This study reports statistically non-significant lower levels ($p \geq 0.05$) of CYP450, but significantly lower ($p \leq 0.05$) levels of caspase-3, p53, and sVEGFR in liver of rats of Group 2 compared with Group 1 as shown in Table 1, and Figures 3 and 4. In addition, results showed statistically significant lower levels ($p \leq 0.05$) of CYP450, caspase-3, p53, and sVEGFR in liver of rats of Group 2 compared with Group 3.

Similarly, there were statistically significant lower levels ($p \leq 0.05$) of CYP450, caspase-3, p53, and sVEGFR in livers of rats in Group 2, compared with Group 4. Furthermore, there were statistically non-significant lower levels ($p \geq 0.05$) of caspase-3 and p53, but significant lower levels ($p \leq 0.05$) of CYP450 and sVEGFR in rat liver of Group 2 compared with Group 6.

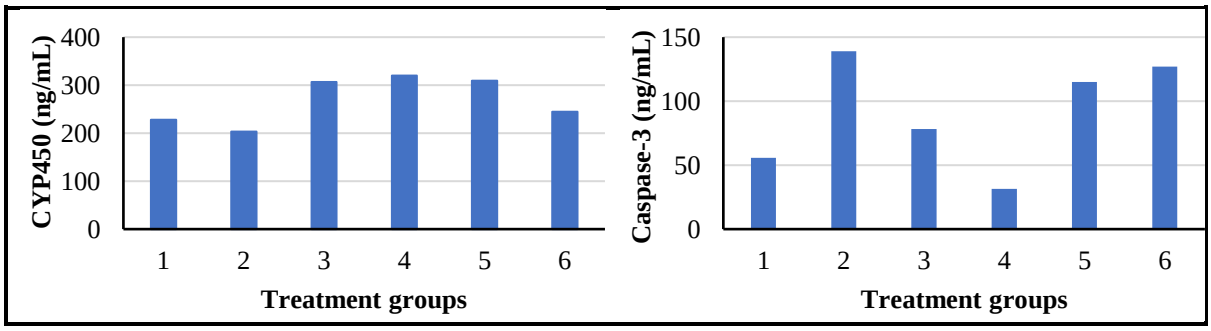

Figure 3: Concentrations of cytochromes p450 (CYP450) and caspase-3 in rat liver homogenates.

The reported CYP450 levels in this study suggest decreased clearance and detoxification of Cd content of the liver in rats of Group 2. These observations imply that MO11, MS06, and doxorubicin may have ameliorated CdCl-induced decreased

clearance and detoxification of Cd content in the livers of treated rats. In addition, the observed levels of caspase-3, p53, and sVEGFR suggest that Cd-induced toxicity resulted in carcinogenesis with accompanied increased angiogenesis in Group 2. Further, the results indicate that treatment with MO11, MO11+MS06, and doxorubicin downregulated caspase-3, p53 and sVEGFR levels and ameliorated CdCl-induced hepatotoxicity, hepatic angiogenesis, and carcinogenesis. Hence, MO11, MS06, and doxorubicin possess anti-angiogenesis, anticancer and anti-metastasis potentials.

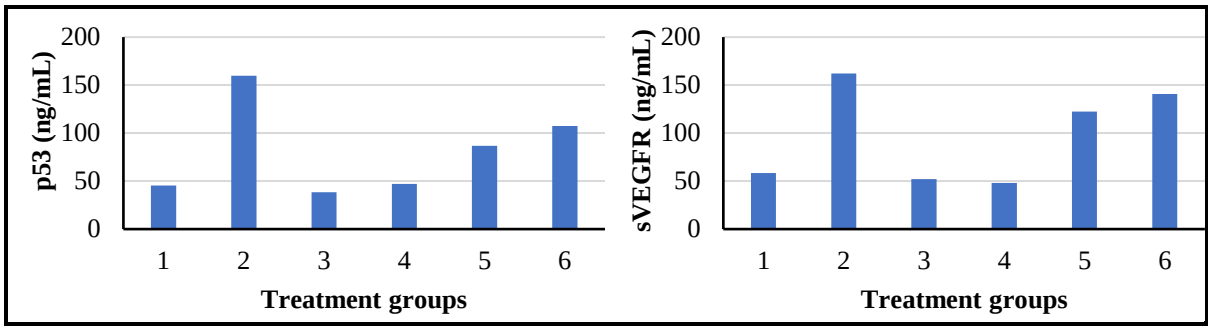

Figure 4: Concentrations of p53 and sVEGFR in rat liver homogenates.

CONCLUSION

The findings of this study suggest that treatment with MO11, MS06, and doxorubicin conferred hepatoprotection against CdCl-induced hepatotoxicity, dysregulation of neurotransmitter levels, demyelination, angiogenesis, and metastasis in the rat liver. Hence, MO11 and MS06 are recommended for further evaluations as potential drug candidates for the treatment of hepatotoxicity, dysregulation of neurotransmitter-cancer interactions, demyelination, angiogenesis, and metastasis.

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