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ANTI-CANCER AND TISSUE-NEUROPROTECTIVE POTENTIALS OF MORINGA OLEIFERA AND MUSA SAPIENTUM AGAINST IN-VIVO CADMIUM CHLORIDE-INDUCED SKIN TOXICITY

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ABSTRACT

Background: Cadmium is an established carcinogen. Cadmium exposure resulted in skin carcinogenesis in rats. Furthermore, neurotransmitter-cancer interaction, tissue innervation, and angiogenesis influence cancer prognosis, metastasis, and survival. In this study, we evaluated the anticancer and neuro-protective potentials of MO11 (isolated from *Moringa oleifera* leaves) and MS06 (isolated from *Musa sapientum* suckers) against *in-vivo* Cadmium Chloride ($CdCl_2$)-induced skin toxicity. **Methods:** Twenty-four adult male wistar rats were randomly divided into six groups (n = 4). Group 1 was control. Groups 2-4 and 6 received a single intraperitoneal administration of 1.5 mg/kg $CdCl_2$ on Day 1. Thereafter, Groups 3, 4, and 6 were post-treated with 15 mg/kg MO11, 15 mg/kg MO11 + 7 mg/kg MS06, and 3.35 mg/kg Doxorubicin respectively (Days 1-17). Group 5 received an olive oil dose (vehicle) (Days 1-17). Skin histopathology and tissue-enzyme-linked-immunosorbent assays (Tissue-ELISA) of neurotransmitters (Dopamine and Glutamate), biomarkers of myelination (Myelin Basic Protein), drug metabolism and carcinogenesis (Cytochrome-p450), apoptosis (Caspase-3 and p53), proliferation (Ki67), and angiogenesis (sVEGFR) were evaluated in skin homogenates. Data were statistically analyzed using the Mann-Whitney-U test at $P \leq 0.05$. **Results:** Histopathological analyses revealed normal skin histology (Groups 3 and 4), mild skin histo-alterations (Group 6), and gross skin histopathological alterations (Group 2). Tissue-ELISA analyses showed upregulations of dopamine, Glutamate and Cytochrome-p450, but downregulations of Myelin Basic Protein, Caspase-3, Ki67, p53, and sVEGFR in groups 3, 4, and 6, compared with group 2. MO11 and MS06 achieved better anticancer effects than doxorubicin. **Conclusion:** Overall, MO11 and MS06 possess histo-protective, neuro-protective, re-myelination, anti-proliferation, anti-angiogenesis, and anti-cancer potentials.

Keywords: anticancer potentials; anti-toxic principles; cadmium; *moringa oleifera; musa sapientum;* skin carcinogenesis

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INTRODUCTION

Cadmium (Cd) is employed industrially in the production of products such as lasers, television screens and batteries (Huff et al., 2007; Wang and Du, 2013; Andjelkovic et al., 2019). Cd is classified as a human carcinogen, and is one of the 10 chemicals or groups of chemicals of concern for human health according to the World Health Organization (Huff et al., 2007; Wang and Du, 2013; Andjelkovic et al., 2019). Cd-exposure (at 1.0, 0.1, and 0.01% weight/volume for 10 days) resulted in alopecia, hyperkeratosis, acanthosis, ulceration, and increased mitotic index in the skin of rats and mice (Lansdown and Sampson, 1996). In addition, exposure of surgically induced skin wounds to 1.0% $CdCl_2$ results in sustained edema, epidermal hyperplasia, inflammatory cell infiltration, and significantly increased skin metallothionein content in rats (Lansdown et al., 2001). Furthermore, human skin penetration of 12.7% (water) and 8.8% (soil) of 5 microliters/cm^2 of Cd dissolved in water were reported (Wester et al., 1992). Significant increase in blood Cd content in humans correlated with severe psoriasis following adjustment for covariates and control of confounding factors (Liaw et al., 2017). The mechanism of Cd-induced skin carcinogenesis in animal models, however, remains poorly understood.

Cancer cells comprise cancer stem cells, macrophages, and vascular endothelial cells, which aid metastasis (Lan et al., 2017; Akinlolu et al., 2021). Cancer cells interact with neighboring cells and extracellular components such as nerve fibers, secretory elements of nerve fibers, stroma cells, immune cells, and endothelial cells, which collectively exert a strong influence on cancer prognosis, metastasis, and survival (Lan et al., 2017). Dopamine is a neurotransmitter involved in regulation of processes such as motor control, arousal, motivation and reward (Noori et al., 2015). Induction of Dopamine causes apoptosis via the Cytochrome C/Caspase-dependent pathway and inhibition of tumor growth (Lan et al., 2017). In contrast, dopamine levels are downregulated in tumors (Lan et al., 2015; Kuol et al., 2018). Glutamate is the major excitatory neurotransmitter of the central nervous system which is involved in regulation of metabolism, and whose levels must be rightly maintained (Zhou and Danbolt, 2014). Metabolism-related genes are mutated in cancers, making them glutamate-dependent. Hence, dysregulation of glutamate levels promotes tumor growth (Yi et al., 2019).

The roles of Dopamine, Glutamate and neuropeptides released by nerve fibers within the tumor microenvironment in cancer prognosis, metastasis, and survival via complex neuro-cancer interactions have led neurotransmitters to be considered as targets for therapeutic interventions (Lan et al., 2017). In addition, adequate myelination of nerve fibers is needed for the proper functioning of nerve fiber-supplying tissues (Akinlolu et al., 2020a). Hence, neurobiological examinations of tissue innervation is of importance in understanding metastasis.

Moringa oleifera (MO) and *Musa sapientum* (MS) are ethno-medicinal plants and dietary supplements which are well grown and widely available in Nigeria (Akinlolu et al., 2021). MOF6, a sub-fraction of ethanolic extracts of MO leaves showed significant neuroprotective potentials against cuprizone-induced cerebellar damage (Omotoso et al., 2018a) and dysregulated acetylcholinesterase concentrations in sodium arsenite-induced neurotoxicity (Akinlolu et al., 2020b) in rats. MOF6 equally showed hepatoprotective, anti-proliferation, and anti-drug resistance potentials in 7,12-Dimethylbenz(a)anthracene-induced hepatotoxicity in rats (Akinlolu et al., 2021). Furthermore, MSF1, which was fractionated from MS suckers, showed hepatoprotective,

anti-proliferation, and anti-drug resistance potentials in 7,12-Dimethylbenz(a)anthracene-induced hepatotoxicity in rats (Akinlolu et al., 2021).

Therefore, in-order to further determine their anticancer and tissue-neuroprotective potentials, this study evaluated the effects of MO11 (isolated from *Moringa oleifera* leaves) and MS06 (isolated from *Musa sapientum* suckers) on skin concentrations of neurotransmitters (Dopamine and Glutamate), biomarkers of myelination (Myelin Basic Protein), drug metabolism and carcinogenesis (cytochrome p450), apoptosis (Caspase-3 and p53), proliferation (Ki67), and angiogenesis (sVEGFR) in $CdCl_2$-induced skin toxicity in adult male Wistar rats.

MATERIALS AND METHODS

Ethical approval and compliance with ethical standards

Ethical approval for this study was obtained from the Ethical Review Committee of the University of Ilorin, Nigeria. Appropriate measures were taken to ensure minimal pain and discomfort in the 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 September 22, 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 MO leaves and MS suckers

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

Evaluations of antioxidant and anti-microbial activities of MO and MS fractions

The antioxidant activities of the plant extracts were evaluated using the modified 2,2- diphenyl-1-picrylhydrazyl method as previously described by Chaves et al. (2020). In addition, the anti-microbial activities of the plant extracts were evaluated by testing the cytotoxic potential of each fraction against E. coli and Salmonella typhimurium, as previously described by Elisha et al. (2017).

Extraction, partitioning of fractions and isolations of MO11 from MO leaves and MS06 from MS suckers

MO11 and MS06 were isolated from *Moringa oleifera* leaves and *Musa sapientum* suckers, respectively, following a series of antioxidant analyses (2,2-diphenyl-1-picrylhydrazyl method), anti-microbial analyses (anti-Escherichia-coli and anti-Salmonella-tiphimurium tests), chromatography, and spectroscopic fractionation techniques, as described previously (Akinlolu et al., 2022a; Ameen and Akinlolu, 2023).

Animal care and feeding

24 adult male Wistar rats (average weight, 155 g; age, 2 months) were purchased from a colony breed at Badagry in Lagos State, Nigeria. The rats were randomly divided into six groups with four rats per group. The rats were acclimatized for a week at the animal house of the Faculty of Pharmacy of Olabisi Onabanjo University, Nigeria, before

beginning the experimental procedures. The rats were maintained under standard conditions and permitted unlimited access to food and drinking water ad libitum. The body weights of the rats were computed on a daily basis using an electronic compact scale (SF-400C weighs in grams) (Valid Enterprise, Kalbadevi, Mumbai, India).

Grouping of rats and extracts/drugs administration

MO11 and MS06 were dissolved in Olive Oil (the vehicle). Rats in Control Group 1 (Baseline Control) received physiological saline for only 17 Days (Days 1-17). Each rat in Experimental Groups 2-4 and 6 received a single intraperitoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ (Sigma-Aldrich, Japan Co.) on Day 1. Rats in group 2 (Negative Control) were left untreated throughout the experimental procedure for 17 Days (Days 1-17). Thereafter, rats in group 3 were post-treated with oral administration of 15 mg/kg body weight of MO11 for 17 Days (Days 1-17). Rats in group 4 were post-treated with oral administration of a combined mixture of 15 mg/kg body weight of MO11 and 7 mg/kg bodyweight of MS06 for 17 Days (Days 1-17). Rats in group 5 received only oral administration of Olive Oil (vehicle, 1 mL/kg body weight) for 17 Days (Days 1-17). Rats in Group 6 were post-treated with tail intravenous administration of 3.35 mg/Kg bodyweight of doxorubicin (standard anticancer drug – Positive Control) for 17 Days (Days 1-17).

Completion of experimental procedures

Twenty-four hours after the last day of drug and extract administration on day 17, the experimental procedures were completed and the rats were sacrificed on day 18 by cervical dislocation, as previously described by Omotoso et al. (2018b). For animal sacrifice, no anesthesia was used, as approved by the University Ethical Review Committee, after their evaluation of the experimental protocol based on the fact that the biomarkers to be examined include enzymes such as Caspase-3 and metabolic agents such as cytochrome p450, which may be endogenously altered by anesthetic agents requiring post-experimental control of confounding factors.

Histo-pathological evaluations of the skin

The excised normal morphological skin portions (Groups 1 and 3 – 6) and alopecia (hairless) skin portions (Group 2) of rats were processed for light microscopy using conventional histological procedures. Histopathological evaluations of the skin were conducted using the Hematoxylin and Eosin technique, as previously described by Akinlolu et al. (2021 and 2022b). Photomicrographs of slides were captured with the aid of Olympus binocular microscope (Tokyo, Japan) which was connected to a 5.0 megapixel Amscope camera (Amscope Inc., Irvine, CA, USA). Tissue sections were photographed at 100X magnification.

Tissue-Enzyme Linked Immunosorbent Assays (Tissue-ELISA) of concentrations of biomarkers in skin homogenates of rats

Normal morphological skin portions (Groups 1 and 3 – 6) and hairless skin portions (Group 2) of the back of all rats were excised and isolated. Thereafter, 1 g of the excised skin portion of each rat was thoroughly homogenized using a porcelain mortar and pestle in 4 ml of ice-cold 0.25 M sucrose solution. The tissue homogenates were additionally filled up to 5 ml with sucrose in a 5 ml serum bottle. Skin homogenates were centrifuged at 3000 rpm for 15 min (Model 90-1). The supernatant was collected with Pasteur pipettes, placed in a freezer at -20 °C, and assayed for concentrations of Dopamine, Glutamate, Myelin Basic Protein (MBP), cytochrome p450, Caspase-3, Ki67, p53, and sVEGFR in the skin of all rats of

Control Group 1 and Experimental Groups 2 – 6 using ELISA, as previously described by Akinlolu et al., 2021.

Statistical analysis

The computed data of the concentrations of each biomarker are expressed as arithmetic means ± standard deviation. The Mann-Whitney U test (Wilcoxon-Mann-Whitney Test, 2016) was used for statistical comparison of the concentration of each biomarker between the two groups, because the sample size of 24 was less than 30. Significant difference was confirmed at 95% confidence interval, with associated p - value of less than 0.05 ($P \leq 0.05$).

RESULTS

Morphological observations of the skin

No alopecia was noted in the skin of rats in Groups 1 and 3 – 6 (Figures 1a and 1c – 1f). However, morphological observations showed severe skin alopecia in rats in $CdCl_2$-only treated group 2 (Figure 1b).

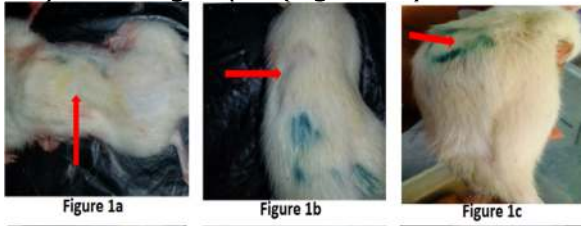

Figure 1a: Photomicrograph of the skin of the back of rats of Group 1 which received physiological saline. Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 1.

Figure 1b: Photomicrograph of the skin of the back of rats of Group 2 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ (Negative Control). Red solid arrow indicates site of skin alopecia of the back of rat. Morphological observations showed morphological anomaly indicated by skin alopecia of the skin of back of rats of Group 2.

Figure 1c: Photomicrograph of the skin of the back of rats of Group 3 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ and were post-treated with 15 mg/Kg bodyweight of MO11. Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 3.

Figure 1d: Photomicrograph of the skin of the back of rats of Group 4 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ and were post-treated with 15 mg/Kg bodyweight of MO11 and 7 mg/Kg bodyweight of MS06. Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 4.

Figure 1e: Photomicrograph of the skin of the back of rats of Group 5 which received only 1 ml/Kg bodyweight of Olive Oil (vehicle). Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 5.

Figure 1f: Photomicrograph of the skin of the back of rats of Group 6 which received single intra-peritoneal administration of 1.5 mg/Kg bodyweight $CdCl_2$ and were post-treated with 3.35 mg/Kg bodyweight of Doxorubicin (standard anticancer drug – Positive Control). Red solid arrow indicates skin of the back of rat. Morphological observations showed normal skin morphology of rats of Group 6.

Histo-pathological evaluations of the skin

Histopathological evaluations showed normal skin histo-architecture in rats of Groups 1 (Figure 2a) and 3-5 (Figures 2c – 2e). In contrast, histopathological evaluations showed depletion of keratinocytes of the stratum corneum of epidermal layer in the hairless skin of rats of $CdCl_2$-only treated Group 2 (Figure 2b). In addition, histopathological analyses showed mild depletion of the stratum corneum of the epidermal layer of the hairless skin of rats in Group 6 (Figure 2f).

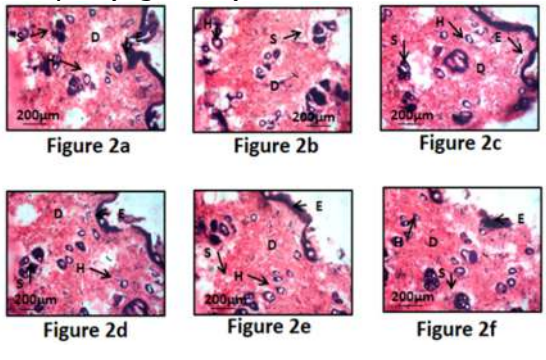

Figure 2a: Representative photomicrograph of the Skin of rats of Group 1 which received physiological saline. Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Staining intensity, cellular and morphological delineation appear normal. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2b: Representative photomicrograph of the Skin of rats of Group 2 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride. Haematoxylin and Eosin. Histological analyses show anomalies of the histoarchitecture of the Skin components such as the stratum corneum, epidermal layer, hair follicle (H) and sebaceous gland (S). Distortion of the stratum corneum and epidermal layer is apparent. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2c: Representative photomicrograph of the Skin of rats of Group 3 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride and were post-treated with 15 mg/Kg bodyweight of *Moringa oleifera*. Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Staining intensity, cellular and morphological delineation appear normal. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2d: Representative photomicrograph of the Skin of rats of Group 4 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride and were post-treated with 15 mg/Kg bodyweight of *Moringa oleifera* and 7mg/Kg bodyweight of *Musa sapientium* (dissolved in 1 ml of Olive oil). Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Staining intensity, cellular and morphological delineation appear normal. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2e: Representative photomicrograph of the Skin of rats of Group 5 which received 1 ml/Kg bodyweight of Olive oil only. Haematoxylin and Eosin. Histological analyses show normal histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Mild distortion of the epidermal layer is apparent. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Figure 2f: Representative photomicrograph of the Skin of rats of Group 6 which received intraperitoneal administration of 1.5 mg/Kg bodyweight of Cadmium Chloride and were post-treated with 3.35 mg/Kg bodyweight of Doxorubicin. Haematoxyline and Eosin. Histological analyses show mild anomalies of the histoarchitecture of the Skin components such as the hair follicle (H) and sebaceous gland (S). Mild distortion of the stratum corneum and epidermal layer is apparent. (S – Sebaceous gland, H – Hair follicle, D – Dermis, E - Epidermis).

Concentrations of Dopamine, Glutamate and Myelin Basic Protein (MBP) in skin of rats: Group 2 versus Group 1

The results showed significantly lower ($P \leq 0.05$) levels of Dopamine and Glutamate, but significantly higher ($P \leq 0.05$) levels of MBP in homogenates of skin of rats in Group 2, when compared with Control Group 1 (Table 1).

Concentrations of Dopamine, Glutamate and MBP in skin of rats: Group 2 versus Groups 3 and 4

The results showed statistically significant lower ($P \leq 0.05$) levels of dopamine in the skin homogenates of Group 2 rats than in Groups 3 and 4, as presented in Table 1. In addition, the results showed statistically significantly lower ($P \leq 0.05$) levels of glutamate, but statistically non-significant lower ($P \geq 0.05$) levels of glutamate in the homogenates of skin of rats in Group 2, compared with Groups 3 and 4, respectively, as presented in Table 1. Furthermore, the results showed statistically significant higher ($P \leq 0.05$) levels of MBP in homogenates of the skin of rats in Group 2 than in Groups 3 and 4, as presented in Table 1.

Concentrations of Dopamine, Glutamate and MBP in skin of rats: Group 2 versus Group 6

The results showed statistically significant lower ($P \leq 0.05$) levels of dopamine in the skin homogenates of Group 2 rats than in Group 6 rats, as presented in Table 1. In addition, the results showed statistically non-significant lower ($P \geq 0.05$) levels of glutamate, but statistically non-significant higher ($P \geq 0.05$) levels of MBP in homogenates of rat skin in Group 2 than in Group 6, as presented in Table 1.

Concentrations of Caspase-3, Ki67, p53, Cytochrome p450 and sVEGFR in skin of rats: Group 2 versus Groups 1, 3 and 4

The results showed statistically significant lower ($P \leq 0.05$) levels of Caspase-3, Ki67, p53, cytochrome p450, and sVEGFR in the skin homogenates of rats in Group 2 when compared with Control Group 1 and Groups 3 and 4, as presented in Table 2.

Concentrations of Caspase-3, Ki67, p53, Cytochrome p450 and sVEGFR in skin of rats: Group 2 versus Group 6

The results showed statistically significant lower ($P \leq 0.05$) levels of Caspase-3, Ki67, Cytochrome p450, and sVEGFR in the skin homogenates of rats in Group 2 than in Group 6, as presented in Table 2. However, the results showed statistically non-significant lower ($P \geq 0.05$) levels of p53 in homogenates of the skin of rats in Group 2 compared with Group 6, as presented in Table 2.

Table 1: Concentrations of Dopamine, Glutamate and MBP in skin of rats.

Drug/Extract →	**Normal Saline only Group 1**	**$CdCl_2$ only Group 2**	**$CdCl_2$-exposure + MO11 post-treated Group 3**	**$CdCl_2$-exposure + MO11 + MS06 post-treated Group 4**	**Olive Oil only Group 5**	**$CdCl_2$-exposure + Doxorubicin post-treated Group 6**
Dopamine (pg/ml)	14.86±0.67	5.97±0.11	11.44±0.21	12.68±0.16	10.95±0.38	10.94±0.27
P – value	*P* < 0.01**		*P* < 0.01**	*P* < 0.01**	*P* < 0.01**	*P* < 0.01**
Glutamate (ng/ml)	41.59±3.03	16.28±1.22	22.81±1.26	33.35±0.28	23.60±0.32	23.55±1.87
P – value	*P* < 0.01**		0.29	*P* < 0.01**	0.19	0.13
Myelin Basic Protein (ng/ml)	4.65±0.02	5.45±0.07	4.47±0.01	3.44±0.34	5.09±0.02	5.05±0.03
P – value	0.05		*P* < 0.01*	*P* < 0.01*	0.47	0.28

P - value at $P \leq 0.05$: Group 2 versus Groups 1 and 3 – 6; ** = significant increase; * = significant decrease.

Table 2: Concentrations of Caspase-3, Ki67, p53, Cytochrome p450 and sVEGFR in skin of rats.

Drug/Extract →	**Normal Saline only Group 1**	**$CdCl_2$ only Group 2**	**$CdCl_2$-exposure + MO11 post-treated Group 3**	**$CdCl_2$-exposure + MO11 + MS06 post-treated Group 4**	**Olive Oil only Group 5**	**$CdCl_2$-exposure + Doxorubicin post-treated Group 6**
Caspase-3 (ng/ml)	169.69 ±1.56	356.89 ±26.88	101.04 ±10.21	45.21±1.99	147.81 ±1.56	293.44±2.19
P – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	*P* < 0.01*
Ki67 (ng/ml)	6.21 ±0.03	14.82±0.47	2.73±0.33	2.05±0.04	4.40±0.19	10.07±0.40
p – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	*P* < 0.01*
p53 (ng/ml)	26.42 ±0.56	37.68±1.04	28.08 ±0.28	23.22±1.25	30.86 ±0.01	34.09±1.56
P – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	0.41
Cytochrome p450 (ng/ml)	121.88 ±1.40	41.65±0.93	96.85 ±1.73	118.28 ±2.20	80.32 ±3.20	78.52±3.00
P – value	*P* < 0.01**		*P* < 0.01**	*P* < 0.01**	*P* < 0.01**	*P* < 0.01**

sVEGFR (ng/ml)	362.92 ±1.25	578.33 ±17.50	302.50 ±1.67	288.89 ±2.22	418.33 ±5.83	498.33±7.50
P – value	*P* < 0.01*		*P* < 0.01*	*P* < 0.01*	*P* < 0.01*	*P* < 0.01*

P - value at *P* ≤ 0.05: Group 2 versus Groups 1 and 3 – 6; ** = significant increase; * = significant decrease).

DISCUSSION

The observed skin alopecia (Figure 1b) and histopathological alterations in rats of $CdCl_2$-only treated group 2 (Figure 2b) confirmed $CdCl_2$-induction of skin carcinogenesis, necrosis, and inflammation, as reported (Akinlolu et al., 2022b). These observations are in agreement with those of previous studies (Lansdown and Sampson, 1992; Lansdown et al., 2001), which reported Cd induction of skin alopecia, ulceration, epidermal hyperplasia, inflammatory cell infiltration, and sustained edema in rats and mice. Furthermore, the observations of this study showed amelioration of Cd-induction of skin alopecia and histo-alteration in rats post-treated with MO11 and MS06 (Figures 2c and 2d) as reported (Akinlolu et al., 2022b). In contrast, post-treatment with doxorubicin (Figure 2f) resulted in a lower degree of amelioration of Cd-induced skin histo-alterations in rats, as reported (Akinlolu et al., 2022b).

The findings of this study showed significant downregulations of Dopamine and Glutamate in $CdCl_2$-only treated group 2 when compared with Normal Saline-only Control Group 1, as presented in Table 1. These results confirmed the dysregulation of Dopamine and Glutamate concentrations in the hairless skin area. These observations are in agreement with the previously reported Cd-induced downregulations of dopamine (Gupta et al., 2018) and glutamate (Lafuente et al., 2002) in rats. In addition, Dopamine levels are downregulated in tumors (Lan et al., 2015; Kuol et al., 2018), while dysregulation of glutamate levels aids tumor growth (Yi et al., 2019). Hence, the observed downregulations of Dopamine and Glutamate in rats of Group 2 indicate that these neurotransmitters are involved in neuro-cancer interaction mechanism of $CdCl_2$-induced carcinogenesis.

Post-treatments with MO11, MO11+MS06 and Doxorubicin in Groups 3, 4, and 6 resulted in the upregulations of Dopamine and Glutamate when compared with $CdCl_2$-only treated Group 2 (Table 1), suggesting that MO11, MS06 and Doxorubicin ameliorated $CdCl_2$-induced adverse effects on neurotransmitter-cancer interactions and possess anticancer potential.

In addition, the results of this study showed significant upregulation of MBP levels in the skin of $CdCl_2$-only treated Group 2 when compared with Groups 1, 3, and 4, as presented in Table 1. MBP is a membrane actin-binding protein (Muller et al., 2013) which is upregulated in response to demyelination, axonal degeneration and oxidative stress (Akinlolu et al., 2020a; Afifi and Embaby, 2016). Hence, observed MBP-upregulation suggests possible Cd-induction of de-myelination of nerve fibers supplying the hairless skin area of rats in Group 2.

Post-treatments with MO11 and MO11+MS06 in Groups 3 and 4 resulted in significant downregulation of MBP levels when compared with $CdCl_2$-only treated Group 2, as presented in Table 1. However, post-treatment with doxorubicin resulted in non-significant MBP downregulation in group 6 compared with that in group 2. These observations suggest that MO11, MS06 and Doxorubicin ameliorated $CdCl_2$-induced de-myelination of nerve fibers in the skin of rats, although MO11and MS06 possess better re-myelination potential than doxorubicin.

Furthermore, the results of this study showed significant downregulation of cytochrome p450 levels in the homogenates of hairless skin of $CdCl_2$-only treated Group 2 when compared with normal hairy skin tissues of Groups 1, 3, 4, and 6, as presented

in Table 2. Cytochrome p450 is part of monooxygenases that oxidize fatty acids, steroids, and xenobiotics, thereby enhancing water solubility and expulsion of foreign compounds, and further clearance of drugs/compounds (Rodriguez-Antona et al. 2006; Afifi and Embaby, 2016; Manikandan and Nagini, 2018). Therefore, our observations suggest a possible decrease in clearance and detoxification of Cd content of the skin in rats of Group 2. In addition, these observations suggest that MO11, MS06 and Doxorubicin ameliorated the $CdCl_2$-induced decreased clearance and detoxification of Cd in the skin in the skin of rats of Groups 3, 4 and 6.

It must be noted that there is a significant increase in blood Cd content in humans with severe psoriasis (Liaw et al., 2017). Hence, the observed increase in cytochrome p450 concentrations in $CdCl_2$-exposed rats post-treated with MO11 and MS06 implied that these drug compounds could be considered for further evaluations in the treatment of psoriasis in humans suspected of significant cadmium exposure.

The findings of this study showed significant upregulations of Caspase-3, Ki67, p53, and sVEGFR in hairless skin of $CdCl_2$-only treated Group 2, when compared with Groups 1, 3, and 4 (Table 2). The significant upregulation of Caspase-3 is associated with the activation of apoptosis following induction of necrosis (Akinlolu et al., 2020a), and it is also positively correlated with p53-induction of apoptosis towards resolution of induced necrosis (Akinlolu et al., 2020a). Ki67 is an established biomarker of proliferation, which is significantly upregulated in hyperplasia (Akinlolu et al., 2020a), while VEGF is an established angiogenic factor (Mahoney et al., 2021). Therefore, our observations suggest $CdCl_2$-induction of hyperplasia, necrosis, and angiogenesis in the hairless skin of rats in Group 2.

Post-treatments with MO11 and MO11+MS06 in Groups 3 and 4 resulted in significant downregulations of Caspase-3, Ki67, p53, and sVEGFR levels, respectively, when compared with $CdCl_2$-only treatment in Group 2, as presented in Table 2. In addition, post-treatment with doxorubicin in Group 6 resulted in significant downregulations of Caspase-3, Ki67, and sVEGFR levels, but non-significant p53 downregulation when compared with $CdCl_2$-only treatment in Group 2, as presented in Table 2. These observations suggest that MO11, MS06 and Doxorubicin ameliorated $CdCl_2$-induced skin hyperplasia, necrosis, and angiogenesis and possess anticancer and anti-angiogenic potentials. However, MO11 and MS06 possess better anticancer potentials than doxorubicin.

LC-MS and spectroscopic analyses confirmed the presence of Guanine, Glutamic acid, Leucine and Phenylalanine, and other anticancer compounds in MO11 and MS06 isolates, as reported by Ameen and Akinlolu (2023). Guanine, Glutamic acid, and phenylalanine are established anticancer, antioxidant, and anti-inflammatory compounds (Dutta, Ray, and Nagaran, 2013; Dasari and Tchounwou, 2014; Lee et al. 2017; Lieu et al. 2020; Ziyu et al. 2020). Therefore, the significant and better anticancer potentials of MO11 (Group 3) and MO11+MS06 (Group 4) than doxorubicin (Group 6) following $CdCl_2$-induced skin carcinogenesis (Table 1 and Table 2) could have been due to the presence of Guanine, Glutamic acid, Leucine and Phenylalanine, and other anticancer compounds in MO11 and MS06 isolates.

CONCLUSIONS

Overall, the findings of this study suggest that post-treatments with MO11, MS06 and Doxorubicin conferred cyto-/histo-protection against $CdCl_2$-induced toxicity, carcinogenesis (alopecia, necrosis, angiogenesis and neuro-cancer interaction) and demyelination via the upregulations of

Dopamine, Glutamate and Cytochrome p450, but downregulations of MBP, Ki67, p53, Caspase-3 and sVEGFR in the skin of rats. In addition, the findings of this study showed that MO11 and MS06 achieved better re-myelination, anti-necrosis, and anticancer potentials than doxorubicin. Hence, MO11 and MS06 are recommended for further evaluations as potential drug candidates and dietary supplements for the treatment of skin alopecia and cancers.

DATA AVAILABILITY STATEMENT

The datasets computed and/or analyzed in this study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTEREST*:* Authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Conception and design: AA and MA.
Critical revision of the article for intellectual content: AA, MA, GE, NA, RA and BF.
Final approval of the article: AA, MA, GE, NA, RA and BF.
Obtaining of funding: AA, MA, GE, NA, RA and BF.
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Collection and assembly of data: AA, MA, GE, NA, RA and BF.

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