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ANTI-TOXIC PRINCIPLES FROM *MUSA SAPIENTUM* AND *MORINGA OLEIFERA* AMELIORATED SKIN HISTO-PATHOLOGY, DECREASED LIPID PEROXIDATION AND PROMOTED MELATONIN/TNF-ALPHA/ P53-MEDIATED APOPTOSIS IN CADMIUM CHLORIDE-INDUCED CARCINOGENESIS IN RATS

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ABSTRACT

This study evaluated anticancer potentials of Musa sapientum (MS) and Moringa oleifera (MO) in cadmium chloride ($CdCl_2$)-induced skin and liver carcinogenesis. Musa sapientum fraction-1 (MSF1) and Moringa oleifera fraction-6 (MOF6) were extracted from MS suckers and MO leaves, respectively, using column-chromatography methods. Forty-five adult male rats were randomly divided into 11 groups (n = 5). Cancer-induction was via single intraperitoneal administration of 1.25 mg/kg $CdCl_2$. Groups 1 and 2 received physiological saline and $CdCl_2$dose, respectively. Groups 3–5 received $CdCl_2$dose on day 1 but were post-treated on days 15–56 with 15 mg/kg MOF6, 30 mg/kg MOF6 and 10 mg/kg MSF1, respectively. Group 6 received $CdCl_2$dose on day 1 and were post-treated on days 15–28 with doxorubicin + cisplatin doses. Groups 7–9 received MOF6 dose and MSF1 dose, respectively (days 1–56). Groups 10 and 11 received $CdCl_2$dose on day 1 and were post-treated with 30 mg/kg MOF6 and 10 mg/kg MSF1, respectively (days 1–56). Doxorubicin and extracts doses were administered orally. Skin histo-pathology (haematoxylin and eosin), sera melatonin and tumour necrosis factor-alpha (TNFα) (enzyme linked immunosorbent

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assays [ELISA]) levels were evaluated. Malondialdehyde (thiobarbituric acid assay) and p53 (ELISA) levels were evaluated in liver homogenates. Data were statistically analysed using ANOVA at $p \leq 0.05$. Results showed skin histo-alterations in Group 2, compared with normal skin histology in Groups 1 and 3–11. Statistical analyses showed significant downregulation of p53, non-significant downregulations of malondialdehyde and TNFα, and non-significant upregulation of melatonin in Groups 3–11 compared with Group 2. Overall, post-treatments with MOF6 and MSF1 exhibited possible anticancer potentials of these plants extracts via degrees of amelioration of $CdCl_2$-induced skin histo-pathology and carcinogenesis.

Keywords: Cadmium, Melatonin, *Musa sapientum*, *Moringa oleifera*, p53, TNFα

INTRODUCTION

Musa sapientum (MS) and *Moringa oleifera* (MO) are ethno-medicinal plants with possible anticancer potentials (Welch and Tietje 2017). MS sucker exhibited anti-ulcer (Akinlolu *et al*. 2013), antioxidant (Akinlolu *et al*. 2013) and anti-diabetic (Akinlolu *et al*. 2015) potentials. In addition, the fraction, *Musa sapientum* fraction-1 (MSF1), which was isolated from MS sucker show-cased hepato-protective, anti-drug resistance and anti-proliferation potentials (Akinlolu *et al*. 2021). Similarly, *Moringa oleifera* fraction-6 (MOF6) (which was fractionated from MO leaves) presented significant antioxidant and neuro-protective potentials (Omotoso *et al*. 2018; Akinlolu *et al*. 2020), hepato-protective potentials (Akinlolu *et al*. 2020), anti-proliferation and anti-drug resistance potentials (Akinlolu *et al*. 2021).

The WHO listed cadmium as one of the 10 chemicals or groups of chemicals of concern for human health (Andjelkovic *et al*. 2019). The liver and the kidney are the major deposit sites of cadmium, receiving approximately 30% of cadmium deposits. The remaining cadmium deposits are distributed across body systems leading to systemic dysfunctions such as skin alopecia and ulceration (Lansdown *et al*. 2001), inflammation and hepato-toxicity (Bernhoft 2013; Wang and Du 2013; Angjelkovic *et al*. 2019). Cadmium-induced carcinogenesis in animal-models are similar in features to those of human cancers, making it a reliable model for evaluating anticancer drugs (Wang and Du 2013; Angjelkovic *et al*. 2019).

Lipid peroxidation is implicated in inflammation and carcinogenesis due to its causative production of mutagenic reactive aldehydes such as malondialdehyde, defective cell membrane and cellular damage (Akinlolu *et al*. 2012). Melatonin which is majorly metabolised in the liver (Mohammadi *et al*. 2016) is an established antioxidant, anti-inflammatory and anti-cancer candidate (Akinlolu *et al*. 2012; Lampiao and Du Plessis 2013; Zamfir *et al*. 2014; Mohammadi *et al*. 2016). This makes melatonin to be of importance in the treatments of hepatic oxidative stress and carcinogenesis. In addition, tumour necrosis factor-alpha (TNFα) is a pro-inflammatory cytokine that plays inductive roles in immune- and inflammatory-responses, and cancer-associated chronic inflammation across body systems (Liu *et al*. 2004; Zahr *et al*. 2010; Chu 2013). Furthermore, p53 which is usually referred to as the guardian of the genome is involved in the induction of apoptotic and cell cycle arrest genes when the body system is exposed to inflammation, DNA damage and carcinogenesis (Chang *et al*. 2010; Toshinori and Akira 2011; Xiao *et al*. 2013).

The skin is the most external and first protective body layer (Moore *et al*., 2014). The liver, on the other hand impact strongly on systemic processes such as drug metabolism, drug detoxification and efficient systemic functioning of the body (Moore *et al*. 2014). Cadmium usually exists as a divalent cation, complexed with other elements, such as cadmium chloride ($CdCl_2$) (Bernhoft 2013; Angjelkovic *et al*. 2019). Therefore, in order to further determine the anticancer potentials of MSF1 (isolated from MS sucker) and MOF6 (isolated from MO leaves), this study evaluated the effects of MSF1 and MOF6 on skin histology, hepatic oxidative stress, and immuno-modulations of melatonin, TNFα and p53 proteins in $CdCl_2$-induced carcinogenesis in rats.

MATERIALS AND METHODS

Ethical Approval

The ethical approval for this study was obtained from the Ethical Review Committee of the University of Ilorin, Nigeria via Ethical Approval number: UERC/ASN/2018/1161. In addition, all experimental procedures of this research were carried out in compliance with the international guidelines for laboratory animal use and care such as the US guidelines (NIH publication #85-23, revised in 1985) and the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Authentication of MO Leaves and MS Suckers

MO leaves and MS suckers were obtained freshly from local forest reserves in Ilorin of Kwara State, Nigeria. The samples were identified and authenticated by a Botanist in the Department of Plant Biology, of University of Ilorin, Nigeria. Thereafter, the plant materials were deposited at the herbarium of the Department of Plant Biology, University of Ilorin. The obtained MS suckers and MO leaves were assigned Herbarium identification numbers: UILH/002/1182 and UILH/001/1249, respectively.

Preparations and Ethanolic Extractions of MO Leaves and MS Suckers

MO leaves and MS suckers were shade-dried at the laboratory unit of the Department of Chemistry, University of Ilorin, Nigeria. A total weight of 5.2 kg of MS suckers and 4.0 kg of MO leaves were pounded, powdered, extracted with distilled ethanol and concentrated with the aid of a rotary evaporator at 40°C–60°C. Ethanol was used to remove impurities while the extracted product was heated in a conical flask. The conical flask contained boiling chips/anti-bumping granules which were used to prevent liquid ethanol from ‘bumping’ into the condenser while flowing into a container. The procedure was continuously recycled to ensure that it keeps running.

Twenty-four hours later, the mixture was decanted and sieved. New distilled ethanol was added to the sieved MO leaves and MS suckers following decantation; and left for another 24 h. The procedure was stopped once the colour quality and texture of the dissolved MO leaves and MS suckers in ethanol became evidently low in comparison with previous decanted solutions. Column chromatography was carried out to obtain different fractions of MO leaves and MS suckers following ethanol-separation.

Column Chromatography Fractionation of Ethanol Extracts of MS Suckers and MO Leaves

The ethanol extract of MO leaves was fractionated in a silica gel open column, using nhexane, dichloromethane, ethyl acetate and methanol in an increasing order of polarity (nhexane; nhexane:nichloromethane [3;1, 3:2, 1:1, 1:2, 1:3]; dichloromethane; dichloromethane:ethylacetate [3:1, 3;2, 1:1, 1:2, 1;3]; ethylacetate; ethylacetate:methanol [3:1, 3:2, 1:1, 1:2, 1:3] and methanol, to afford 36 eluents of 250 mL each. Similarly, the ethanol extract of MS suckers was fractionated in a silica gel open column, using nhexane, dichloromethane, ethyl acetate and methanol in an increasing order of polarity (nhexane; nhexane:dichloromethane [3;1, 3:2, 1:1, 1:2, 1:3]; dichloromethane; dichloromethane:ethylacetate [3:1, 3;2, 1:1, 1:2, 1;3]; ethylacetate; ethylacetate:methanol [3:1, 3:2, 1:1, 1:2, 1:3] and methanol, to afford 13 eluents of 250 mL each. The obtained eluents were pooled together based on the colour of the eluting solvents to produce a total of five combined MS fractions and nine combined MO fractions. All MS and MO fractions were tested for their antioxidant potentials. MSF1 and MOF6 fractions, possessed the best preliminary antioxidant potentials and were further evaluated for their anticancer potentials in this study.

Animals Care and Feeding

Fifty-five male Wistar rats with an average weight of 200 g were acclimatised for 5 days in the animal house of Faculty of Basic Medical Sciences of University of Ilorin, Nigeria. Following acclimatisation, the rats were randomly divided into 11 groups (Control Group 1 and Experimental Groups 2–11) with five rats per group. Each group of rats was kept in a wire gauzed cage. All rats were kept under standard conditions and fed water and food *ad libitum*. Bodyweights (g) of all rats were measured on day 1 of experimental procedure and at the end of each week.

Induction of Carcinogenesis

A single dose of 1.25 mg/kg bodyweight $CdCl_2$ was injected into each rat of Experimental Groups 2–6 via intra-peritoneal administration on day 1 to induce carcinogenesis. Each rat was monitored daily from days 1–14. Induction of cancer (carcinogenesis) was confirmed after 14 days via morphological appearance of alopecia and ulcers on the skin of the rat.

Experimental Procedures and Drugs Administration

The 11 groups of rats employed in this study were categorised into six groups as below and as highlighted in Tables 1–4.

Baseline Control Group 1: Rats of Control Group 1 received only physiological saline for 56 days (days 1–56).

Negative or Toxic Control Group: Rats of Experimental Group 2 received only single intraperitoneal administration of 1.25 mg/kg bodyweight of $CdCl_2$ on day 1 but were left untreated following $CdCl_2$.induced skin carcinogenesis for 42 days (days 14–56).

Anti-Cancer Treatment Groups: Rats of Experimental Groups 3–5 received single intraperitoneal administration of 1.25 mg/kg bodyweight of $CdCl_2$ on day 1, and were post-treated with oral administrations of 15 mg/kg bodyweight of MOF6, 30 mg/kg bodyweight of MOF6 and 10 mg/kg bodyweight of MSF1, respectively, for 42 days (days 14–56).

Positive Control Group: Rats of Experimental Group 6 received single intraperitoneal administration of 1.25 mg/kg bodyweight of $CdCl_2$ on day 1, and were post-treated with intravenous injection of 0.5 mL/200 g of cisplatin and oral administration of 3.35 mg/kg bodyweight of doxorubicin for 14 days (days 15–28). Post-treatment with 0.5 mL/200 g of cisplatin and oral administration of 3.35 mg/kg bodyweight of doxorubicin was stopped on day 28, because the rats could not tolerate further treatment with cisplastin and doxorubicin beyond 14 days.

Toxicological Profiling Groups: Rats of Groups 7 and 8 received only oral administrations of 15 mg/kg and 30 mg/kg bodyweight of MOF6, respectively, for 56 days (days 1–56). Rats of Group 9 received only oral administration of 10 mg/kg bodyweight of MSF1 for 56 days (days 1–56).

Hepato-protective and Cancer Prevention Groups: Rats of Experimental Group 10 received single intraperitoneal administration of 1.25 mg/kg bodyweight of $CdCl_2$ on day 1 and were post-treated with oral administration of 30 mg/kg bodyweight of MOF6 for 56 days (from days 1 to 56). Rats of Experimental Group 11 received single intraperitoneal administration of 1.25 mg/kg bodyweight of $CdCl_2$ on day 1 and were post-treated with oral administration of 10 mg/kg bodyweight of MSF1 for 56 days (from days 1 to 56). Therefore, in-order to determine if extracts-doses could prevent $CdCl_2$-induced carcinogenesis, there was no confirmation of induction of cancer in rats of Groups 10 and 11 as treatment with extracts-doses commenced on day 1 after intraperitoneal administration of $CdCl_2$-dose.

Animal Sacrifice

All rats were sacrificed by cervical dislocation following completion of experimental procedures either on day 28 (Experimental Group 6) or on day 56 (Control Group 1 and Experimental Groups 2–5 and 7–11).

Histo-Pathological Evaluations of the Skin of Rats

The dorsal skin area of each rat in all Groups was scraped, resected and fixed in 10% formal saline of at least five times of its volume. The fixed skin tissues were prepared for light microscopy by employing standard histological methods. Tissue sections were stained via haematoxylin and eosin stain method as previously described (Akinlolu *et al.* 2017).

Evaluations of Lipid Peroxidation

The thiobarbituric acid assay method was employed for quantification of malondialdehyde levels in liver homogenates of all rats as previously described (Akinlolu *et al.* 2012).

Sera Melatonin, Sera TNFα and Liver Tissues' p53 Proteins Concentrations Using Enzyme Linked Immunosorbent Assay (ELISA)

The thoracic cavity of each rat was exposed and 5 mL blood sample collected via the ventricles of the heart into lithium heparinised bottles. The blood samples were centrifuged to obtain the sera. Thereafter, the sera were used for quantitative histochemical enzyme linked immunosorbent assays (ELISA) of levels of melatonin and TNFα proteins in rats of Groups 1–11.

In addition, liver tissues were isolated immediately following animal sacrifice and thoroughly homogenised with the aid of porcelain mortar and pestle in ice-cold 0.25 M sucrose, in the ratio of 1 g to 4 mL of 0.25 M sucrose solution. Skin and liver tissue homogenates were filled up to 5 mL with additional sucrose and collected in a 5 mL serum bottle. The resulting homogenates were centrifuged at 3,000 revolutions per minute for 15 min using a centrifuge (Model 90-1). Supernatants were obtained from the liver tissue homogenates using pasteur pipettes and stored in a freezer at −20°C. Thereafter, quantitative histochemical ELISA of levels of p53 protein in obtained supernatants of rats of Groups 1–11 were conducted. Obtained data acquired from the micro-plate ELISA results were recorded and computed.

Statistical Analyses

Statistical analyses of data and comparisons between groups were conducted for any significant difference using one-way analysis of variance (ANOVA). Tukey post-hoc test was further employed for groups' comparisons. Statistical level of significance was set at $p \leq 0.05$.

RESULTS

Histo-Pathological Evaluations of the Skin of Rats

Morphological observations showed dorsal skin ulceration in rats of $CdCl_2$-treated Group 2 (Figure 1), compared with normal skin morphology in rats of Control Group 1; and normal skin morphology with minimal distortions in Groups 3–11 (Figures 4–8). Histo-pathological evaluations of the skin showed poorly outlined and thinner epidermis, hypo-dense dermis with sparse hair follicles and non-distinct hypodermis in rats of Group 2, when compared with Control Group 1 (Figures 2 and 3).

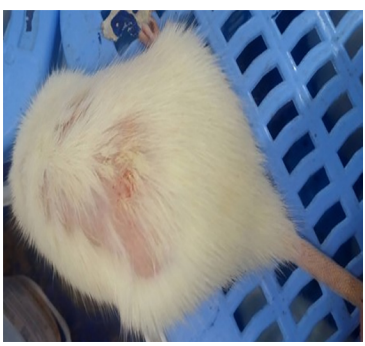

Figure 1: Photograph of skin of rat of Experimental Group 2, which received single intra-peritoneal administration of 1.25 mg/kg bodyweight $CdCl_2$ (day 1) only. Morphological observations show skin ulceration and visible hair loss.

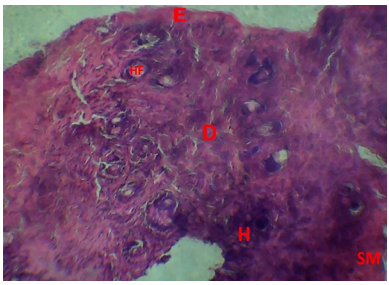

Figure 2: Photomicrograph of skin of rat of Control Group 1, which received physiological saline from days 1–56. Histological observations show well outlined epidermis. The dermis is distinct and contains numerous and visible hair follicles.
Haematoxylin and eosin stain × 120; Scale bar: 50 µm; E = epidermis; HF = hair follicle; D = dermis; H = hypodermis; SM = skeletal muscle.

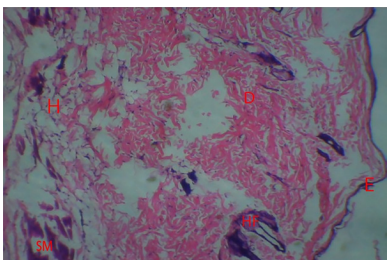

Figure 3: Photomicrograph of skin of rat of Experimental Group 2, which received single intra-peritoneal administration of 1.25 mg/kg bodyweight of $CdCl_2$ (day 1) only. Histo-pathological observations show poorly outlined and thinner epidermis compared with Control Group 1. The dermis is hypo-dense with sparse hair follicles and the hypodermis appears not distinct.
Haematoxylin and eosin stain × 120; Scale bar: 50 µm; E = epidermis; HF = hair follicle; D = dermis; H = hypodermis; SM = skeletal muscle.

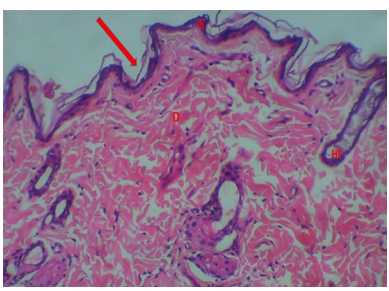

Figure 4: Photomicrograph of skin of rat of Experimental Group 4, which received single intra-peritoneal administration of 1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 15–56). Histo-pathological observations show well outlined, but infolded epidermis. The dermis with its hair follicles appears normal.
Haematoxylin and eosin stain × 120; Scale bar: 50 µm; E = epidermis; HF = hair follicle; D = dermis; H = hypodermis; SM = skeletal muscle.

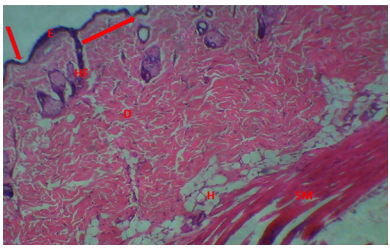

Figure 5: Photomicrograph of skin of rat of Experimental Group 5, which received single intra-peritoneal administration of 1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 15–56). Histo-pathological observations show well outlined, but slightly infolded epidermis. The dermis with its hair follicles appears normal, and it has distinct hypodermal and muscular layers.
Haematoxylin and eosin stain × 120; Scale bar: 50 µm; E = epidermis; HF = hair follicle; D = dermis; H = hypodermis; SM = skeletal muscle.

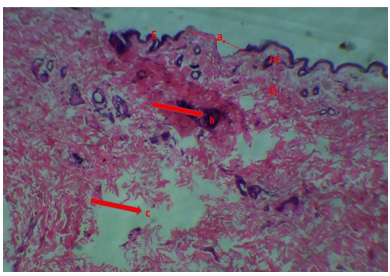

Figure 6: Photomicrograph of skin of rat of Experimental Group 6, which received single intra-peritoneal administration of 1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 0.5 mL/200 g cisplastin + 3.35 mg/kg bodyweight doxorubicin (days 15–56). Histo-pathological observations showed that: a = the epidermis is slightly discontinuous. b = the dermis contains distinct hair follicles.
Haematoxylin and eosin stain × 120; Scale bar: 50 µm; E = epidermis; HF = hair follicle; D = dermis; H = hypodermis; SM = skeletal muscle.

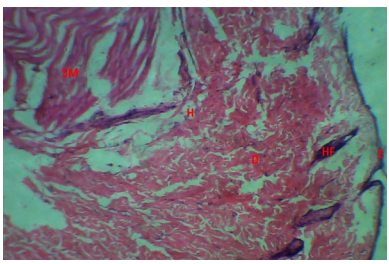

Figure 7: Photomicrograph of skin of rat of Experimental Group 8, which received only 30 mg/kg bodyweight MOF6 (days 1–56). Histo-pathological observations show well outlined and continuous epidermis. The dermis with its hair follicles appears normal.
Haematoxylin and eosin stain × 120; Scale bar: 50 µm; E = epidermis; HF = hair follicle; D = dermis; H = hypodermis; SM = skeletal muscle.

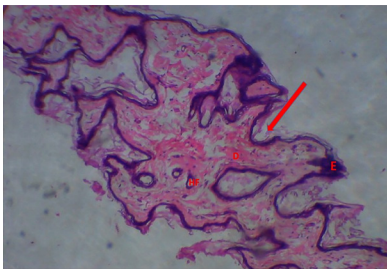

Figure 8: Photomicrograph of skin of rat of Experimental Group 9, which received only 10 mg/kg bodyweight MSF1 (days 1–56). Histo-pathological observations show well outlined, but grossly in-folded epidermis. The dermis appears distinct.
Haematoxylin and eosin stain × 120; Scale bar: 50 µm; E = epidermis; HF = hair follicle; D = dermis; H = hypodermis; SM = skeletal muscle.

Malondialdehyde Levels in Liver Homogenates of Rats

Results showed no significant difference ($p \geq 0.05$) of malondialdehyde levels in rats of Group 2, when compared with Control Group 1 and Groups 3–11 (Table 1).

Table 1: Malondialdehyde concentrations (µmol/mL) in liver tissues of rats.

Groups of rats	Doses of drug/extract administered	MDA (mean ± SEM) (µmol/mL)	$p \leq 0.05$: Group 2 versus Groups 1 and 3–12
1	Physiological saline (56 days)	2.18 ± 1.32	0.28
2	1.25 mg/kg bodyweight $CdCl_2$ (day 1 only)	3.91 ± 1.44	–
3	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 15 mg/kg bodyweight MOF6 (days 15–56)	3.09 ± 1.36	0.59
4	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 15–56)	2.58 ± 1.03	0.27
5	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 15–56)	2.40 ± 1.02	0.21
6	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 0.5 mL/200 g cisplastin + 3.35 mg/kg bodyweight doxorubicin (days 15–29)	2.44 ± 1.03	0.22
7	15 mg/kg bodyweight MOF6 (56 days)	2.50 ± 1.33	0.37
8	30 mg/kg bodyweight MOF6 (56 days)	2.49 ± 1.33	0.36
9	10 mg/kg bodyweight MSF1 (56 days)	2.38 ± 1.32	0.33
10	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 1–56)	2.37 ± 1.02	0.21
11	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 1–56)	2.95 ± 1.54	0.67

Sera Melatonin Levels in Rats

Results showed no significant difference of melatonin levels ($p \geq 0.05$) in Group 2 when compared with Control Group 1 and Groups 3–11 (Table 2).

Table 2: Melatonin concentrations (mean ± SEM) (ng/mL) in sera of rats.

Groups of rats	Doses of drug/extract administered	Melatonin (mean ± SEM) (ng/mL)	$p \leq 0.05$: Group 2 versus Groups 1 and 3–11
1	Physiological saline (56 days)	3.80 ± 1.47	0.50
2	1.25 mg/kg bodyweight $CdCl_2$ (day 1 only)	3.40 ± 1.87	–
3	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 15 mg/kg bodyweight MOF6 (days 15–56)	4.24 ±.453	0.65
4	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 15–56)	5.20 ± 1.60	0.87
5	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 15–56)	4.00 ± 1.52	0.59
6	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 0.5 mL/200g cisplastin + 3.35 mg/kg bodyweight doxorubicin (days 15–29)	4.60 ± 2.40	0.90
7	15 mg/kg bodyweight MOF6 (56 days)	5.41 ± 2.23	0.86
8	30 mg/kg bodyweight MOF6 (56 days)	5.16 ± 1.04	0.91
9	10 mg/kg bodyweight MSF1 (56 days)	4.97 ± 1.23	0.95
10	1.25 mg/kg bodyweight CdCl (day 1) + 30 mg/kg bodyweight MOF6 (days 1–56)	4.84 ± 1.31	0.93
11	1.25 mg/kg bodyweight CdCl (day 1) + 10 mg/kg bodyweight MSF1 (days 1–56)	4.50 ± 3.11	0.41

Sera TNFα Levels in Rats

Results showed no significant difference of TNFα levels ($p \geq 0.05$) in rats of Group 2, compared with Control Group 1 and Groups 3–6 and 8–11 (Table 3). However, there was a pattern of lower TNFα in rats of Group 4 when compared to Group 2 although it is not statistically significant (Table 3).

Table 3: TNFα concentrations (mean ± SEM) (ng/mL) in sera of rats.

Groups of rats	Doses of drug/extract administered	TNFα (mean ± SEM) (ng/mL)	$p \leq 0.05$: Group 2 versus Groups 1 and 3–11
1	Physiological saline (56 days)	4.16 ± 1.49	0.54
2	1.25 mg/kg bodyweight $CdCl_2$ (day 1 only)	5.19 ± 0.11	–
3	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 15 mg/kg bodyweight MOF6 (days 15–56)	5.01 ± 1.27	0.90

(continued on next page)

Table 3: (*continued*)

Groups of rats	Doses of drug/extract administered	TNFα (mean ± SEM) (ng/mL)	$p \leq 0.05$: Group 2 versus Groups 1 and 3–11
4	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 15–56)	7.18 ± 1.89	0.35
5	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 15–56)	4.36 ± 1.19	0.53
6	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 0.5 mL/200 g cisplastin + 3.35 mg/kg bodyweight doxorubicin (days 15–29)	4.94 ± 1.99	0.91
7	15 mg/kg bodyweight MOF6 (56 days)	4.19 ± 1.84	0.30
8	30 mg/kg bodyweight MOF6 (56 days)	3.11 ± 3.85	0.57
9	10 mg/kg bodyweight MSF1 (56 days)	4.95 ± 1.28	0.86
10	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 1–56)	4.29 ± 1.18	0.49
11	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 1–56)	4.85 ± 1.02	0.76

Levels of p53 in Liver Homogenates of Rats

Results showed statistically significant higher ($p \leq 0.05$) levels of p53 in rats of Experimental Group 2, when compared with Control Group 1 and Groups 3–8, 10 and 11 (Table 4).

Table 4: Concentrations of p53 (mean ± SEM) (ng/mL) in liver tissues of rats.

Groups of rats	Doses of drug/extract administered	p53 (mean ± SEM) (ng/mL)	$p \leq 0.05$: Group 2 versus Groups 1 and 3–11
1	Physiological saline (56 days)	24.79 ± 4.23	0.04*
2	1.25 mg/kg bodyweight $CdCl_2$ (day 1 only)	41.82 ± 0.11	–
3	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 15 mg/kg bodyweight MOF6 (days 15–56)	10.71 ± 5.97	0.04*
4	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 15–56)	15.77 ± 4.22	0.02*
5	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 15–56)	25.46 ± 4.19	0.04*
6	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 0.5 mL/200g cisplastin + 3.35 mg/kg bodyweight doxorubicin (days 15–29)	12.45 ± 3.45	0.01*
7	15 mg/kg bodyweight MOF6 (56 days)	15.21 ± 3.42	0.01*
8	30 mg/kg bodyweight MOF6 (56 days)	28.61 ± 3.45	0.04*
9	10 mg/kg bodyweight MSF1 (56 days)	30.89 ± 4.86	0.15

(continued on next page)

Table 4: (*continued*)

Groups of rats	Doses of drug/extract administered	p53 (mean ± SEM) (ng/mL)	$p \leq 0.05$: Group 2 versus Groups 1 and 3–11
10	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 30 mg/kg bodyweight MOF6 (days 1–56)	20.12 ± 3.65	0.02*
11	1.25 mg/kg bodyweight $CdCl_2$ (day 1) + 10 mg/kg bodyweight MSF1 (days 1–56)	16.14 ± 3.49	0.01*

Note: * = Statistical significant difference.

DISCUSSION

The observed dorsal skin ulceration and histo-pathological anomalies of the skin of rats of $CdCl_2$-treated Group 2 suggest that observed $CdCl_2$-induced skin alopecia and ulcers are associated with inhibited wound healing, dystrophic hair papillae, depigmentation and defective hair root sheath. These findings are in agreement with those of Lansdown *et al*. (2001) which reported that treatment of surgically induced skin wound with 1% $CdCl_2$ did not restore and re-epithelialise skin ulceration in rats, and there were non-resolved swelling, abnormal epidermal cell growth, wound waste and sustained mass of inflammatory cell penetration. In addition, the observed dermal atrophy with sparse hair follicles in this study was possibly induced by decreased number of cellular elements, loss of intercellular substance and degeneration of fibrous structures as posited by Abraham and Roga (2014).

Histo-pathological evaluations showed normal histo-architectures of the skin components, though with minimal distortions in rats of Groups 3–11, when compared with Control Group 1 (Figures 2 and 4–8). These observations indicate that anticancer post-treatments or chemo-preventive co-administrations with MOF6 high-dose and MSF1-dose ameliorated and prevented $CdCl_2$-induced skin skin ulceration, histo-pathology and carcinogenesis.

Malondialdehyde is a by-product of lipid peroxidation. Thus, upregulation of tissue level of malondialdehyde indicates induction of un-restrained oxidative stress (Akinlolu *et al.* 2012). The computed higher levels but non-significant upregulation of malondialdehyde levels in rats of Group 2 when compared with Group 1 (Table 1) implies mild induction of oxidative stress following $CdCl_2$-exposure in rats of Group 2.

Post-treatments of $CdCl_2$-induced oxidative stress with MOF6 low-dose, MOF6 high-dose and MSF1-dose resulted in non-significant downregulations of malondialdehyde levels in rats of Groups 3–6, when compared with Group 2 (Table 1). In addition, the commencement of treatment of $CdCl_2$-induced carcinogenesis with MOF6 high-dose and MSF1-dose on day 1 and continued from days 2 to 56 resulted in non-significant downregulations of malondialdehyde levels in Groups 10 and 11, when compared with Group 2 (Table 2). These observations implied that MSF1 and MOF6 did not offer significant antioxidant potentials against $CdCl_2$-induced oxidative stress.

Melatonin is an established antioxidant which targets the mitochondria for induction of its antioxidant effects (Russel *et al.* 2016). Melatonin is, therefore, able to inhibit lipid peroxidation via its antioxidant activities (Lampiao and Du Plessis 2013; Zamfir *et al*. 2014; Mohammadi *et al*. 2016). Furthermore, tissue-inflammation is associated with and accompanied by production of reactive oxygen species and free radicals as well

as carcinogenesis. Therefore, the anti-inflammatory effects of Melatonin increases its antioxidant capability (Lampiao and Du Plessis 2013; Zamfir *et al*. 2014; Mohammadi *et al*. 2016; Russel *et al*. 2016). Thus, upregulation of melatonin levels promotes resolutions of oxidative stress, DNA and cellular damage, inflammation and carcinogenesis. The non-significant downregulation of melatonin levels in rats of Group 2 when compared with Group 1 (Table 2) indicated that $CdCl_2$-exposure led to mild depletion of the antioxidant system in rats of Group 2.

Post-treatments of $CdCl_2$-induced reduction in the antioxidant system with doses of MSF1 and MOF6 resulted in non-significant upregulation of melatonin levels in rats of Groups 3–6, when compared with Group 2 (Table 2). Similarly, the commencement of treatment of $CdCl_2$-induced carcinogenesis with MOF6 high-dose and MSF1-dose on day 1 and continued fromd 2 to 56 resulted in non-significant increased melatonin levels in Groups 10 and 11, when compared with Group 2 (Table 2). These observations implied that MSF1 and MOF6 did not offer significant pro-melatonin potentials against $CdCl_2$-induced depletion of the antioxidant system.

Tissue-inflammation is associated with releases of pro-inflammatory cytokines such as TNFα (Liu *et al*. 2004; Zahr *et al*. 2010; Chu 2013). Hence, the defense system of the body ensures inhibition of upregulation of pro-inflammatory cytokines but promotion of upregulation of anti-inflammatory cytokines. Thus, the non-significant upregulation of TNFα levels in rats of Group 2 when compared with Group 1 (Table 3) indicated that $CdCl_2$-exposure resulted in mild induction of cancer-associated inflammation in rats of Group 2.

Post-treatments of $CdCl_2$-induced inflammation with MOF6 low-dose and MSF1-dose resulted in non-significant downregulations of TNFα in rats of Groups 3 and 5, when compared with Group 2 (Table 3). Similarly, the co-administration of $CdCl_2$ with MOF6 high-dose and MSF1-dose on day 1, and further administrations of extracts only from days 2–56, resulted in non-significant downregulation of TNFα in rats of Groups 10 and 11, when compared with Group 2 (Table 3). Thus, MSF1 and MOF6 MSF1 and MOF6 did not offer significant anti-inflammatory potentials against $CdCl_2$-induced inflammation.

Tumour protein p53 is a pro-apoptotic and tumour suppressor gene. Hence, p53 is referred to as the guardian of the genome. p53 exists at a low level in its quiescent normal state and in normal conditions. Contrariwise, p53-upregulation occurs in cancer-associated chronic inflammation, DNA/cellular damage and carcinogenesis (Chang *et al*. 2010; Toshinori and Akira 2011; Xiao *et al*. 2013). Once upregulated, p53 becomes converted into its active state in-order to implement the repairs of DNA and cellular damages resulting in induction of arrest of the cell cycle. Following successful DNA and cellular procedures, normal cell cycle resumes and p53 level goes back to its low level.

The reported significant upregulation of p53 levels in rats of Group 2 when compared with Control Group 1 (Table 4) indicated that $CdCl_2$-exposure led to induction of DNA and cellular damage with accompanied carcinogenesis in rats of Group 2. The observed consistent upregulation of p53 levels in rats of Group 2 at the end of the 8-week experimental procedure indicated sustained $CdCl_2$-induced carcinogenesis in the absence of cancer treatment. This condition promotes inhibition of the capacity of p53 to repair DNA and cellular damage ensuring that remediating roles of p53 are used up and leading to un-restrained carcinogenesis. This state of anomaly will aid survival of cancer cells and promote tumorigenesis. The observed $CdCl_2$-induction of carcinogenesis and toxicity in this study are in agreement with those of previous studies which reported cadmium-induction of oxidative stress (Wang and Du 2013; Mohamed *et al*. 2019), inflammation (Liu *et* al. 2009; Bernhoft, 2013; Wang and Du 2013), apoptosis and carcinogenesis (Júnior *et al*. 2020).

Post-treatments of $CdCl_2$-induced carcinogenesis with MOF6 low-dose, MOF6 high-dose and MSF1-dose resulted in significant downregulations of p53 levels in rats of Groups 3–5, when compared with Group 2 (Table 4). These results indicate that MSF1 and MOF6 have significant capacities to promote the DNA repair mechanism of p53 and have rapid and very significant pro-apoptotic, tumor suppression and anticancer effects ensuring the return of p53 to normal levels following fast amelioration of $CdCl_2$-induced carcinogenesis.

The co-administrations of $CdCl_2$ with MOF6 high-dose and MSF1-dose on day 1, and further administrations of extracts only from days 2–56, resulted in downregulations of p53 in rats of Groups 10 and 11, when compared with Group 2 (Table 4). Thus, MSF1 and MOF6 conferred chemo-prevention, cyto-protective, pro-apoptotic and anticancer capacities against $CdCl_2$-induced carcinogenesis in rats.

In addition, cancer stem cells (CSCs) are evasive and are not destroyed by different cancer treatment procedures due to their capacities to escape DNA repair mechanism of p53 (Chen *et al*. 2013; Plaks *et al*. 2015). Thus p53 evaluation is of relevance in the production of anticancer drugs which have the capacity to destroy CSCs. The noted significant anticancer effects of MOF6 and MSF1 on p53 levels in $CdCl_2$-induced carcinogenesis indicate that these plant fractions possibly contain anti-cancer drug candidates which can specifically target CSCs.

Furthermore, the anticancer potentials of MSF1 and MOF6 on p53 levels observed in this study are as good as post-treatments with a combined administration of cisplatin and doxorubicin (Table 4). These findings indicate that MSF1 and MOF6 possibly possess anticancer potentials that merit further assessments for the developments of anticancer drug candidates that can specifically target CSCs.

The non-significant downregulations of malondialdehyde, melatonin and TNFα in rats post-treated with doses of MSF1 and MOF6 against $CdCl_2$-induced oxidative stress and inflammation possibly imply that higher doses of these plant fractions or extension of treatment days may be required for significant effects to be elicited.

CONCLUSION

Overall, the observations of this study suggest that post-treatments of $CdCl_2$-induced carcinogenesis with MOF6 low-dose, MOF6 high-dose and MSF1-dose ameliorated skin histo-pathology and resulted in significant downregulations of p53 levels. These observations indicate that MSF1 and MOF6 possibly possess cyto- and histo-protective, hepato-protective and anticancer potentials against $CdCl_2$-induced carcinogenesis. Thus, MSF1 and MOF6 are recommended for further evaluation as anticancer drug candidates.

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