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Original Paper

MORINGA OLEIFERA (MOF6) AND MUSA SAPIENTUM (MSF1) AMELIORATED 7,12-DIMETHYLBENZ[A]ANTHRACENE-INDUCED SKIN HISTO-PATHOLOGY, INFLAMMATION, HEPATIC OXIDATIVE STRESS AND MUTAGENESIS IN RATS

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Abstract

Moringa oleifera and Musa sapientum are ethno-medicinal plants, while 7,12-Dimethylbenz[a] anthracene is a carcinogen. This study evaluated anticancer potentials of MOF6 (extracted from Moringa oleifera leaves) and MSF1 (extracted from Musa sapientum suckers) in 7,12-Dimethylbenz[a]anthracene-induced mutagenesis in rats. Forty-five adult male rats were randomly divided into 9 groups (n = 5). Group 1 was Control. Groups 2 - 6 received single intra-peritoneal administration of 15 mg/Kg bodyweight of Dimethylbenz[a]anthracene on Day 1. Groups 3 - 6 were post-treated with 15 and 30 mg/Kg bodyweight MOF6 (Days 15 - 56), 10 mg/Kg bodyweight MSF1 (Days 15-56) and Doxorubicin/Cisplatin-dose (Days 15 - 29) respectively. Groups 7 - 9 received only MOF6-doses and MSF1-dose respectively. Doses of 7,12-Dimethylbenz[a]anthracene and extracts were administered orally. Skin histo-pathology (Haematoxylin and Eosin), thiobarbituric acid assay of Malondialdehyde (in Liver homogenates), and ELISA concentrations of sera TNFα and Liver homogenates' p53 were evaluated. Data were statistically analyzed ($p \leq 0.05$). Histopathological evaluations showed normal skin histology in Groups 1, 4, 8 and 9. Significant skin histo-alteration was observed in Group 2. Mild skin histo-alterations were observed in Groups 5 and 6. Statistical analyses showed decreased levels of TNF-alpha, Malondialdehyde and p53 in Groups 3 - 9 compared with Group 2. Overall, MOF6 and MSF1 ameliorated 7,12-Dimethylbenz[a]anthracene-induced skin histo-pathology, inflammation, hepatic oxidative stress and mutagenesis.

Keywords: *7,12-dimethylbenz[a]anthracene, lipid peroxidation, Moringa oleifera, Musa sapientum, p53, TNF-alpha*

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Introduction

Cancers are functionally comprised of cancer stem cells (CSCs), macrophages and vascular endothelial cells, with CSCs having tumourigenic capacity while others do not [*Chen et al., 2013; Plaks et al., 2015*]. Cancer treatment regimens kill most cancer cells, but do not eliminate CSCs, which have protective and resistance mechanisms via down-regulation of pro-apoptotic genes such as p53; as well as de-regulations of biomarkers of immune and inflammatory responses such as TNF-alpha (TNFα) [*Chen et al., 2013; Plaks et al., 2015*]. The majority of genes associated with human diseases such as TNFα and p53 seem to be highly conserved across mammalian evolution, and have counterparts in the rat genome [*Simon, 2004; Akinlolu and Shokunbi, 2010*]. This provides the bases for the use of rat models in cancer studies, towards the understanding of cancer pathology and the development of anticancer drugs.

TNFα is a pro-inflammatory cytokine that is involved in the resolution of immune, general inflammatory responses and cancer-associated chronic inflammation [*Liu et al., 2004; Zahr et al., 2010; Chu, 2013*]. This makes TNFα a biomarker of interest in evaluation of tumor progression and cancer cells survival [*Liu et al., 2004; Zahr et al., 2010; Chu, 2013*). p53 is a nuclear transcription factor that trans-activates several apoptotic and cell cycle arrest-induction genes in response to inflammation, DNA damage, chromosomal aberrations, mutagenesis and carcinogenesis. p53 is usually expressed as a functionally latent form at a very low level in normal condition. However, in response to DNA damage, there is upregulation and accumulation of p53 in the cell nucleus resulting in the conversion of p53 from a latent to an active form [*Tong et al., 2010; Toshinori and Akira, 2011; Xiao et al., 2013*]. Functionally active p53 activates apoptosis and cell cycle arrest in-order to allow for the repair of damaged DNA. Thereafter, cells re-enter the normal cell cycle following completion of DNA repair while p53 level returns to its normal low status [*Tong et al., 2010; Toshinori and Akira, 2011; Xiao et al., 2013*].

7,12-Dimethylbenz[a]anthracene (DMBA), is a polycyclic aromatic hydrocarbon, which is produced during the incomplete combustion of carbon-containing compounds, and mainly found in the environment as components of tobacco smoke and exhaust emissions of vehicles. The presence of DMBA in tobacco smoke and exhaust emissions makes it an environmental pollutant of global health concern [*Anderson et al., 1999; Veerasamy and Shanmugam, 2011; Aroyo-Acevedo et al., 2015*]. When metabolically activated, DMBA results in production of a reactive metabolite, dihydrodiolepoxide, which promotes mutagenesis and carcinogenesis by binding to adenine and guanine residues of DNA [*Anderson et al., 1999; Veerasamy and Shanmugam, 2011; Aroyo-Acevedo et al., 2015*]. DMBA-induced mutagenesis is via the production of reactive oxygen species (ROS) resulting in lipid peroxidation, DNA damage and depletion of cell antioxidant defense systems. After exposure to DMBA, stable DNA carcinogen adducts are found in target or affected tissues and protein adducts of serum albumin and haemoglobin are also formed [*Anderson et al., 1999; Veerasamy and Shanmugam, 2011; Aroyo-Acevedo et al., 2015*].

The plant *Moringa oleifera* Lam. (MO), is the most widely cultivated species of the monogeneric family Moringaceae [*Akinlolu et al., 2017; Welch and Tietje, 2017*]. MO is a plant of ethno-medicinal importance which is rich in compounds containing the simple sugar (rhamnose), glucosinolates, isothiocyanates, vitamins, minerals and carotenoids. MO leaves have been reported to have anticancer potentials [*Akinlolu et al., 2017*]. In addition, MOF6, which was fractionated and isolated from *Moringa oleifera* leaves showed significant antioxidant and neuro-protective potentials [*Omotoso et al., 2018; Akinlolu et al., 2020*].

Musa sapientum (MS) or banana belongs to the family musaceae and is a food crop well grown in Nigerian communities [*Akinlolu et al., 2013; Akinlolu et al., 2015*], and it is a plant of ethno-medicinal importance [*Akinlolu et al., 2013; Dahham et al., 2015*]. Scientific studies reported that MS pulps and unripe bananas have anti-ulcer properties while its seeds possess antioxidant, anti-diarrheal and anti-microbial activities [*Akinlolu et al., 2013; Dahham et al., 2015*]. Peel extracts, inflorescence and stalk of MS have also been reported to have antioxidant potentials [*Kumar et al., 2012; Akinlolu et al., 2013*]. MS fruit possesses anticancer potentials [*Dahham et al., 2015*], while aqueous extract of MS sucker was reported to have an-

tioxidant [*Akinlolu et al., 2013*], anti-ulcer [*Akinlolu et al., 2013*] and anti-diabetic [*Akinlolu et al., 2015*] potentials.

The skin forms the first external protective layer of the body, while the liver plays significant roles in drug metabolism, detoxification and the functionality of the body systems [*Moore et al., 2014*]. Therefore, this study evaluated the mechanisms underlying DMBA-induced mutagenesis and also evaluated the anticancer potentials of isolated compounds from *Moringa oleifera* leaves (MOF6) and *Musa sapientum* sucker (MSF1) on skin histology, hepatic oxidative stress, and immuno-modulations of TNFα and p53 genes in 7,12-Dimethylbenz[a]anthracene-induced mutagenesis in rats.

Materials and methods

Ethical approval: Ethical approval for this study (Ethical Committee No: UERC/ASN/2018/1161) was provided by the University of Ilorin Ethical Review Committee, Nigeria on 5th May, 2018. 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) and the US guidelines (NIH publication #85-23, revised in 1985).

Collection, authentication and deposition of* Moringa oleifera *leaves and* Musa sapientum suckers*: Freshly cut leaves of *Moringa oleifera* (MO) leaves and *Musa sapientum* (MS) suckers were obtained locally from forest reserves in Ilorin and samples identified and authenticated by a Pharmaceutical Botanist of the Department of Botany, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria. MO leaves and MS suckers were deposited at the herbarium of the Department of Botany, Faculty of Life Sciences, University of Ilorin, and assigned Herbarium Identification Numbers UILH/001/1249 and UILH/002/1182 respectively.

Preparations and ethanolic extractions of MO leaves and MS suckers: MO leaves and MS suckers were air-dried at the laboratory unit of the Department of Chemistry, University of Ilorin, Ilorin, Nigeria. The dried MO leaves and MS suckers were grinded to powder form to enable proper absorption of solvent and weighed using the electronic compact scale. Extraction was carried out using distilled ethanol in-order to remove impurities, and the resultant product was put in a conical flask and heated. Liquid ethanol flowed from the condenser into a container and was continuously recycled to keep the process running. Boiling chips/anti-bumping granules were put in the conical flask to prevent liquid ethanol from 'bumping' into the condenser [Omotoso *et al*., 2018; Akinlolu *et al*., 2020].

The mixture was decanted and then sieved after 24 hours. After decantation, another distilled ethanol was added to the sieved MO leaves and MS suckers; and left for another 24 hours. When the colour quality and texture of the dissolved MO leaves and MS suckers in ethanol became evidently low (compared to previous solutions decanted), the procedure was halted. Ethanol was separated from MO leaves and MS suckers; and Column chromatography was done to get different fractions of MO leaves and MS suckers [*Omotoso et al., 2018; Akinlolu et al., 2020*].

Column chromatography fractionation of ethanol extract of MO leaves: The ethanol extract of MO leaves was fractionated in a silica gel open column, using n-hexane, dichloromethane, ethyl acetate and ethanol in an increasing order of polarity (N-hexane: 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 thirty-six eluents of 250ml each. The resulting eluents were pooled based on the colour of the solvents that elute them to give a total of nine combined fractions [*Omotoso et al., 2018; Akinlolu et al., 2020*]. The fraction MOF6 which had the best preliminary antioxidant potential out of the 9 fractions, and which we had previously reported to possess antioxidant [*Omotoso et al., 2018*] and neuro-protective [Omotoso *et al*., 2018; Akinlolu *et al*., 2020] potentials was used in this study.

Column chromatography fractionation of ethanol extract of MS suckers: The ethanol extract of MS suckers was fractionated in a silica gel open column, using n-hexane, dichloromethane, ethyl acetate and ethanol in an increasing order of polarity (N-hexane: 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 thirteen eluents of 250ml each. The resulting eluents were pooled based on the colour of the solvents that elute them to give a total of five combined fractions [*Omotoso et al., 2018; Akinlolu et al., 2020*]. The fraction MSF1 which had the best preliminary antioxidant potential out of the 5 fractions was used in this study.

Animal care and feeding: A total number of forty-five (45) male Wistar rats with an average weight of 200 *g* were used in this study. The rats were acclimatized for 5 days, received water ad libitum and kept in the animal house located in the Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, Nigeria. The animals were fed daily with pelletized grower feed from Kusa Ventures, Ilorin, Kwara State, Nigeria. The animals were grouped into nine with five animals each in a wire gauzed cage. The animals were kept under a normal room temperature of 37 ^{0}C and double-crossed ventilation. The number of rats employed in this study was determined based on the guidelines and approval of the University Ethical Review Committee (UERC) of University of Ilorin, Nigeria.

Chemicals and reagents: 7,12-Dimethylbenz[a] anthracene (DMBA) was a product of Sigma-Aldrich Japan Co. (Tokyo, Japan), and was purchased from Bristol Scientific Company, Lagos State, Nigeria. Normal Saline was obtained from MOM-ROTA pharmaceutical company in Ilorin, Kwara State, Nigeria.

Experimental procedures and drugs administration: The experimental procedure and drugs administration were in 5 categories as below. 7,12-Dimethylbenz[a]anthracene (DMBA)-induced carcinogenesis was confirmed by the morphological appearance of skin alopecia and ulcers in rats of Groups 2 – 6 after two weeks (14 days) of experimental procedure.

Rats of Control Group 1 received physiological saline from Days 1 - 56.

Negative Control Group: Rats of Experimental Group 2 received single intra-peritoneal administration of 15 *mg/Kg* bodyweight DMBA, monitored for 2 weeks to confirm cancer-induction, and left untreated for the 56 days (8 weeks) of experimental procedure.

Anti-Cancer Treatment Groups: Rats of Group 3 received single oral administration of 15 *mg/Kg* bodyweight DMBA, monitored for 14 days to confirm cancer-induction, and were treated with oral administration of 15 *mg/Kg* bodyweight of MOF6 from Days 15 - 56 (6 weeks). Rats of Group 4 received single oral administration of 15 *mg/Kg* bodyweight DMBA, monitored for 2 weeks to confirm cancer-induction, and were treated with oral administration of 30 *mg/Kg* bodyweight of MOF6 from Days 15 – 56 (6 weeks). Rats of Group 5 received single oral administration of 15 *mg/Kg* bodyweight DMBA, monitored for 2 weeks to confirm cancer-induction, and were treated with oral administration of 10 *mg/Kg* bodyweight of MSF1 from Days 15 – 56 (6 weeks).

Positive Control Group: Rats of Group 6 received single oral administration of 15 *mg/Kg* bodyweight DMBA, monitored for 2 weeks to confirm cancer-induction, and were treated with intravenous injection of 0.5 *ml*/200 *g* of Cisplatin and oral administration of 3.35 *mg/Kg* bodyweight of Doxorubicin from Days 15 - 29 (2 weeks). This was because the rats could not tolerate more than two-week administrations of Cisplastin and Doxorubicin.

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

Bodyweights (*g*) of all rats were measured on Day 1 of experimental procedure and at the end of each week. The dose of DMBA used in this study was determined from previous studies [*Anderson et al., 1999; Veerasamy and Shanmugam, 2011; Aroyo-Acevedo et al., 2015*]. The MOF6 doses were determined from our previous studies on cyto-protective potentials of *Moringa oleifera* leaves [*Omotoso et al., 2018; Akinlolu et al., 2020*], while the MSF1 doses were determined from our previous studies on cyto-protective potentials of *Musa sapientum* suckers [*Akinlolu et al., 2013; Akinlolu et al., 2015*].

Animal sacrifice: At the end of experimental procedures, all rats were sacrificed by cervical dislocation.

Histo-pathological evaluations of the skin: The dorsal skin area of each rat in all Groups was scraped, excised and fixed in 10 % formal saline of at least five times of its volume. Skin tissues were

processed for light microscopy using conventional histological procedures. Tissue sections were stained via Haematoxylin and Eosin method as previously described by Akinlolu *et al.*, 2017).

Evaluations of lipid peroxidation: The thiobarbituric acid assay (TBARS assay) method was used to quantify Malondialdehyde concentrations in liver homogenates of rats of Groups 1 - 9 as previously described by Akinlolu *et al.*, 2012).

Sera TNFα and liver tissues' p53 proteins concentrations using Enzyme Linked Immunosorbent Assay (ELISA)

The thoracic cavity of each rat was exposed and 5mls blood sample collected via the ventricles of the heart into Lithium heparinized bottles. The blood samples were centrifuged and the serum was used for ELISA analyses of concentrations of TNFα protein (Sigma-Aldrich: RAB0479) in rats of Groups 1 – 9 using ELISA technique. In addition, Liver tissues were isolated immediately after animal sacrifice and then subjected to thorough homogenization using porcelain mortar and pestle in ice-cold 0.25 *M* sucrose, in the proportion of 1 *g* to 4 *ml* of 0.25 *M* sucrose solution. The tissue homogenates were filled up to 5 *ml* with additional sucrose and collected in a 5 *ml* serum bottle. Homogenates were thereafter centrifuged at 3000 revolution per minute for 15 minutes using a centrifuge (Model 90-1). The supernatant was collected with Pasteur pipettes and placed in a freezer at -20 ^{O}C, and thereafter assayed for concentrations of p53 protein (Sigma-Aldrich: MABC1167M) in the liver tissues of rats of Groups 1 – 9 using ELISA technique.

The ELISA assay technique uses the quantitative sandwich enzyme immunoassay technique. Antibodies specific for TNFα and p53 proteins were pre-coated onto a microplate. Standards and samples were pipetted into the wells, and TNFα and p53 proteins present were bound by the immobilized antibodies. After removing any unbound substances, biotin-conjugated antibodies specific for TNFα and p53 proteins were added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of TNFα and p53 proteins bound in the initial step. The colour development was stopped and the intensity of the colour was measured.

Statistical analyses: Statistical analyses were conducted using the 2019 Statistical Package for the Social Science software Version 23.0. Computed data of concentrations of each of TNFα, Malondialdehde and p53 were expressed as arithmetic means ± standard error of mean, and were subjected to statistical analyses using One-way Analysis of Variance to test for significant difference amongst Groups 1 – 9. Significant difference was confirmed at 95% confidence interval with associated p-value of less than 0.05 ($p \leq 0.05$). In addition, Scheffe Post-hoc analysis was used for separation of Mean values between each of Groups 1 – 9. The statistical comparison of the concentration of TNFα, Malondialdehyde and p53 between two groups was considered significant only at $p \leq 0.05$.

Rats of Group 2 received only the carcinogen (DMBA) used for the induction of toxicity in this study, and without further treatment with plants' extracts. Hence, Scheffe Post-hoc analysis was used for comparison between Group 2 and Control Group 1 to establish the adverse effects of DMBA on TNFα, Malondialdehyde and p53 levels. Similarly, Groups 3 – 9 were compared with Group 2 using Scheffe Post-hoc analysis to confirm the degree of ameliorative potentials of doses of MOF6 and MSF1 on the effects of DMBA on TNFα, Malondialdehyde and p53 levels.

Results

Gross morphological observations of the skin: Morphological observations showed dorsal skin ulceration in rats of Group 2, which received only single intra-peritoneal administration of 15 *mg/Kg* bodyweight of DMBA (Figure 1), compared with normal skin morphology in rats of Control Group 1 and Experimental Groups 3 – 9.

Histo-pathological evaluations of the skin: Histopathological evaluations showed normal skin histology in Groups 1, 4, 8 and 9 as presented in Figures 2, 4, 7 and 8. Significant skin histo-alteration was observed in Group 2 as presented in Figure 3. Mild skin histo-alterations were observed in Groups 5 and 6 (Figures 5 and 6).

Malondaldehyde concentrations in liver tissues of rats: Results showed statistically signifi-

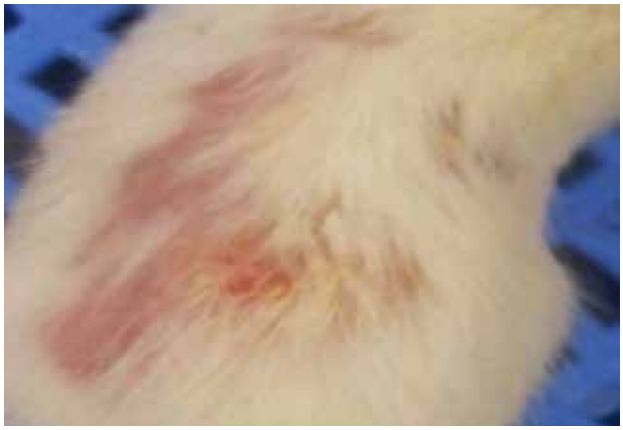

Figure 1. *Photograph of skin of rat of Experimental Group 2, which received single intra-peritoneal administration of 15 mg/Kg bodyweight of 7,12-Dimethylbenz[a]anthracene (DMBA) (Day 1) only. Morphological observations show skin ulceration and visible hair loss (alopecia).*

cant higher ($p \leq 0.05$) Malondialdehyde levels in rats of Group 2 when compared with Groups 1 and 3 – 9 (Table 1).

Sera TNFα concentrations in rats: Results showed statistically significant higher ($p \leq 0.05$) level of TNFα in rats of Group 2 when compared with Groups 1, 3 - 4 and 7 – 8 (Table 2). However, there was non-significant higher ($p \geq 0.05$) level of TNFα in rats of Group 2 when compared with Groups 5 – 6 and 9 (Table 2).

p53 concentrations in liver tissues of rats: Results showed statistically significant higher ($p \leq 0.05$) level of p53 in rats of Group 2 when compared with Groups 1, 3 - 4 and 7 – 8 (Table 2). However, there was non-significant higher ($p \geq 0.05$) level of p53 in rats of Group 2 when compared with Groups 5 – 6 and 9 (Table 2).

Discussion

The observed DMBA-induced skin alopecia and ulcers in rats of Group 2 are possibly associated with inhibited wound healing, dystrophic hair papillae, depigmentation and defective hair root sheath. The observed dermal atrophy with sparse hair follicles in rats of Group 2 possibly resulted from decrease in the number of cellular elements, loss of intercellular substance and degeneration of fibrous structures as opined in a previous study (Abraham and Roga, 2014). In addition, histopathological observations showed non-distinct and dis-continuous epidermis (Group 5), hypo-dense dermis with sparse hair follicles (Groups 5 and 6), and hypertrophied skeletal muscle (Group 6) in rats of Groups 5 and 6 (Figures 1, 5 and 6). These observations suggest that post-treatments with 10 *mg/Kg* bodyweight of MSF1 and standard drugs (Cisplastin and Doxorubicin) partially restored DMBA-induced skin histo-pathology. In contrast, post-treatments with 30 *mg/kg* bodyweight of MOF6 ameliorated DMBA-induced skin histo-pathology in rats of Group 4 (Figure 4).

Lipid peroxidation results in increased oxidative stress, compromised cell membranes and cellular damage; and it is increased in inflammatory and cancer conditions [Akinlolu *et al*., 2012]. Fur-

Table 1.

Malondialdehyde (MDA) concentrations (Mean±SEM) (*μmol/ml*) in liver tissues of rats.

Groups of rats	Doses of drug/extract administered	MDA (μmol/ml)	P-value‡
1	Physiological saline (56 Days)	2.18 ± 1.32	0.02*
2	15 *mg/l=kg* bodyweight DMBA (Day 1 only)	4.24 ± 1.26	
3	15 *mg/kg* bodyweight DMBA (Day 1) + 15 *mg/kg* bodyweight MOF6 (Days 15 - 56)	2.39 ± 0.65	0.04*
4	15 *mg/kg* bodyweight DMBA (Day 1) + 30 *mg/kg* bodyweight MOF6 (Days 15 - 56)	2.34 ± 0.46	0.04*
5	15 *mg/kg* bodyweight DMBA (Day 1) + 10 *mg/kg* bodyweight MSF1 (Days 15 - 56)	2.50 ± 0.48	0.05*
6	15 *mg/kg* bodyweight DMBA (Day 1) + 0.5 *ml*/200 *g* Cisplastin + 3.35 *mg/kg* bodyweight Doxorubicin (Days 15 - 56)	2.49 ± 0.48	0.04*
7	15 *mg/kg* bodyweight MOF6 (56 Days)	2.50 ± 1.33	0.05*
8	30 *mg/kg* bodyweight MOF6 (56 Days)	2.49 ± 1.33	0.04*
9	10 *mg/kg* bodyweight MSF1 (56 Days)	2.38 ± 1.32	0.04*

Notes: ** - Statistical significant difference. DMBA - 7,12-Dimethylbenz[a]anthracene., ‡ - p≤0.05: Group 2 versus Groups 1 and 3 – 12*

TABLE 2.

Sera TNFα and Liver p53 concentrations (Mean±SEM) (*ng/ml*) in rats.

Groups of rats	Doses of drug/extract administered	TNFa (ng/ml) Mean ± SEM	P-value‡	p53 (ng/ml) Mean ± SEM	P-value‡
1	Physiological saline (56 Days)	4.16 ± 1.49	0.03*	24.79 ± 4.23	0.04*
2	15 *mg/ kg* bw DMBA (Day 1)	8.27 ± 0.21		40.82 ± 0.11	
3	15 *mg/ kg* bw DMBA (Day 1) + 15 *mg/ kg* bw MOF6 (Days 15-56)	4.86 ± 1.28	0.05*	12.68 ± 2.47	0.03*
4	15 *mg/ kg* bw DMBA (Day 1) + 30 *mg/ kg* bw MOF6 (Days 15 - 56)	4.82 ± 1.63	0.04*	10.71± 0.26	<0.01*
5	15 *mg/ kg* bw DMBA (Day 1) + 10 *mg/ kg* bw MSF1 (Days 15 - 56)	6.04 ± 1.69	0.11	32.25 ± 3.19	0.45
6	15 *mg/ kg* bw DMBA (Day 1) + 0.5 *ml*/200 *g* Cisplastin + 3.35 *mg/ kg* bw Doxorubicin (Days 15 -56)	7.84 ± 2.33	0.21	36.82 ± 2.04	0.61
7	15 *mg/ kg* bw MOF6 (56 Days)	4.19 ± 1.84	0.04*	15.21 ± 3.42	0.01*
8	30 *mg/ kg* bw MOF6 (56 Days)	3.11 ± 3.85	0.02*	28.61 ± 3.45	0.05*
9	10 *mg/ kg* bw MSF1 (56 Days)	4.95 ± 1.28	0.06	30.89 ± 4.86	0.15

NOTES: ** - Statistical significant difference. SEM - Standard Error of Mean. DMBA - 7,12-Dimethylbenz[a]anthracene. bw - Bodyweight, ‡ -p≤0.05: Group 2 versus Groups 1, and 3 – 9*

thermore, Malondialdehyde is a resultant mutagenic aldehyde product of lipid peroxidation. Hence, increased Malondialdehyde levels imply increased oxidative stress. The observed significant Malondialdehyde level in rats of Group 2 implied DMBA-induction of increased oxidative stress. Do MOF6 and MSF1 have antioxidant potentials against DMBA-induced oxidative stress? Post-treatments of DMBA-induced oxidative stress with 15 and 30 *mg/Kg* bodyweight of MOF6 and 10 *mg/Kg* bodyweight of MSF1 resulted in significant decreased Malondialdehyde levels in rats of Groups 3 - 6, when compared with Group 2 (Table 1). Hence, MOF6 and MSF1 possess antioxidant potentials.

When cells are exposed to chemical or physical injury, anoxia or starvation, there is activation of cellular immune response, inflammation and induction of release of cytokines. Inflammation results in cell damage via necrosis [*Liu et al., 2004; Zahr et al., 2010; Chu, 2013*], resulting in the release of cellular contents into the extracellular space and in the induction of apoptosis of adjacent cells. Hence, in chemicals-induced mutagenesis, the upregulation of TNFα is a characteristic immune response mecha-

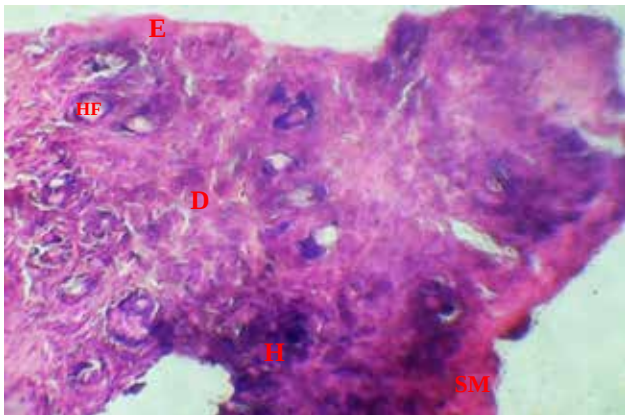

FIGURE 2. *Photomicrograph of skin of rat of Control Group 1, which received physiological saline from Days 1 - 56. Haematoxylin and Eosin X 120. E = Epidermis, HF = Hair follicle, D = Dermis, H = Hypodermis and SM = Skeletal muscle. Histo-pathological observations show well-outlined epidermis and distinct dermis containing numerous and visible hair follicles.*

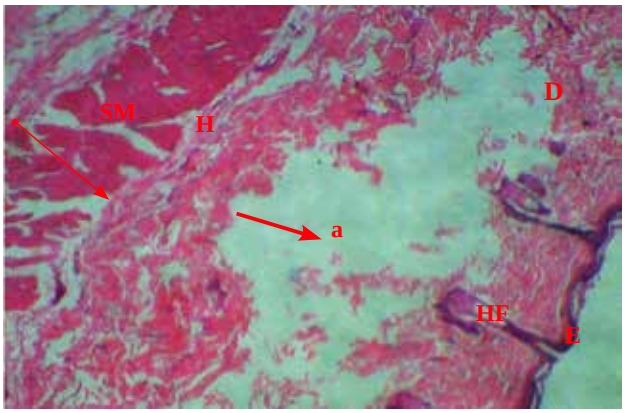

FIGURE 3. *Photomicrograph of skin of rat of Experimental Group 2, which received 15 mg/Kg bodyweight of DMBA (Day 1) only. Haematoxylin and Eosin X 120. E = Epidermis, HF = Hair follicle, D = Dermis, H = Hypodermis and SM = Skeletal muscle. Histo-pathological observations show well-outlined epidermis, hypodense dermis, non-distinct hypodermis and hyper-trophied skeletal muscle.*

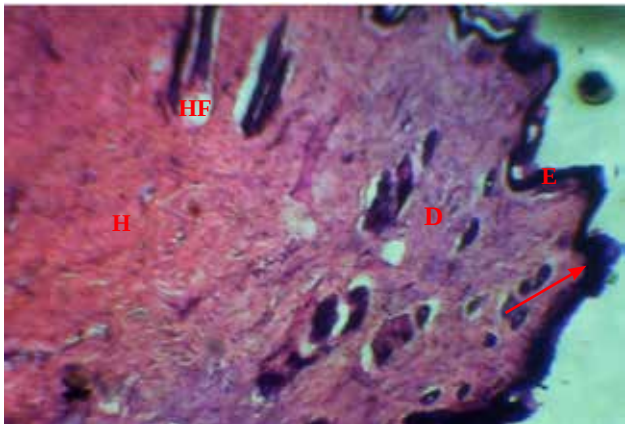

FIGURE 4. *Photomicrograph of skin of rat of Experimental Group 4, which received 15 mg/Kg bodyweight DMBA (Day 1) + 30 mg/Kg bodyweight MOF6 (Days 15 - 56). Haematoxylin and Eosin X 120. E = Epidermis, HF = Hair follicle, D = Dermis, H = Hypodermis and SM = Skeletal muscle. Histo-pathological observations show well-outlined epidermis and dermis.*

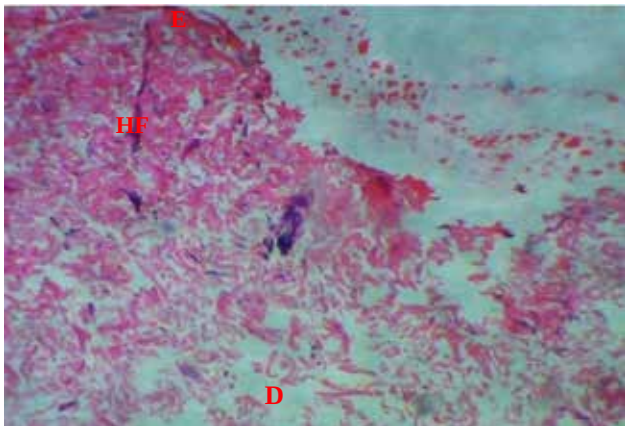

FIGURE 5. *Photomicrograph of skin of rat of Experimental Group 5, which received 15 mg/Kg bodyweight DMBA (Day 1) + 10 mg/Kg bodyweight MSF1 (Days 15 - 56). Haematoxylin and Eosin X 120. E = Epidermis, HF = Hair follicle, D = Dermis, H = Hypodermis and SM = Skeletal muscle. Histo-pathological observations show non-distinct and dis-continuous epidermis, and hypo-dense dermis.*

nism for the resolution of cancer-associated chronic inflammation, resulting in induction of necrosis and consequent apoptosis [*Liu et al., 2004; Zahr et al., 2010; Chu, 2013*]. The upregulation of TNFα in rats of Group 2 implied DMBA-induction of cancer-associated inflammation and upregulation of TNFα in rats of Group 2.

Do MOF6 and MSF1 have anticancer potentials against DMBA-induced inflammation? Post-treatments of DMBA-induced inflammation with 15 and 30 *mg/Kg* bodyweight of MOF6 and 10 *mg/Kg* bodyweight of MSF1 resulted in significant (Groups 3 and 4) and non-significant (Group 5) downregulations of TNFα levels when compared with Group 2 (Table 2). Hence, MOF6 and MSF1 possess cyto-protective and anti-inflammatory potentials.

p53 is a pro-apoptotic gene which is upregulated while functioning to induce cell cycle arrest in cells with damaged DNA and chromosomal aberrations, and it returns to its normal status consequent to completion of repair of damaged DNA [*Tong et al., 2010; Toshinori and Akira, 2011; Xiao et al., 2013*]. The significant upregulation of p53 in rats of Group 2 implied DMBA-induction of mutagenesis. The characteristic expected immune response to mutagenesis is upregulation of p53 pro-apoptotic gene resulting in p53-induction of apoptosis. p53 upregulation is, however, as long as it takes for p53 activated apoptotic mechanism to complete the repair of damaged DNA/cells. Thereafter, p53 expression returns to normal status as the cells re-enter the normal cell cycle. In the absence of cancer treatment, the sustained upregulation of p53 in rats of Group 2 implied on-going DMBA-induced mutagenesis. This will gradually inhibit and prolong the DNA repair mechanism of p53 until its actions are spent out in-order to allow for un-inhibited mutagenesis. Consequently, cancer cells survival and tumorigenesis will prevail.

Do MOF6 and MSF1 have anticancer potentials against DMBA-induced mutagenesis? Post-treatments of DMBA-induced mutagenesis with 15 and

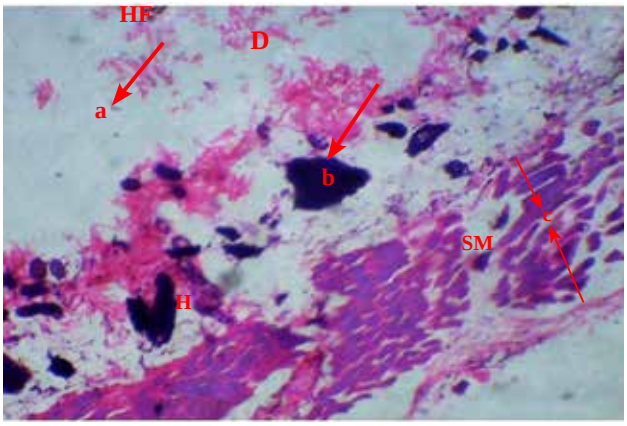

FIGURE 6. *Photomicrograph of skin of rat of Experimental Group 6, which received 15 mg/Kg bodyweight DMBA (Day 1) + 0.5 ml/200 g Cisplastin + 3.35 mg/Kg bodyweight Doxorubicin (Days 15 - 56). Haematoxylin and Eosin X 120. HF = Hair follicle, D = Dermis, H = Hypodermis and SM = Skeletal muscle. Histo-pathological observations show hypo-dense dermis, hyper-trophied and distorted skeletal muscle.*

30 *mg/Kg* bodyweight of MOF6 and 10 *mg/Kg* bodyweight of MSF1 resulted in significant (Groups 3 and 4) and non-significant (Group 5) downregulations of p53 levels when compared with Group 2 (Table 2). The downregulations of p53 in rats of Groups 3 - 5, when compared with Group 2 (Table 2) imply that MOF6 and MSF1 possess significant pro-apoptotic and anticancer potentials bringing p53 to normal levels after quick resolution of mutagenesis.

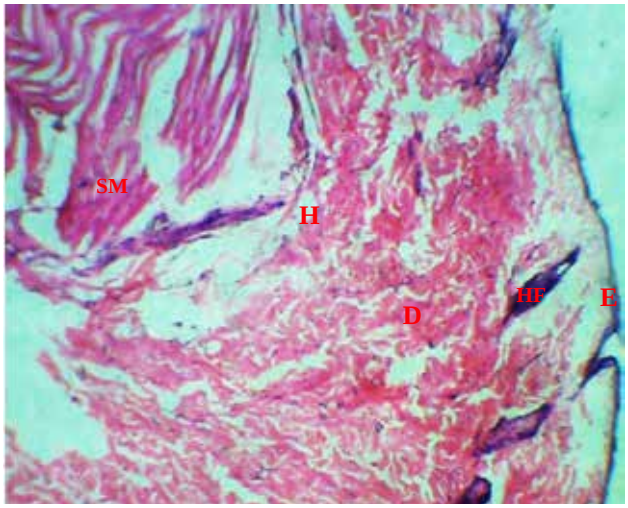

Figure 7. *Photomicrograph of skin of rat of Experimental Group 8, which received only 30 mg/Kg bodyweight MOF6 (Days 1 - 56). Haematoxylin and Eosin X 120. HF = Hair follicle, D = Dermis, H = Hypodermis and SM = Skeletal muscle. Histo-pathological observations show well outlined and continuous epidermis. The dermis with its hair follicles appears normal.*

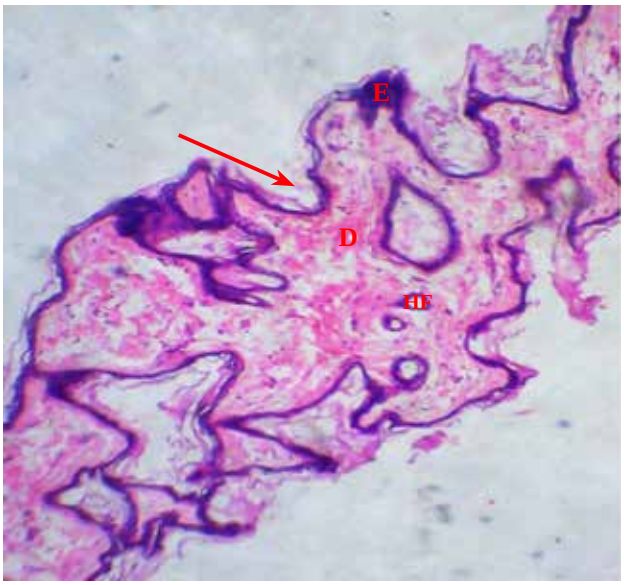

Figure 8. *Photomicrograph of skin of rat of Experimental Group 9, which received only 10 mg/Kg bodyweight MSF1 (Days 1 - 56). Haematoxylin and Eosin X 120. E = Epidermis, HF = Hair follicle, D = Dermis, H = Hypodermis and SM = Skeletal muscle. Histo-pathological observations show well outlined, but grossly infolded epidermis. The dermis appears distinct.*

p53 is regarded as the guardian of the genome. Therefore, cancer stem cells (CSCs) evade apoptosis via dysregulation and induction of loss of function mutation of the p53 apoptotic gene. Furthermore, there are dys-regulations of antioxidant defense mechanism and TNFα in cancer cells survival, hence the relevance of these biomarkers in the development of drugs that can eliminate CSCs. Hence, our findings indicate that MOF6 and MSF1 possibly possess anti-cancer compounds that can specifically target and eliminate CSCs.

Are the histo-protective, cyto-protective, anti-inflammatory, pro-apoptotic and anti-cancer potentials of MOF6 and MSF1 comparable to standard anticancer drugs? Our findings showed that MOF6 offered better histo-protective (Figures 2 – 6), cyto-protective, pro-apoptotic and anticancer potentials than treatments with a combination of Cisplatin and Doxorubicin (Tables 1 and 2) against DMBA-induced mutagenesis. On the other hand, MSF1 offered similar histo-protective (Figures 2 – 6), cyto-protective, antioxidant, pro-apoptotic and anticancer potentials in comparison with Cisplastin and Doxorubicin against DMBA-induced mutagenesis (Tables 1 and 2). These findings implied that 15 and 30 *mg/Kg* bodyweight of MOF6 possess better anticancer potentials than 10 *mg/Kg* bodyweight of MSF1, and deserve further evaluations towards the discovery of anticancer drug compounds that can eliminate CSCs. The anticancer potentials of MSF1 should, however, be further evaluated using higher doses than the 10 *mg/Kg* bodyweight employed in this study.

The observed anticancer potentials of MOF6 in this study are in agreement with those of Welch and Tietje, 2017 and Tiloke *et al*., 2018, which reported anticancer potentials of *Moringa oleifera* against cancer cells and chemicals-induced carcinogenesis. Similarly, our observed anticancer potentials of MSF1 are in agreement with those of Kumar *et al*., 2012 and Dahham *et al*., 2015, which reported anticancer potentials of *Musa sapientum* in in vitro and in vivo models.

Conclusion

Overall, our findings suggest that DMBA-induced mutagenesis is associated with skin ulceration, skin histo-pathology, lipid peroxidation, inflammation and necrosis with accompanied p53-induction of apoptosis in rats. In addition, post-treatments with doses of MOF6 (extracted from *Moringa oleifera* leaves) and MSF1 (extracted from *Musa sapientum* suckers) conferred a degree of histo-protection and hepato-protection against 7,12-Dimethylbenz[a]anthracene-induced skin ulceration, skin histo-pathology, hepatic oxidative stress, inflammation and mutagenesis, and are recommended for further evaluation as potential drug candidates for the elimination of cancer stem cells and for the treatment of cancers.

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