Moringa oleifera and *Musa sapientum* ameliorated 7,12-Dimethylbenz[a]anthracene-induced upregulations of Ki67 and multidrug resistance 1 genes in rats

**A. A. Akinlolu[1],
A. O. Oyewopo[2], R. E. Kadir[2],
A. Lawal[2], J. Ademiloye[2],
A. Jubril[2], M. O. Ameen[3],
G. E. Ebito[4]**

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun State, Nigeria, [2]Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria, [3]Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria, [4]Department of Anatomy, Faculty of Basic Medical Sciences, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria

Address for correspondence:
Dr. A. A. Akinlolu, Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun State, Nigeria.
Phone: +2348062765308.
E-mail: adelaja.akinlolu.454@my.csun.edu

ABSTRACT

Objectives: *Moringa oleifera* (MO) and *Musa sapientum* (MS) are plants of ethnomedicinal importance. We evaluated the effects of MOF6 (extracted from MO leaves) and MSF1 (extracted from MS suckers) on immunomodulations of Ki67 (proliferation biomarker) and multidrug resistance 1 (MDR1) genes in the liver of rats in 7,12-Dimethylbenz[a]anthracene (DMBA)-induced hepatotoxicity and mutagenesis to determine their antiproliferation, anti-drug resistance, and anticancer potentials.

Methods: Forty-five adult male rats were randomly divided into nine groups ($n = 5$). Groups 1 and 2 received physiological saline and 15 mg/kg bodyweight of DMBA, respectively. Groups 3 and 4 received 15 mg/kg bodyweight DMBA and were treated with 15 and 30 mg/kg bodyweight of MOF6, respectively. Group 5 received 15 mg/kg bodyweight DMBA and was treated with 10 mg/kg bodyweight of MSF1. Group 6 received 15 mg/kg bodyweight DMBA and was treated with 3.35 mg/kg bodyweight of doxorubicin and intravenous injection of 0.5 ml/200 g of cisplatin. Groups 7–9 received only 15 and 30 mg/kg bodyweight of MOF6 and 10 mg/kg bodyweight of MSF1, respectively. DMBA, doxorubicin, and extracts doses were administered orally. The duration of our experimental procedure was 8 weeks. Consequently, liver histopathology (hematoxylin and eosin technique) and enzyme-linked immunosorbent assay homogenates' concentrations of Ki67 and MDR1 were evaluated. Computed data were statistically analyzed ($P \leq 0.05$).

Results: Results showed normal histoarchitectures of the liver in all groups. Statistical analyses showed significant ($P \leq 0.05$) and non-significant decreased concentrations ($P \geq 0.05$) of Ki67 and MDR1 in Groups 3–9 compared with Group 2. Therefore, MOF6 and MSF1 ameliorated DMBA-induced hepatotoxicity, abnormal proliferation, and drug resistance.

Conclusion: MOF6 and MSF1 possess antiproliferation, anti-drug resistance, and anticancer potentials.

Keywords: 7,12-Dimethylbenz[a]anthracene, Ki67, *Moringa oleifera*, Multidrug resistance 1, *Musa sapientum*

Introduction

Cancer is a group of diseases characterized by unrestricted growth and metastasis of abnormal cells.[1,2] It was ranked the second leading cause of death behind cardiovascular diseases since 2013,[1,2] and this imposes a huge burden on societies.[3] Cancer can be caused by internal factors (inherited genetic mutations, hormones, and immune conditions) and/or external factors (tobacco, infectious agents, environmental carcinogens, and unhealthy diet). Cancer treatments include surgery, radiation, hormone, immune, and chemical therapy, which interfere with cancer cell growth or destroy cancer tissues.[1,2]

Cancers comprise cancer stem cells (CSCs), macrophages, and vascular endothelial cells, with CSCs having tumorigenic capacity while others do not.[1-3] This is due to the fact that CSCs are cancer cells that mimic embryonic stem cells and thus possess the capacity to self-renew, to differentiate into new progenies, and to initiate and sustain tumourigenesis and tumor growth.[1-3] Cancer treatment regimens (including surgery, radiation, hormone, immune, and chemical therapy) kill most cancer cells, but do not eliminate CSCs, which possess protective and resistance mechanisms through the upregulation of specific factors such as biomarkers of proliferation (Ki67) and drug resistance (multidrug resistance

1 or P-glycoprotein).[2-4] The characteristic survival of CSCs provides explanation for the failures of cancer treatments, hence, the need to search for drug sources that can target CSCs from plants or other sources.

Ki-67 protein is detected during all the active phases of the cell cycle and it is usually used as a complement to grading systems that include mitotic counting as a sign of proliferation.[5,6] It is one of the five genes (out of 16 cancer-associated genes) of proliferation that plays a key role in Oncotype scoring. Ki-67 is not expressed by quiescent or resting cells in the G0phase, hence, it is an excellent operational marker for the evaluation of the proliferation of a given cell population and the aggressiveness of malignancies.[5-7]

The multidrug resistance 1 (MDR1) gene or P-glycoprotein is localized in the cell membrane and it functions pharmacologically as an active drug efflux transporter protein of various substances including drugs and toxins. [4,8,9] The MDR1 protein is physiologically expressed at the bile canalicular membrane of the liver functioning in biliary excretion of lipophilic drugs.[10] The MDR1 protein has affinity for hydrophobic compounds and efforts have been made to bypass its efflux effect using reversal agents such as R-verapamil, Tween-80, and Cremophor EL. These reversal agents have, however, been reported to induce significant toxicity at required doses for MDR1's inhibition.[4,8,9]

The plant *Moringa oleifera* Lam. (MO) is the most widely cultivated species of the monogeneric family Moringaceae (order Brassicales), which includes 13 species of trees and shrubs distributed in sub-Himalayan ranges of India, Sri Lanka, and Northeast and West Africa (including Nigeria).[11-14] MO is a plant of ethnomedicinal importance and has been used traditionally to treat many diseases such as cancer, ulcer, diabetes, and hypertension. MO is rich in compounds containing the simple sugar (rhamnose), glucosinolates, isothiocyanates, vitamins, minerals, and carotenoids (including β-carotene or pro-Vitamin A). MO leaves have been reported to have anticancer[12], neuroprotective,[13,14] and antioxidant[13] potentials.

Musa sapientum (MS) or banana belongs to the family Musaceae and is a food crop well grown in Nigerian communities.[15,16] MS is a plant of ethnomedicinal importance and its various parts of have traditionally been used for the treatment of diseases such as ulcer, diabetes, and hypertension.[15-18] Scientific studies have equally observed that MS pulps and unripe bananas have anti-ulcer properties while its seeds possess antioxidant, anti-diarrheal, and antimicrobial activities. Peel extracts, inflorescence, and stalk of MS have also been reported to have significant antioxidant potentials.[15,16] MS fruit was reported to have anticancer potentials[18], while MS sucker was reported to have antioxidant[15], anti-ulcer,[15] and antidiabetic[16] potentials.

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 a chemical agent of global health concern.[19-22] When metabolically activated, DMBA results in production of a reactive metabolite, dihydrodiol epoxide, which promotes mutagenesis and carcinogenesis by binding to adenine and guanine residues of DNA.[19-22] DMBA-induced mutagenesis and carcinogenesis are through the production of reactive oxygen species resulting in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems. DMBA experimentally induces cancer in endodermal (liver), mesodermal (kidney), and ectodermal (skin and mammary gland) derivatives in animal models. After exposure to DMBA, stable DNA carcinogen adducts are found in target or affected tissues and protein adducts of serum albumin and hemoglobin are also formed.[19-22]

The characteristic abnormal cellular proliferation with accompanied increased expressions of Ki67 and MDR1 by CSCs makes the treatment of cancers a very challenging task. It is, therefore, very relevant to evaluate plants sources toward the isolation of drugs compounds that can specifically target CSCs and reduce or eliminate drug resistance. We have previously reported that MOF6 (fractionated and isolated from MO leaves) showed significant antioxidant potentials against cuprizone-induced increased superoxide dismutase levels[13], while the aqueous extract of MS sucker significantly reduced indomethacin-induced increased catalase, superoxide dismutase, and malondialdehyde (lipid peroxidation) levels[15] in rats.

The liver is the largest body organ, and it plays significant roles in drug metabolism, detoxification, and the functionality of the body systems.[23] Therefore, this study evaluated the effects of fractionated and isolated compounds from MO leaves (MOF6) and MS sucker (MSF1) on immunomodulations of Ki67 and MDR1 genes in the liver of rats in DMBA-induced mutagenesis to further determine their hepatoprotective, antiproliferation, anti-drug resistance, and anticancer potentials.

Materials and Methods

Ethical approval

Ethical approval for this study was sought and received from the Ethical Review Committee of the University of Ilorin, Nigeria, where the study was primarily conducted. The ethical approval number is UERC/ASN/2018/1161. 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 MO leaves and MS suckers

Freshly cut leaves of MO leaves and 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 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

- The mixture was decanted and then sieved after 24 h. After decantation, another distilled ethanol was added to the sieved MO leaves and MS suckers; and left for another 24 h. When the color 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.

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:ethyl acetate [3:1, 3:2, 1:1, 1:2, 1:3]; ethyl acetate; ethyl acetate:methanol [3:1, 3:2, 1:1, 1:2, 1:3] and methanol), to afford 36 eluents of 250 ml each. The resulting eluents were pooled based on the color of the solvents that elute them to give a total of nine combined fractions. The fraction MOF6 which had the best preliminary antioxidant potential out of the nine fractions and which we had previously reported to possess antioxidant[13] and neuroprotective[13,14] potentials was used in this study to evaluate the effects of MO leaves on DMBA-induced hepatotoxicity and mutagenesis in rats.

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:ethyl acetate [3:1, 3:2, 1:1, 1:2, 1;3]; ethyl acetate; ethyl acetate:methanol [3:1, 3:2, 1:1, 1:2, 1:3] and methanol), to afford 13 eluents of 250 ml each. The resulting eluents were pooled based on the color of the solvents that elute them to give a total of five combined fractions. The fraction MSF1 which had the best preliminary antioxidant potential out of the five fractions was used in this study to evaluate the effects of MS on DMBA-induced hepatotoxicity and mutagenesis in rats.

Animal care and feeding

A total number of 60 (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°C and double-crossed ventilation.

Chemicals and reagents

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 MOMROTA pharmaceutical company in Ilorin, Kwara State, Nigeria.

Experimental procedures and drugs administration

The experimental procedure and drugs administration were in six categories as below to evaluate the anticancer and hepatoprotective potentials of MOF6 (extracted from MO leaves) and MSF1 extracted from MS suckers on DMBA-induced toxicity. Rats of control Group 1 received physiological saline for 8 weeks.

Negative control group
Rats of experimental Group 2 received single intraperitoneal administration of 15 mg/kg bodyweight DMBA, monitored for 2 weeks to confirm cancer induction, and left untreated for the 8 weeks of experimental procedure.

Anticancer treatment groups
Rats of Group 3 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 15 mg/kg bodyweight of MOF6 for another 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 for another 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 for another 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 for another 6 weeks.

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.

Bodyweights (g) of all rats were measured on day 1 of experimental procedure and at the end of each week.

Animal sacrifice

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

Histopathological evaluations of the liver

The liver of all rats was excised and a lobe fixed in 10% formal saline of at least 5 times of its volume. Liver tissues were processed for light microscopy using conventional histological procedures. Tissue sections were stained through hematoxylin and eosin method as previously described.[11]

Enzyme-linked immunosorbent assay (ELISA) of concentrations of Ki67 and MDR1 genes in liver tissues of rats

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–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 min using a centrifuge (Model 90-1). The supernatant was collected with Pasteur pipettes and placed in a freezer at −4°C, and thereafter assayed for concentrations of Ki67 and MDR1 protein in the liver tissues of all rats of control and experimental groups using ELISA technique.

Statistical analyses

All data obtained were expressed as arithmetic means ± standard error of mean and were subjected to statistical analyses using *t*-test to compare Group 2 with Groups 3–9. Differences were tested and considered statistically significant when $P \leq 0.05$ using GraphPad Prism software package (GraphPad Software Inc., San Diego, CA, USA; version 7 for Windows) and Microsoft Excel 2016.

Results

### Histopathological evaluations of the liver

Histopathological evaluations showed normal histoarchitectures of the liver in rats of Groups 1–9 [Figures 1–7]. There were normal cellular density and staining characteristics of hepatocytes, hepatic sinusoids, and central veins. The nuclei of hepatocytes were well characterized with no apparent large vacuolation around them.
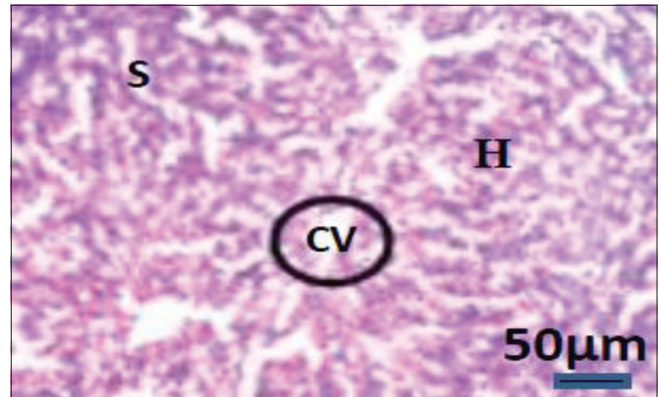

Figure 1: Photomicrograph of liver of rat of control Group 1, which received normal saline for 8 weeks. Hematoxylin and eosin X 100. Scale bar: 50μm. H = Hepatocytes, S = Sinusoid, and CV = Central vein. Histopathological evaluations showed normal histoarchitecture of the liver components
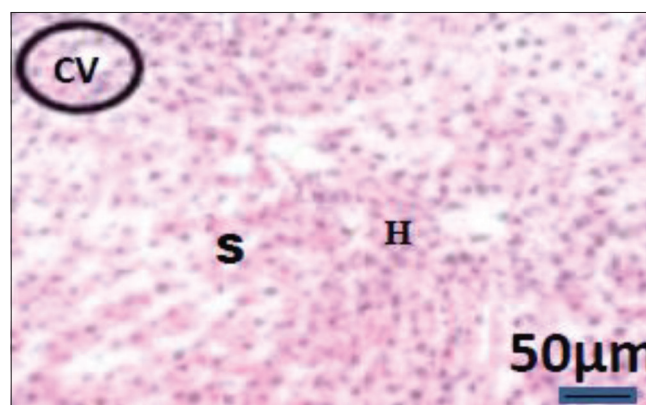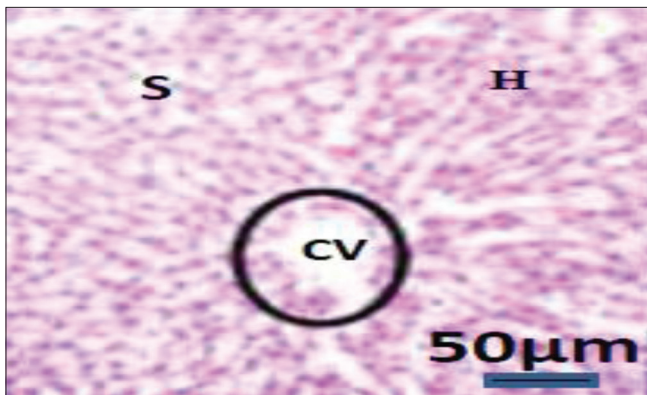

Figure 2: Photomicrograph of liver of rat of experimental Group 2, which received single administration of 15 mg/Kg bodyweight DMBA only. Hematoxylin and eosin X 100. Scale bar: 50 μm. H = Hepatocytes, S = Sinusoid, and CV = Central vein. Histopathological evaluations showed normal histoarchitecture of the liver components
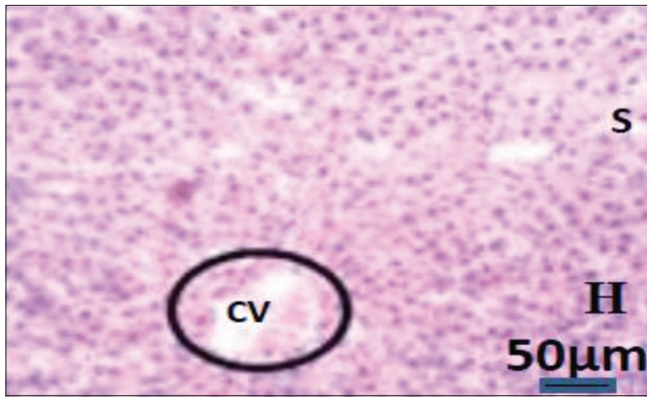
Figure 3: Photomicrograph of liver of rat of experimental Group 4, which received 15 mg/Kg bodyweight DMBA (2 weeks) + 30 mg/Kg bodyweight MOF6 (6 weeks). Hematoxylin and eosin X 100. Scale bar: 50 μm. H = Hepatocytes, S = Sinusoid, and CV = Central vein. Histopathological evaluations showed normal histoarchitecture of the liver components

Figure 6: Photomicrograph of liver of rat of experimental Group 8, which received only 30 mg/Kg bodyweight MOF6 (8 weeks), respectively. Hematoxylin and eosin X 100. Scale bar: 50 μm. H = Hepatocytes, S = Sinusoid, and CV = Central vein. Histopathological evaluations showed normal histoarchitecture of the liver components
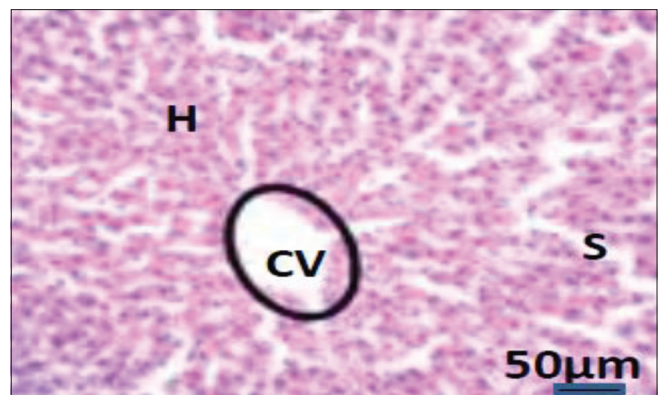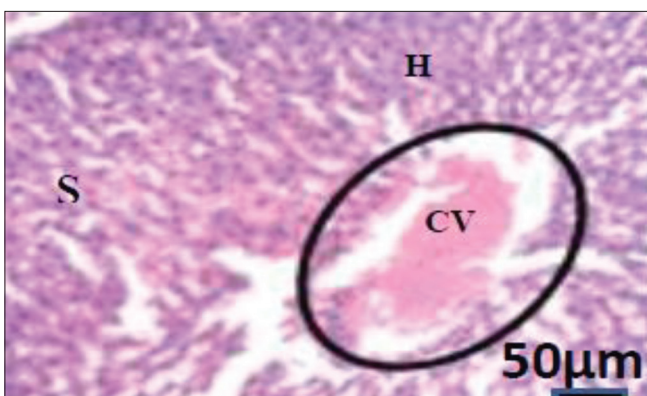

Figure 4: Photomicrograph of liver of rat of experimental Group 5, which received 15 mg/Kg bodyweight DMBA (2 weeks) + 10 mg/Kg bodyweight MSF1 (6 weeks). Hematoxylin and eosin X 100. Scale bar: 50 μm. H = Hepatocytes, S = Sinusoid, and CV = Central vein. Histopathological evaluations showed normal histoarchitecture of the liver components

Figure 7: Photomicrograph of liver of rat of experimental Group 9, which received 10 mg/Kg bodyweight MSF1 (8 weeks). Hematoxylin and eosin X 100. Scale bar: 50 μm. H = Hepatocytes, S = Sinusoid, and CV = Central vein. Histopathological evaluations showed normal histoarchitecture of the liver components

ELISA concentrations of Ki67 in Liver tissues of rats

Results showed statistically significant higher ($P \leq 0.05$) levels of Ki67 in rats of Group 2 ($P = 0.04$) when compared with Group 1 [Table 1]. There were statistically non-significant lower ($P \geq 0.05$) levels of Ki67 in rats of Groups 3 ($P = 0.39$) and 4 ($P = 0.06$), when compared with Group 2 [Table 1]. In addition, results showed statistically significant lower ($P \leq 0.05$) levels of Ki67 in rats of Groups 5 ($P = 0.02$), 6 ($P = 0.01$), 7 ($P = 0.01$), 8 ($P = 0.01$), and 9 ($P = 0.01$), when compared with Group 2 [Table 1].

ELISA concentrations of MDR1 in liver tissues of rats

Results showed statistically non-significant higher ($P \geq 0.05$) levels of MDR1 in rats of Group 2 when compared with Group 1 ($P = 0.11$) [Table 2]. In addition, results showed statistically

Figure 5: Photomicrograph of liver of rat of experimental Group 6, which received 15 mg/Kg bodyweight DMBA (2 weeks) + 0.5 ml/200 g cisplatin + 3.35 mg/Kg bodyweight doxorubicin (6 weeks). Hematoxylin and eosin X 100. Scale bar: 50 μm. H = Hepatocytes, S = Sinusoid, and CV = Central vein. Histopathological evaluations showed normal histoarchitecture of the liver components

Table 1: Ki67 concentrations (Mean±SEM) (ng/ml) in liver tissues of rats

Groups of rats	Doses of drug/extract administered	Ki67 (Mean±SEM) (ng/ml)	P≤0.05: Group 2 versus Groups 1 and 3–12
1	Physiological saline (8 weeks)	8.03±0.62	0.04*
2	15 mg/kg bodyweight DMBA	18.15±0.11	
3	15 mg/kg bodyweight DMBA (2 weeks) + 15 mg/kg bodyweight MOF6 (6 weeks)	16.46± 1.55	0.39
4	15 mg/kg bodyweight DMBA (2 weeks) + 30 mg/kg bodyweight MOF6 (6 weeks)	12.40± 1.53	0.06
5	15 mg/kg bodyweight DMBA (2 weeks) + 10 mg/kg bodyweight MSF1 (6 weeks)	12.95± 0.67	0.02*
6	15 mg/kg bodyweight DMBA (2 weeks) + 0.5 ml/200 g cisplatin + 3.35 mg/kg bodyweight doxorubicin (6 weeks)	8.49± 0.69	0.01*
7	15 mg/kg bodyweight MOF6 (8 weeks)	10.79± 0.58	0.01*
8	30 mg/kg bodyweight MOF6 (8 weeks)	11.53± 0.57	0.01*
9	10 mg/kg bodyweight MSF1 (8 weeks)	11.28± 0.79	0.01*

Table 2. MDR1 concentrations (Mean±SEM) (ng/ml) in liver tissues of rats

Groups of rats	Doses of drug/ extract administered	MDR1 (Mean±SEM) (ng/ml)	P≤0.05: Group 2 versus Groups 1 and 3–12
1	Physiological saline (8 weeks)	22.86± 3.98	0.11
2	15 mg/kg bodyweight DMBA	30.91±3.16	
3	15 mg/kg bodyweight DMBA (2 weeks) + 15 mg/kg bodyweight MOF6 (6 weeks)	16.03± 3.14	0.03*
4	15 mg/kg bodyweight DMBA (2 weeks) + 30 mg/kg bodyweight MOF6 (6 weeks)	15.47±3.16	0.03*
5	15 mg/kg bodyweight DMBA (2 weeks) + 10 mg/kg bodyweight MSF1 (6 weeks)	27.16± 3.95	0.22
6	15 mg/kg bodyweight DMBA (2 weeks) + 0.5 ml/200 g cisplatin + 3.35 mg/kg bodyweight doxorubicin (6 weeks)	20.19± 3.82	0.07
7	15 mg/kg bodyweight MOF6 (8 weeks)	20.91± 3.31	0.06
8	30 mg/kg bodyweight MOF6 (8 weeks)	10.80± 3.49	0.02*
9	10 mg/kg bodyweight MSF1 (8 weeks)	14.43± 4.57	0.07

significant lower ($P \leq 0.05$) levels of MDR1 in Groups 3 ($P = 0.03$), 4 ($P = 0.03$), and 6 ($P = 0.07$), but statistically non-significant lower ($P \geq 0.05$) levels of MDR1 in Groups 5 ($P = 0.22$), 7 ($P = 0.06$), 8 ($P = 0.02$), and 9 ($P = 0.07$), when compared with Group 2 [Table 2].

Discussion

Cancer treatment regimens kill most cancer cells, but do not eliminate CSCs, which possess protective and resistance mechanisms through the upregulation of specific factors such as biomarkers of proliferation (Ki67) and drug resistance (MDR1 or P-glycoprotein).[2-4] In addition, DMBA is a polycyclic aromatic hydrocarbon, which is present in tobacco smoke and exhaust emissions of vehicles. Hence, DMBA is a chemical agent of global health concern.[19-22]

Histopathological evaluations showed normal histoarchitectures of the components of the liver in rats of Groups 1–9 [Figures 1–7]. This implied that administrations of 15 mg/kg bodyweight of DMBA, 15 and 30 mg/kg bodyweight of MOF6 (extracted from MO leaves), and 10 mg/kg bodyweight of MSF1 (extracted from MS suckers) to rats did not result in evident histopathology of the liver after 8 weeks of exposure. This is possibly due to the fact that chemicals-induced

cytotoxicity and mutagenesis are dose and exposure dependent; and are usually first elicited on molecular markers, while further exposure or increase in doses will result in evident histopathology at tissue level.

Ki-67 protein is detected during all the active phases of the cell cycle, and it is one of the five genes (out of 16 cancer-associated genes) of proliferation that plays a key role in Oncotype scoring.[5-7] Ki-67 is not expressed by quiescent or resting cells in the G0phase, hence, it is an excellent operational marker for evaluation of the proliferation of a given cell population and the aggressiveness of malignancies.[5-7]

The exposures of rats of Group 2 to oral administration of 15 mg/kg bodyweight of DMBA resulted in upregulation of Ki67, when compared with control Group 1 [Table 1], implying DMBA induction of increased and abnormal proliferation. This observation is in agreement with previous observation that all proliferating cells tested expressed Ki67 and that there is no evidence to the contrary that proliferating cells do not express Ki67.[7-9] In addition, our finding on DMBA induction of upregulation of Ki67 levels is in agreement with previous observation that DMBA-induced toxicity resulted in tumor data multiplicity in female Sprague-Dawley rats.[24]

Do MOF6 (extracted from MO) and MSF1 (extracted from MS) have cytoprotective and anticancer potentials against DMBA-induced upregulation of Ki67 and abnormal proliferation? Post-treatments of DMBA-induced toxicity with 15 and 30 mg/kg bodyweight of MOF6, and 10 mg/kg bodyweight of MSF1 resulted in decreased and downregulations of Ki67 levels in rats of Groups 3–5, when compared with Group 2 [Table 1]. These results implied that MOF6 and MSF1 ameliorated DMBA-induced abnormal proliferations, and therefore, possess cytoprotective, anti-proliferation, and anticancer potentials. Our observations are in agreement with those of the previous studies which reported that MO[12] and MS[16] possess anticancer properties. Furthermore, Ki67 is a biomarker of CSCs, hence, our findings indicate that MOF6 and MSF1 possibly possess anticancer compounds that can specifically target and eliminate CSCs.

Is there any adverse effects on Ki67 levels following exposures of rats to only the evaluated doses of MOF6 and MSF1? The exposures of rats to oral administrations of 15 and 30 mg/kg bodyweight of MOF6, and 10 mg/kg bodyweight of MSF1 for 8 weeks resulted in significant downregulations of Ki67 levels in rats of Groups 7–9, when compared with Group 2 [Table 1], implying that MOF6 and MSF1 have no adverse effects on Ki67 levels and possess antiproliferative potentials.

MDR1 gene or P-glycoprotein is a cell membrane protein, which by its pharmacological function as an active drug efflux transporter protein enhances drug resistance capacity of CSCs.[4,8-10] Hence, significant upregulation of MDR1 is characteristic of drug resistant tumors and has been associated with cancer cells survival.[4,8-10] The administration of 15 mg/kg bodyweight of DMBA resulted in upregulation of MDR1 in rats of Group 2 when compared with control Group 1 [Table 2], implying DMBA induction of increased drug resistance.

Do MOF6 and MSF1 have cytoprotective and anticancer potentials against DMBA-induced upregulation of MDR1 and increased drug resistance? Post-treatments of DMBA-induced toxicity with 15 and 30 mg/kg bodyweight of MOF6, and 10 mg/kg bodyweight of MSF1 resulted in decreased and downregulations of MDR1 levels in rats of Groups 3–5, when compared with Group 2 [Table 1]. These results implied that MOF6 and MSF1 ameliorated DMBA-induced upregulation of MDR1F and offered cytoprotective, anti-drug resistance, and anticancer potentials against DMBA-induced drug resistance in rats. Furthermore, MDR1 is a biomarker of CSCs, hence, our findings indicate that MOF6 and MSF1 possibly possess anticancer compounds that can specifically target and eliminate CSCs.

Is there any adverse effects on MDR1 levels following exposures of rats to only the evaluated doses of MOF6 and MSF1? The exposures of rats to oral administrations of 15 and 30 mg/kg bodyweight of MOF6, and 10 mg/kg bodyweight of MSF1 for 8 weeks resulted in decreased and downregulations

of MDR1 levels in rats of Groups 7–9, when compared with Group 2 [Table 1], implying that MOF6 and MSF1 have no adverse effects on MDR1 levels, and possess anti-drug resistance potentials.

Are the antiproliferation and anti-drug resistance potentials of MOF6 and MSF1 comparable to those of standard anticancer drugs? Our findings showed that MOF6 and MSF1 conferred anti-proliferation and anti-drug resistance potentials that are well comparable to a combination of cisplatin and doxorubicin against DMBA-induced toxicity in rats [Tables 1 and 2]. These findings implied that MOF6 and MSF1 have anticancer potentials that deserve further evaluations toward the discovery of anticancer drug compounds that can eliminate CSCs.

Conclusion

Our findings in this study implied that post-treatments with MOF6 (extracted from MO leaves) and MSF1 (extracted from MS suckers) conferred a degree of hepatoprotective, antiproliferation, anti-drug resistance, and anticancer potentials against DMBA-induced toxicity and upregulations of biomarkers of cancer stem cells (Ki67 and MDR1) in rats. Hence, MOF6 and MSF1 possibly contain chemical components that may target cancer stem cells, and are recommended drug candidates for further evaluations for the treatments and cure of cancers.

Limitations of the study

This study is limited to the evaluations of the anticancer potentials of MO and MS in *in vivo* DMBA toxicity model in rats. Future studies shall evaluate the effects of MO and MS on the viability, cohesion, and growth of human cancer cell lines.

Authors' Declaration Statements

Ethics approval and consent to participate

All experimental protocols were approved and performed in accordance with the guiding principles of the UERC.

Availability of data and materials

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

Authors' Contributions

AAA, AOO, REK, and MOA designed, performed the project work, analyzed, and interpreted the data. AAA was the main contributor in writing the manuscript. AAA, AOO, REK, AL, JA, AJ, MOA, and GEE contributed to analyzing, interpretation of the data, and writing of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

References

1. Global Burden of Disease Collaboration; Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, *et al*. The global burden of cancer. JAMA Oncol 2013;1:505-27.

2. Chen K, Huang YH, Chen JI. Understanding and targeting cancer stem cells: Therapeutic implications and challenges. Acta Pharmacol Sin 2013;34:732-40.

3. Plaks VL, Kong N, Werb Z. The cancer stem cell niche: How essential is the niche in regulating stemness of tumor cells? Cell Stem Cell 2015;16:225-38.

4. Alfarouk KO, Stock CM, Taylor S, Walsh M, Muddathir AK, Verduzco D, *et al*. Resistance to cancer chemotherapy: Failure in drug response from ADME to P-gp. Cancer Cell Int 2015;15:71.

5. Luporsi E, Andre F, Spyratos F, Martin PM, Jacquemier J, Penault-Llorca F, *et al*. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: Analytical and critical review. Breast Cancer Res Treat 2012;132:895-915.

6. Scholzen T, Gerdes J. The Ki-67 protein: From the known and the unknown. J Cell Physiol 2000;182:311-22.

7. Xiao L, Shuang Z, En-Hong Z, Xin Z, Wen-Feng G, Yasuo T, *et al*. Clinicopathological and prognostic significance of Ki-67, caspase-3 and p53 expression in gastric carcinomas. Oncol Lett 2013;6:1277-84.

8. Dwibhashyam VS, Nagappa AN. Strategies for enhanced drug delivery to the central nervous system. Indian J Pharm Sci 2008;70:145-53.

9. Dong X. Current strategies for brain drug delivery. Theranostics 2018;8:1481-93.

10. Ming Y, Weiguang Z, Lihua Q, Long T, Changman Z. Enhancement of P-glycoprotein expression by hepatocyte transplantation in carbon tetrachloride-induced rat liver. Anatomical Rec (Hoboken) 2010;293:167-74.

11. Akinlolu AA, Bayode OE, Ghazali OK, Ameen OM. The effects of *Moringa oleifera* on lipid profile status, heart histology and liver histochemistry in adult Wistar rats. CHRISMED J Health Res 2017;4:104-9.

12. Welch HR, Tietje AH. Investigation of *Moringa oleifera* leaf extract and its cancer-selective antiproliferative properties. J South Carolina Acad Sci 2017;15:13.

13. Omotoso GO, Kadir ER, Lewu SF, Gbadamosi IT, Akinlolu AA, Adunmo GO, *et al*. *Moringa oleifera* ameliorates cuprizone-induced cerebellar damage in adult female rats. Res J Health Sci 2018;6:13-25.

14. Akinlolu AA, Ameen M, Quadri T, Odubela O, Omotoso G, Yahya R, *et al*. Extraction, isolation and evaluation of anti-toxic principles from *Moringa oleifera* (MOF6) and *Myristica fragrans* (Trimyristin) upregulated acetylcholinesterase concentrations in sodium arsenite-induced neurotoxicity in rats. J Phytomed Ther 2020;19:503-19.

15. Kumar KP, Bhowmik D, Duraivel S, Umadevi M. Traditional and medicinal uses of Banana. J Pharmacogn Phytochem 2012;1:51-63.

16. Akinlolu A, Ghazali OK, Salau BA, Otulana JO, Omowunmi M. *Musa sapientum* attenuates total antioxidant and lipid profile values in rats with indomethacin-induced gastric ulceration. J Cell Membr Free Radic Res 2013;5:236-43.

17. Akinlolu AA, Salau BA, Ekor M, Otulana JO, Ameen MO. *Musa sapientum* with exercises attenuates hyperglycemia and pancreatic islet cells degeneration in alloxan-diabetic rats. J Intercul Ethnopharmacol 2015;4:202-7.

18. Saad SD, Mohamad TA, Yasser MT, Amin MA. Antioxidant activities and anticancer screening of extracts from banana fruit (*Musa sapientum*). Acad J Cancer Res 2015;8:28-34.

19. Anderson LE, Boorman GA, Morris JE, Sasser LB, Mann PC, Grumbein SL, *et al*. Effect of 13 week magnetic field exposures on DMBA-initiated mammary gland carcinomas in female Sprague-Dawley rats. Carcinogen 1999;20:1615-20.

20. Veerasamy V, Shanmugam M. Chemopreventive efficacy of geraniol against 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis. Redox Rep 2011;16:91-100.

21. Acevedo JA, Asmat RJ, Guzmán AA, Donaires R, Gonzáles JR. Protective effect of Piper aduncum capsule on DMBA-induced breast cancer in rats. Breast Cancer (Auckl) 2015;9:41-8.

22. National Center for Biotechnology Information; 2020. https://www.compound/7_12Dimethylbenz_a_anthracene#section=2D-structure. [Last accessed on 2020 Dec 14].

23. Moore KL, Agur AM, Dalley AF. Clinically Oriented Anatomy. 7th ed. Philadelphia, PA: Lippincott Williams and Wilkins, 2014. p. 98-105.

24. Agung EW, Sriningsih, Puspita EW, Raafqi R. The influence of DMBA (7,12-dimethylbenz-[a]anthracene) regimen in the development of mammae carcinogénesis on Sprague Dawley female rat. Indones J Cancer Chemoprevnt 2010;1:60-6.