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Research Article

Anti-toxic principles from Moringa oleifera and Musa sapientium down-regulated Ki67 and Multidrug resistance1 proteins in Cadmium Chloride-induced hepato-toxicity and mutagenesis in rats.

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ABSTRACT
Moringa oleifera (MO) and Musa sapientium (MS) are ethno-medicinal plants with anticancer potentials. Cadmium is a pro-carcinogen of global health concerns. This study evaluated the anticancer potentials of MOF6 (extracted from MO leaves) and MSF1 (extracted from MS suckers) on immuno-modulations of Ki67 (proliferation biomarker) and Multi-drug resistance1 (MDR1) proteins in the liver of rats in Cadmium Chloride (CdCl)-induced hepato-toxicity and mutagenesis. 55 adult male rats were randomly divided into 11 groups (n = 5). Group 1 received physiological saline. Groups 2-6 and 10-11 received single intra-peritoneal administration of 1.25mg/kg bodyweight of CdCl on Day 1. Groups 3-5 were treated with 15 and 30mg/kg bodyweight of MOF6, and 10mg/kg bodyweight of MSF1 respectively from Days 15-56. Group 6 was treated with 3.35 mg/kg bodyweight of Doxorubicin and intravenous injection of 0.5ml/200g of Cisplatin from Days 15-29. Groups 7-9 received only 15 and 30mg/kg bodyweight of MOF6, and 10mg/kg bodyweight of MSF1 respectively from Days 1-56. Groups 10 and 11 received preventive treatments with 30mg/kg bodyweight of MOF6 and 10mg/kg bodyweight of MSF1 respectively from Days 1-56. Doxorubicin and extracts doses were administered orally. Consequently, Ki67 and MDR1 concentrations in Liver homogenates were evaluated using Enzyme Linked Immunosorbent Assay. Computed data were statistically analyzed ($p \leq 0.05$). Results showed statistically significant ($p \leq 0.05$) and non-significant decreased concentrations ($p \geq 0.05$) of Ki67 and MDR1 in Groups 3-11 comparedwith Group 2. Therefore, MOF6 and MSF1 ameliorated CdCl-induced hepato-toxicity, mutagenesis, hyperplasia and drug resistance. In conclusion, MOF6 and MSF1 possess hepato-protective, anti-proliferation, anti-drug resistance and anticancer potentials.

Keywords: Moringa oleifera, Musa sapientum, Cadmium Chloride, Anti-cancer compound, Ki67, Multidrug resistance1.

INTRODUCTION

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, North-Eastern and Western Africa (including Nigeria)[1-4]. MO is a plant of ethno-medicinal importance and has been used traditionally to treat many diseases such as cancer, such as 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[2], neuro-protective[3,4] and antioxidant[3] potentials.

Musa sapientum (MS) or banana belongs to the family musaceae and is a food crop well grown in Nigerian communities[5,6]. MS is a plant of ethno-medicinal importance and its various parts of have traditionally been used for the treatment of diseases such as ulcer, diabetes and hypertension[5-8]. Scientific studies have equally observed that MS pulps and unripe bananas have anti-ulcer properties while its seeds possess antioxidant, anti-diarrheal and anti-microbial activities. Peel extracts, inflorescence and stalk of MS have also been reported to have significant antioxidant potentials[5,6]. MS fruit was reported to have anticancer potentials[8], while MS sucker was reported to have antioxidant[5], anti-ulcer[5] and anti-diabetic[6] potentials.

Cancer was ranked the second leading cause of death behind cardiovascular diseases since

2013[9,11], and this imposes a huge burden on societies[11]. Cancers comprise of cancer stem cells (CSCs), macrophages and vascular endothelial cells, with CSCs having tumourigenic capacity while others do not[9-11]. Cancer treatment regimens kill most cancer cells, but do not eliminate CSCs, which possess protective and resistance mechanisms[10,11] via the up-regulation of specific factors such as biomarkers of proliferation (Ki67) and drug resistance (Multidrug resistance1 or P- glycoprotein)[12]. 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[13,14]. It is one of the five genes (out of 16 cancer-associated genes) of proliferation that is of important weight to the Oncotype score. Ki-67 is not expressed by quiescent or resting cells in the G0-phase, hence it is an excellent operational marker for evaluation of the proliferation of a given cell population and the aggressiveness of malignancies[13-15].

The multidrug resistance1 (MDR1) gene or P-glycoprotein is localized in the cell-membrane and it functions pharmacologically as an active drug efflux transporter protein of various substancesincluding drugs and toxins[12,16,17]. The MDR1 protein is physiologically expressed at the bile canalicular membrane of the liver functioning in biliary excretion of lipophilic drugs[18]. The MDR1 protein has affinity for hydrophobic compounds and efforts have been made to by-pass 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[12,16,17].

Cadmium (Cd) is one of the 10 chemicals or groups of chemicals of concern for human health, according to the World Health Organization[19]. Commercially, Cd is used in television screens, lasers, batteries, paint pigments, cosmetics, in galvanizing steel and as a barrier in nuclear fission[20]. Exposure to Cd is followed by its absorption and transportation throughout the body, usually bound to a sulfhydryl group-containing protein like metallothionein. Approximately 30% of Cd deposits inthe liver and 30% in the kidneys, with the rest distributed throughout the body. Cd has a clearance half-life of twenty-five years, while the half-life of Cd in the blood has been estimated at 75 to 128 days. Hence, Cd can produce long-term health effects[19,20].

Cd-induced toxicity results in tissue injury mainly through increased cellular oxidative stress and epigenetic changes in DNA expression[19,20]. Due to its transportation to the liver, Cd can cause hepato-toxicity with accompanied histo-pathological disruptions, increased blood enzyme levels and reduced protein synthesis[19,20]. Due to its low rate of excretion from the body, prolonged exposureto Cadmium will cause toxicity via its accumulation over time in a variety of tissues, including testes, kidneys, liver, central and peripheral nervous systems. In addition to its carcinogenic effects, Cd also causes decreased body weight, most probably as a result of lower-diet intake due to Cd-induced inflammation[21].

The characteristic abnormal cellular proliferation with accompanied increased expressions of Ki67 and MDR1 by Cancer Stem Cells (CSCs) makes the treatment of cancers a very challenging task. It is, therefore, very relevant to evaluate plants sources towards 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 Moringa oleifera leaves) showed significant antioxidant potentials against Cuprizone-induced increased superoxide dismutase levels[3], while the aqueous extract of Musa sapientium sucker significantly reduced indomethacin-induced increased catalase, superoxide dismutase and malondialdehyde (lipid peroxidation) levels[5] in rats.

Cd-induced toxicity is via induction of cellular oxidative stress and approximately 30% of Cd deposits in the liver resulting in hepato-toxicity. In addition, Cd-induced carcinogenesis in animal-models grossly mimics and resembles human cases of cancer, thus providing a good avenue for studying chemo-preventive agents. Cd generally exists as a divalent cation, complexed with other elements, such as Cadmium Chloride (CdCl)[19,20]. The liver is the largest body organ, and it plays significant roles in drug metabolism, detoxification and the functionality of the body systems[22].

Therefore, this study evaluated the effects of fractionated and isolated compounds from Moringa oleifera leaves (MOF6) and Musa sapientium sucker (MSF1) on immuno-modulations of Ki67 and Multi-drug resistance1 (MDR1) proteins in the liver of rats in Cadmium Chloride-induced hepato-toxicity and mutagenesis in-order to determine their hepato-protective, anti-proliferation, 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 institution 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 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 Moringa oleifera (MO) leaves and Musa sapientum (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.

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.

Column chromatography fractionation of ethanol extract of Moringa oleifera (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. The fraction MOF6 which had the best preliminary antioxidant potential out of the 9 fractions, and which we had previously reported to possess antioxidant[3] and neuro-protective[3,4] potentials was used in this study to evaluate the effects of MO leaves on Cadmium Chloride (CdCl)-induced hepato-toxicity and mutagenesis in rats.**Column**

chromatography fractionation of ethanol extract of Musa sapientum (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. The fraction MSF1 which had the best preliminary antioxidant potential out of the 5 fractions was used in this study to evaluate the effects of MS on Cadmium Chloride (CdCl)-induced hepato-toxicity and mutagenesis in rats.

Animal Care and Feeding

A total number of fifty-five (55) male Wistar rats with an average weight of 200g 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 37^0C and double-crossed ventilation.

Chemicals and Reagents

Cadmium Chloride (CdCl)-induced 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 6 categories as below in-order to evaluate the hepato-protective potentials of MOF6 (extracted from Moringa oleifera leaves), and MSF1 (extracted from Musa sapientum suckers) in Cadmium Chloride (CdCl)-induced toxicity and mutagenesis.

Rats of Control Group 1 received physiological saline for 56 days.

Negative Control Group: Rats of Experimental Group 2 received single intra-peritoneal administration of 1.25 mg/kg bodyweight CdCl, monitored for 14 days to confirm cancer-induction, and left untreated for the 56 days of experimental procedure.

Anti-Cancer Treatment Groups: Rats of Group 3 received single intra-peritoneal administration of 1.25 mg/kg bodyweight CdCl, monitored for 14 days to confirm cancer-induction, and were treated with oral administration of 15 mg/kg bodyweight of MOF6 for another 42 days. Rats of Group 4 received single intra-peritoneal administration of 1.25 mg/kg bodyweight CdCl, monitored for 14 days to confirm cancer-induction, and were treated with oral administration of 30 mg/kg bodyweight of MOF6 for another 42 days. Rats of Group 5 received single intra-peritoneal administration of 1.25 mg/kg bodyweight CdCl, monitored for 14 days to confirm cancer-induction, and were treated with oral administration of 10 mg/kg bodyweight of MSF1 for another 42 days.

Positive Control Group: Rats of Group 6 received single intra-peritoneal administration of 1.25 mg/kg bodyweight CdCl, monitored for 14 days 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 14 days. This was because the rats could not tolerate more than two weeks of administrations of standard doses of Cisplastin and Doxorubicin.

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

Hepato-protective and Cancer Prevention Groups: Rats of Group 10 received co-administration of single intra-peritoneal 1.25 mg/kg bodyweight CdCl and 30 mg/kg bodyweight of MOF6 on Day 1,
followed by daily oral administration of 30 mg/kg bodyweight of MOF6 for another 55 days. Rats of Group 11 received co-administration of single intra-peritoneal 1.25 mg/kg bodyweight CdCl and 10mg/kg bodyweight of MSF1 on Day 1, followed by daily oral administration of 10 mg/kg bodyweight of MSF1 for another 55 days.

Bodyweights (g) of all rats were measured on Day 1 of experimental procedure and at the end of each week. The model/guidelines on which the experimental procedure used in this study was based, is 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). The experimental procedures used in this studywere modified from our previous studies on cyto-, histo- and pharmaco-protective potentials of Moringa oleifera leaves[3,4] and Musa sapientum suckers[5,6] in rats.

Animal Sacrifice

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

Liver tissues' concentrations of Ki67 and Multidrug Resistance1 (MDR1) proteins using Enzyme Linked Immunosorbent Assay (ELISA) in 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 to 4 ml of 0.25 M sucrose solution. The tissue homogenates were filled up to 5ml 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 -4^OC, and thereafter

assayed for concentrations of Ki67 and MDR1 proteins 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 - 11. Differences were tested and considered statistically significant when ($p \leq 0.05$) using Graph Pad Prism software package (Graph Pad Software Inc., San Diego, CA, USA; version 7 for Windows) and Microsoft Excel 2016.

RESULTS

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 when compared with Group 1 (Table 1). In addition, there were statistically non-significant lower ($p \geq 0.05$) levels of Ki67 in rats of Groups 4 - 11, when compared with Group 2 (Table 1).

ELISA concentrations of MDR1 in liver tissues of rats

Results showed statistically significant higher ($p \leq 0.05$) levels of MDR1 in rats of Group 2 when compared with Group 1 (Table 2). In contrast, there were statistically significant lower ($p \leq 0.05$) levels of MDR1 in rats of Groups 3 - 11, 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[10,11] via the up-regulations of specific factors such as biomarkers of proliferation (Ki67) and drug resistance (Multidrug resistance1 or P-glycoprotein)[12]. In addition, Cadmium (Cd) is one of the 10 chemicals or groups of chemicals of concern for human health, according to the World Health Organization[19]. Cd is used as component part of valuable tools such as television screens, lasers, batteries, paint pigments and cosmetics[20]. Hence, Cd is a chemical agent of global health concern[19-21].

Ki-67 protein is detected during all the active phases of the cell cycle and it is used in mitotic counting grading systems as a sign of proliferation[13,14]. It is one of the five genes of proliferation that is of important weight to the Oncotype score. Ki-67 is not expressed by quiescent or resting cellsin the G0-phase, hence it is an excellent operational marker for evaluation of the proliferation of a given cell population and the aggressiveness of malignancies[13-15]. Results showed statistically significant higher ($p \leq 0.05$) levels of Ki67 in rats of Group 2 when compared with Group 1 (Table 1). This result implied that administration of 1.25 mg/kg bodyweight of Cadmium Chloride (CdCl) resulted in upregulation of Ki67 and induction of hepatocytes hyper-proliferation and hyperplasia in rats of Group 2. This observation is in agreement with previous reports that all proliferating cells tested expressed Ki67, and that there is no evidence to the contrary that proliferating cells do not express Ki67[13-15].

Does MOF6 have anticancer potentials against CdCl-induced abnormal proliferation? Post-treatments of CdCl-induced hepatocytes hyper-proliferation and hyperplasia with 15 and 30 mg/kg bodyweight of MOF6 resulted in the downregulation of Ki67 levels, in rats of Groups 3 and 4, whencompared with Group 2 (Table 1). Hence, MOF6 possesses cyto-protective, anti-proliferation and anticancer potentials. This observation is in agreement with those of[2], which reported anticancer potentials of Moringa oleifera.

Does MSF1 have anticancer potentials against CdCl-induced abnormal proliferation? Post-treatment of 1.25 mg/kg bodyweight of CdCl-induced hepatocytes hyper-proliferation and hyperplasia with 10 mg/kg bodyweight of MSF1 resulted in significant downregulation of Ki67 levels in rats of Group 5, when compared with Group 2 (Table 1). Therefore, MSF1 offered cyto-protective, anti-proliferation and anticancer potentials against CdCl-induced hepatocytes hyper-proliferation and hyperplasia in rats. This observation is in agreement with those of[8], which reported anticancer potentials of Musa sapientum.

Can MOF6 and MSF1 offer cyto-protection against CdCl-induced hepato-toxicity, hepatocytes hyper-proliferation and hyperplasia when the organism becomes exposed to the carcinogen? The concomitant treatments of 1.25 mg/kg bodyweight of CdCl on Day 1 with 30 mg/kg bodyweight of MOF6 and 10 mg/kg bodyweight of MSF1, followed by administrations of 30 mg/kg bodyweight of MOF6 and 10 mg/kg bodyweight of MSF1 from Days 2 – 56, significantly prevented CdCl-induced upregulation of Ki67 and abnormal proliferation in rats of Groups 10 and 11, when compared with Group 2 (Table 1). Hence, MOF6 and MSF1 offered chemo-prevention and cyto- protection against CdCl-induced hepato-toxicity, hepatocytes hyper-proliferation and hyperplasia.

Furthermore, Ki67 is a biomarker of CSCs, hence our findings indicate that MOF6 and MSF1 possibly possess anti-cancer compounds that can specifically target and eliminate CSCs.

Multidrug resistance1 (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[12,16-18]. Hence, significant upregulation of MDR1 is characteristic of drug resistant tumours and has been associated with cancer cells survival[12,16-18]. Results showed statistically significant higher ($p \leq 0.05$) levels of MDR1 in rats of Group 2 when compared with Group 1 (Table 2). This result implied that administration of 1.25 mg/kg bodyweight of CdCl resulted in upregulation of MDR1 and induction of hepatic drug resistance in rats of Group 2. This observation agrees with those of previous studies that mutagenesis is accompanied with increased drug resistance and cancer cells survival[12,16-18].

Does MOF6 have cyto-protective and anticancer potentials against CdCl-induced hepatic drug resistance? Post-treatments of 1.25 mg/kg bodyweight of CdCl-induced hepatic drug resistance with 15 and 30 mg/kg bodyweight of MOF6 resulted in significant downregulation of MDR1 levels in rats of Groups 3 and 4, when compared with Group 2 (Table 2). Hence, MOF6 offered cyto- protective, anti-drug resistance and anticancer potentials against CdCl-induced hepatic drug resistance in rats. This observation is in agreement with those of[2], which reported anticancer potentials of Moringa oleifera.

Does MSF1 have cyto-protective and anticancer potentials against CdCl-induced hepatic drug resistance? Post-treatment of 1.25 mg/kg bodyweight of CdCl-induced hepatic drug resistance with 10 mg.kg bodyweight of MSF1 resulted in downregulation of MDR1 in rats of Group 5, when compared with Group 2 (Table 2). Therefore, MSF1 possesses anti-drug resistance potentials. This observation is in agreement with those of[8], which reported anticancer potentials of Musa sapientum. Furthermore, MDR1 is a biomarker of CSCs, hence our findings indicate that MOF6 and MSF1 possibly possess anti-cancer compounds that can specifically target and eliminate CSCs.

Can MOF6 and MSF1 offer chemo-prevention and cyto-protection against CdCl-induced hepatic drug resistance when the organism becomes exposed to the carcinogen? The concomitant treatments of 1.25 mg/kg bodyweight of CdCl on Day 1 with 30 mg/kg bodyweight of MOF6 and 10 mg/kg bodyweight of MSF1, followed by administrations of 30 mg/kg bodyweight of MOF6 and 10 mg/kg bodyweight of MSF1 from Days 2 – 56, significantly prevented CdCl-induced upregulation of MDR1 and hepatic drug resistance in rats of Groups 10 and 11, when compared with Group 2 (Table 2). Hence, MOF6 and MSF1 offered chemo-prevention and cyto-protection against CdCl- induced hepato-toxicity and drug resistance.

Are the anti-proliferation and anti-drug resistance potentials of MOF6 and MSF1 comparable to standard anticancer drugs? Our findings showed that MOF6 and MSF1 offered anti-proliferation and anti-drug resistance potentials that are well comparable to treatments of CdCl-induced toxicity with a combination of Cisplatin and Doxorubicin (Tables 1 and 2). These findings implied that MOF6 and MSF1 have anticancer potentials that deserve further evaluations towards the discovery of anticancer drug compounds that can eliminate CSCs.

CONCLUSIONS

Our findings suggest that post-treatments with doses of MOF6 (extracted from Moringa oleifera leaves) and MSF1 (extracted from Musa sapientum suckers) following exposure to Cadmium Chloride downregulated and restored the activities of proliferation biomarker (Ki67) and Multidrug Resistance1 genes to normal levels. This indicates that MOF6 and MSF1 conferred a degree of hapatoprotection against Cadmium Chloride-induced proliferation and drug resistance, and are recommended for further evaluation as potential drug candidates for the elimination of Cancer Stem Cells and for the treatment of cancers.

Conflict of Interest

The authors confirm that there is no conflict of interest associated with thisstudy.

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REFERENCES

1. 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 Journal of Health Research, 2017; 4: 104-109.
2. Welch HR, Tietje AH. Investigation of Moringa oleifera leaf extract and its cancer-selective antiproliferative properties. Journal of South Carolina Academy of Science, 2017; 15(2): 13.

3. Omotoso GO, Kadir ER, Lewu SF et al. Moringa oleifera ameliorates cuprizone-induced cerebellar damage in adult female rats. Research Journal of Health Science, 2018; 6(1): 13- 25.
4. Akinlolu AA, Ameen MO, Quadri T 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. Journal of Phytomedicine and Therapeutics, 2020; 19(2): 503-519.
5. Akinlolu AA, Ghazali OK, Salau BA, Otulana JO, Omowunmi M. Musa Sapientum attenuates total antioxidant and lipid profile values in rats with indomethacin–induced gastric ulceration. Journal of Cell Membrane and Free Radical Research, 2013; 5(1): 236-243.
6. 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. Journal of Intercultural Ethnopharmacology, 2015; 4(3): 202-207.
7. Kumar KPS, Bhowmik D, Duraivel S, Umadevi M. Traditional and medicinal uses of Banana. Journal of Pharmacognosy and Phytochemistry, 2012; 1(3): 51-63.
8. Saad SD, Mohamad TA, Yasser MT, Amin MAA. Antioxidant activities and anticancer screening of extracts from Banana fruit (Musa sapientum). Academic Journal of Cancer Research, 2015; 8(2): 28-34.
9. Global Burden of Disease Collaboration. The Global Burden of Cancer. Journal of American Medical Association of Oncology, 2013; 1(14): 505-527.
10. Chen K, Huang Y, Chen JI. Understanding and targeting cancer stem cells: therapeutic implications and challenges. Acta Pharmacologica Sinica, 2013; 34: 732-740.
11. Plaks VI, 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(3): 225-238.
12. Alfarouk KO, Stock C-M, Taylor S et al. Resistance to cancer chemotherapy: failure in drugresponse from ADME to P-gp. Cancer Cell International, 2015; 15: 71. DOI:10.1186/s12935-015-0221-1.
13. Luporsi E, Andre F, Spyratos 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 Resistant Treatment, 2012; 132: 895-915.
14. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. Journal of Cell Physiology, 2000; 182: 311-322.
15. Xiao L, Shuang Z, En-Hong Z et al. Clinicopathological and prognostic significance of Ki- 67, caspase-3 and p53 expression in gastric carcinomas. Oncology Letters, 2013; 6(5): 1277-1284.
16. Dwibhashyam VSNM, Nagappa AN. Strategies for enhanced drug delivery to the central nervous system. Indian Journal of Pharmaceutical Science, 2008; 70(2): 145-153.
17. Dong X. Current strategies for brain drug delivery. Theranostics, 2018; 8(6): 1481-1493.
18. 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 Records. (Hoboken), 2010; 293(7): 167-174.
19. Andjelkovic M, Djordjevic AB, Antonijevic E et al. Toxic effect of acute Cadmium and Leadexposure in rat Blood, Liver, and Kidney. International Journal of Environmental Research and Public Health, 2019; 16: 274. DOI:10.3390/ijerph16020274.
20. Bernhoft RA. Cadmium toxicity and treatment. The Scientific World Journal, 2013; 394652: 1-7. DOI:dx.doi.org/10.1155/2013/394652.
21. Wang B, Du Y. Cadmium and its neurotoxic effects. Oxidative Medicine and Cellular Longevity, 2013; 10(12): 89-102.
22. Moore KL, AMR A, Dalley AF. Clinically oriented anatomy, 7th edition, Williams and Wilkins, Lippincott, 2014.

Table 1: Ki67 concentrations (ng/ml) in Liver tissues of rats of Control and ExperimentalGroups.

Groups of rats	Doses of drug/extract administered	Ki67 (Mean±SEM) (ng/ml)	p≤0.05: Group versus Groups and 3 – 11	2 1
1	Physiological saline (56 Days)	8.03 ± 0.79	0.02*	
2	1.25mg/Kg bodyweight Cadmium Chloride (CdCl)	13.09 ± 0.11		
	(Day 1 only)			
3	1.25mg/Kg bodyweight CdCl (Day 1) + 15mg/Kg	14.19 ± 2.51	0.70	

	bodyweight MOF6 (Days 15 - 56)		
4	1.25mg/Kg bodyweight CdCl (Day 1) + 30mg/Kg	12.39 ± 1.41	0.67
	bodyweight MOF6 (Days 15 - 56)		
5	1.25mg/Kg bodyweight CdCl (Day 1) +	11.02 ± 0.79	0.31
	10mg/Kg bodyweight MSF1 (Days 15 - 56)		
6	1.25mg/Kg bodyweight CdCl (Day 1) + 0.5ml/200g	11.58 ± 0.87	0.22
	Cisplastin + 3.35mg/Kg bodyweight Doxorubicin		
	(Days 15 - 29)		
7	15mg/Kg bodyweight MOF6 (56 Days)	10.79 ± 0.76	0.09
8	30mg/Kg bodyweight MOF6 (56 Days)	11.53 ± 0.75	0.17
9	10mg/Kg bodyweight MSF1 (56 Days)	11.28 ± 0.93	0.19
10	1.25mg/Kg bodyweight CdCl (Day 1) + 30mg/Kg	11.18 ± 7.48	0.13
	bodyweight MOF6 (Days 1 - 56)		
11	1.25mg/Kg bodyweight CdCl (Day 1) +10mg/Kg bodyweight MSF1 (Days 1 - 56)	11.99 ± 0.75	0.28

Where *- represents statistical significance at p≤0.05.

Table 2: MDR1 concentrations (ng/ml) in Liver tissues of rats of Control andExperimental Groups.

Groups of rats	Doses of drug/extract administered	MDR1 (Mean±SEM) (ng/ml)	p≤0.05: Group 2 versus Groups 1 and 3 – 11
1	Physiological saline (56 Days)	10.44 ± 2.45	0.01*
2	1.25mg/Kg bodyweight Cadmium Chloride (CdCl)	33.3 ± 3.16	
	(Day 1 only)		
3	1.25mg/Kg bodyweight CdCl (Day 1) + 15mg/Kg	7.93 ± 3.38	0.01*
	bodyweight MOF6 (Days 15 - 56)		
4	1.25mg/Kg bodyweight CdCl (Day 1) + 30mg/Kg	20.86 ± 0.78	0.01*
	bodyweight MOF6 (Days 15 - 56)		
5	1.25mg/Kg bodyweight CdCl (Day 1) + 10mg/Kg	15.77 ± 0.72	0.01*
	bodyweight MSF1 (Days 15 - 56)		
6	1.25mg/Kg bodyweight CdCl (Day 1) + 0.5ml/200g	16.53 ± 1.03	0.01*
	Cisplastin + 3.35mg/Kg bodyweight Doxorubicin		
	(Days 15 - 29)		
7	15mg/Kg bodyweight MOF6 (56 Days)	12.39 ± 1.05	0.01*
8	30mg/Kg bodyweight MOF6 (56 Days)	22.50 ± 1.54	0.01*
9	10mg/Kg bodyweight MSF1 (56 Days)	15.54 ± 3.33	0.04*
10	1.25mg/Kg bodyweight CdCl (Day 1) + 30mg/Kg	20.64 ± 1.24	0.01*
	bodyweight MOF6 (Days 1 - 56)		
11	1.25mg/Kg bodyweight CdCl (Day 1) + 10mg/Kg	14.51 ± 0.88	0.01*
	bodyweight MSF1 (Days 1 - 56)		

Where *- represents statistical significance at p≤0.05.