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ANTI-TOXIC PRINCIPLES FROM *Morinda lucida* AND *Annona muricata* DOWN-REGULATED KI67 AND MULTI-DRUG RESISTANCE1 GENES IN LEAD-INDUCED HEPATO-TOXICITY IN RATS

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ABSTRACT

Morinda lucida (ML) and Annona muricata (AM) are ethno-medicinal plants with antioxidant potentials. In addition, lead is a toxic pollutant of global health concerns. This study evaluated the effects of column chromatography-extracted ethanolic fractions of ML and AM leaves on immuno-modulations of Ki67 and multi-drug resistance1 (MDR1) proteins in the liver of rats in lead acetate (LA)-induced hepato-toxicity in-order to determine their hepato-protective, anti-proliferation, anti-drug resistance and anti-cancer potentials. Sixty adult female rats were randomly divided into 12 groups (n = 5). Groups 1 and 2 received physiological saline and 100 mg/kg bodyweight of LA, respectively, for 5 weeks. Groups 3–6 received 100 mg/ kg bodyweight LA for 2 weeks followed by post-treatments with 7.5 mg/kg and 15 mg/kg bodyweight of ML, and 7.5 mg/kg and 10 mg/kg bodyweight of AM, respectively, for another 3 weeks. Groups 7–10 received 7.5 mg/kg and 15 mg/kg bodyweight of ML, and 7.5 mg/ kg and10 mg/kg bodyweight of AM, respectively, for 5 weeks. Groups 11 and 12 received co-administrations of 100 mg/kg bodyweight LA simultaneously with 15 mg/kg bodyweight ML and 10 mg/kg bodyweight of AM, respectively, for 5 weeks. Drugs and extracts were administered orally. Consequently, liver histopathology (heamatoxylin and eosin technique) and enzyme linked immunosorbent assay (ELISA) homogenates' concentrations of Ki67 and MDR1 were evaluated. Computed data were statistically analysed (p ≤ 0.05). Results showed normal liver histology in all groups. Post-treatments of LA-induced hepato-toxicity resulted in statistically significant (p ≤ 0.05) and non-significant decreased concentrations (p ≥ 0.05) of Ki67 and MDR1 in Groups 3–12 compared with Group 2. These observations

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indicated that ML and AM ameliorated LA-induced hepato-toxicity, abnormal proliferation, drug resistance and mutagenesis.

Keywords: *Morinda lucida*, *Annona muricata*, Lead, Hepato-toxicity, Anti-cancer potentials

INTRODUCTION

Morinda lucida (ML) is an ethno-medicinal plant that is well-grown in Nigeria. ML is a medium size tree with short crooked branches. ML leaves have been traditionally employed to treat malaria and reported to possess hypoglycaemic, trypanocidal and aortic vaso-relaxant potentials. In addition, the stem bark of ML is also used traditionally to treat severe jaundice (Adejo *et al.* 2014; Adeyemi *et al.* 2014; Adeleye, Ayeni and Ajamu 2018). ML fruits and leaves showed significant *in vitro* anti-cancer potentials against human laryngeal carcinoma, neuroblastoma, breast cancer cells, human cervical carcinoma, human epidermoid carcinoma and human hepatocellular carcinoma. ML contained potential anti-cancer and therapeutic agents such as phenolic compounds (anthraquinones damnacanthal, scopoletin, nordamnacanthal, rubiadin-1-methyl ether, alizarin, morindone, aucubin, rubiadin and asperuloside), vitamins, alkaloids, organic acids, proteins and minerals (Akinlolu *et al.* 2021; Ohiagu *et al.* 2021). In addition, post-treatments of lead acetate (LA)-induced toxicity with ML resulted in melatonin-upregulation, decreased lipid peroxidation and downregulations of malondialdehyde (MDA), tumour necrosis factor-alpha (TNF-α) and p53 in rats (Akinlolu *et al.* 2021).

 Annona muricata (AM) is a member of the Annonaceae family and is an evergreen ethno-medicinal fruit tree that is widely grown in Nigeria. AM fruits are used to prepare syrups and beverages, while the different parts of AM have been employed traditionally to treat different diseases. Furthermore, previous studies have reported the anti-cancer, anti-convulsant, anti-arthritic, anti-parasitic, hepato-protective and anti-diabetic potentials (Moghadamtousi *et al.* 2015; Agu and Okolie 2017). The anti-cancer and cytotoxic effects of AM are via inhibitions of ATP-synthesis and mitochondrial complexes, Bax-upregulation, Bcl2-downregulation, epithelial growth factor receptor (EGFR)-downregulation (in breast cancer cases) and downregulations of oncogenes (Prasad, Varsha and Devegowda 2019). Phytochemical analyses of AM parts showed the presence of essential oils and anti-cancer agents such as annonaceous acetogenins, alkaloids, flavonol triglycosides, megastigmanes, phenolics and cyclopeptides (Prasad, Varsha and Devegowda 2019). Furthermore, post-treatments of LA-induced toxicity with AM resulted in melatonin-upregulation, decreased lipid peroxidation and downregulations of MDA, TNF-α and p53 in rats (Akinlolu *et al.* 2021).

 Lead (Pb) is a highly toxic pollutant of global health concerns, and it is a persistent metal that is present in the environment water, soil and dust. It is widely used in the production of brass plumbing fixtures and paints. Lead is majorly deposited in the liver, followed by the kidney (Nabil *et al.* 2012; Haouas *et al.* 2014; Ahmed 2016). Lead-induced hepatotoxicity is mediated via increased generation of free radicals with consequent disruptions of cholesterol metabolism and hepatocytes hyperplasia (Nabil *et al.* 2012; Haouas *et al.* 2014; Ahmed *et al.* 2016).

 Ki67 protein is detected during all the active phases of the cell cycle and it is an established biomarker of proliferation (Scholzen and Gerdes 2000; Luporsi *et al.* 2012; Xiao *et al.* 2013). In addition, the multi-drug resistance1 (MDR1) gene or P-glycoprotein functions pharmacologically as an active drug efflux transporter protein. MDR1 protein is physiologically expressed at the bile canalicular membrane of the liver functioning in biliary excretion of lipophilic drugs (Ming *et al.* 2010; Alfarouk *et al.* 2015). Ki67 and MDR1 proteins

are up-regulated in carcinogenesis, and are biomarkers of cancer stem cells (CSCs). CSCs are tumourigenic, unlike other cancer cells (macrophages and vascular endothelial cells) (Chen, Huang and Chen 2013; Plaks, Kong and Werb 2015). Hence, CSCs are evasive and not eliminated by cancer treatment regimens.

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 towards the isolation of drugs compounds that can specifically target CSCs and reduce or eliminate drug resistance. ML and AM have been established to have anti-cancer potentials, but there are paucity of studies which specifically evaluated anti-cancer potentials of ML and AM on Ki67 and MDR1 levels in LA-induced hepatoxicity. The liver plays significant roles in detoxification, drug metabolism and the functionality of the body systems. Therefore, this study evaluated the effects of column chromatography-extracted ethanolic fractions of ML and AM leaves on immuno-modulations of Ki67 and MDR1 proteins in the liver of rats in LA-induced hepato-toxicity in order to determine their hepato-protective, anti-proliferation, anti-drug resistance and anti-cancer 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 ML and AM Leaves

Freshly cut leaves of ML and AM 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. ML and AM leaves were deposited at the herbarium of the Department of Botany, Faculty of Life Sciences, University of Ilorin and assigned Herbarium Identification Numbers UITH/004/1103 and UITH/003/1106, respectively.

Preparations and Ethanolic Extractions of ML and AM Leaves

ML and AM leaves were air-dried at the laboratory unit of the Department of Chemistry, University of Ilorin, Ilorin, Nigeria. The dried leaves of ML and AM 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 h. After decantation, another distilled ethanol was added to the sieved ML and AM and left for another 24 h. When the colour quality and texture of the dissolved ML and AM in ethanol became evidently low (compared to previous solutions decanted), the procedure was halted. Ethanol was separated from ML and AM and column chromatography was done to get different fractions of ML and AM*.*

Column Chromatography Fractionation of Ethanol Extracts of ML and AM Leaves

The ethanol extracts of ML and AM were 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 36 eluents of 250 mL each for ML, while 13 eluents of 250 mL each were obtained for AM. The resulting eluents were pooled based on the colour of the solvents that elute them to give a total of 9 combined ML fractions and 5 combined AM fractions**.** The ML fraction 1 (F1) which had the best preliminary antioxidant potential out of the 9 ML fractions was used in this study to evaluate the effects of ML on LA-induced hepato-toxicity in rats. In addition, the AMF1 which had the best preliminary antioxidant potential out of the 5 AM fractions was used in this study to evaluate the effects of ML on LA-induced hepato-toxicity in rats.

Animal Care and Feeding

A total number of 60 female Wistar rats with an average weight of 200 g were used in this study. The rats were acclimatised 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 pelletised grower feed from Ogo-Oluwa Livestock and Aqua Feed Enterprise, Kwara State, Ilorin, Nigeria. The grower feed contains 15% crude protein, 7% fat, 10% crude fibre, 1% calcium, 0.35% phosphorus and 2.55% kcal/kg of metabolised energy as indicated on the pack. The animals were grouped into 12 with five animals each in a wire gauzed cage. The animals were kept under a normal room temperature of 25°C and double-crossed ventilation.

Chemicals and Reagents

LA was a product of Sigma–Aldrich Japan Co. (Tokyo, Japan) and was purchased from Emed Ejeson Enterprises in Ilorin, Kwara State, Nigeria. Normal saline was obtained from MOMROTA pharmaceutical company in Ilorin, Kwara State, Nigeria.

Experimental Procedures and Drugs Administration

Group 1 received physiological saline. Group 2 received 100 mg/kg bodyweight of LA for 5 weeks. Groups 3 and 4 received 100 mg/kg bodyweight LA for 2 weeks followed by treatments with 7.5 mg/kg and 15 mg/kg bodyweight of ML, respectively, for another 3 weeks. Groups 5 and 6 received 100mg/kg bodyweight LA for 2 weeks followed by treatments with 7.5 mg/kg and 10 mg/kg bodyweight of AM, respectively, for another 3 weeks. Groups 7 and 8 received 7.5 mg/kg and 15 mg/kg bodyweight of ML, respectively, for 5 weeks. Groups 9 and 10 received 7.5 mg/kg and 10 mg/kg bodyweight of AM, respectively, for

5 weeks. Group 11 received co-administrations of 15 mg/kg bodyweight ML and 100 mg/kg bodyweight LA for 5 weeks. Group 12 received co-administrations of 10 mg/kg bodyweight of AM and 100 mg/kg bodyweight LA for 5 weeks. All drugs and extract were administered orally. Bodyweights (g) of all rats were measured on Day 1 of experimental procedure and at the end of each week. The doses of ML and AM employed in this study are as determined from our previous study on the anti-cancer potentials of ML and AM LA-induced toxicity (Akinlolu *et al*. 2021).

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 were excised and a lobe fixed in 10% formal saline of at least five times of its volume. Liver tissues were processed for light microscopy using conventional histological procedures. Tissue sections were stained via haematoxylin and eosin method as previously described (Akinlolu *et al.* 2017).

Enzyme Linked Immunosorbent Assay (ELISA) of Concentrations of Ki67 and MDR1 Proteins in Liver Tissues of Rats

Liver tissues were isolated immediately after animal sacrifice and then subjected to thorough homogenisation using porcelain mortar and pestle in ice-cold 0.25 M sucrose, in the proportion of 1 g to 4mL 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 3,000 revolution per min 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 mean ± standard error of mean and were subjected to statistical analyses using *t*-test to compare Group 2 with Groups 3–12. Differences were tested and considered statistically significant when $p ≤ 0.05$ using Graph Pad Prism software package (Graph Pad Software Inc., San Diego, CA, USA; version 7.0 for Windows) and Microsoft Excel 2016.

RESULTS

Histopathological Evaluations

Histopathological evaluations showed normal histoarchitectures of the liver in rats of Groups 1–12 (Figures 1–12). There were normal cellular density and staining characteristics of hepatocytes, hepatic sinusoids and central veins. The nuclei of hepatocytes were well characterised with no apparent large vacuolations around them.
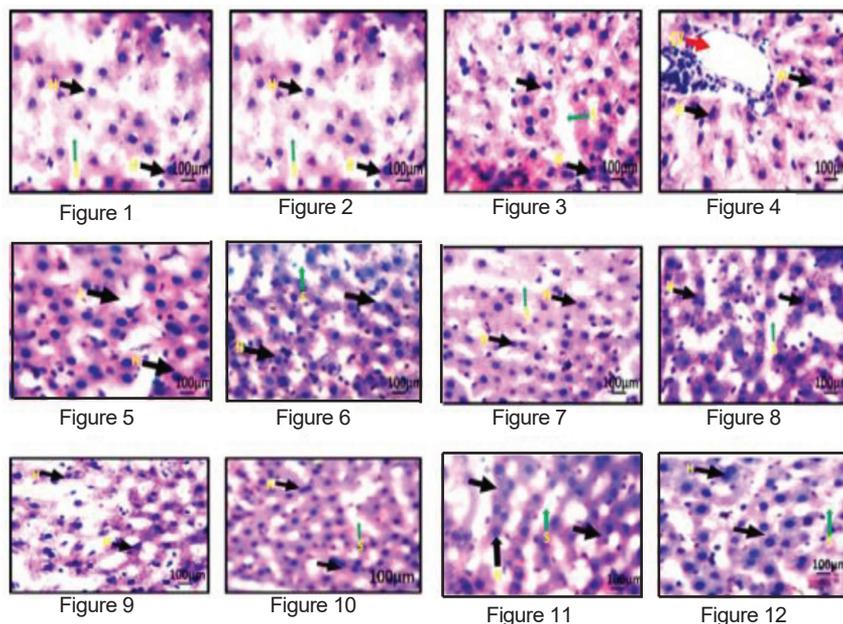
Figures 1–12: Photomicrograph samples of liver tissues of rats of Groups 1–12, which received normal saline only; 100 mg/kg LA only; 100 mg/kg LA + 7.5 mg/kg ML; 100 mg/kg LA + 15 mg/kg ML; 100 mg/kg LA + 7.5 mg/kg AM; 100 mg/kg LA + 10 mg/kg AM; 7.5 mg/kg ML only; 15 mg/kg ML only; 7.5 mg/kg AM only; 10 mg/kg AM only; co-administration of 100 mg/kg LA + 15 mg/kg ML and co-administration of 100 mg/kg LA + 10 mg/kg AM, respectively. Histopathological evaluations showed normal histoarchitectures of the liver components in Groups 1–12.

Notes: Histological technique/magnification: haematoxylin and eosin 400×; scale bar: 100 μm; H = hepatocytes; S = blood sinusoids and CV = central vein.

Ki67 Concentrations in Rats

Results showed statistically non-significant higher Ki67 levels ($p \geq 0.05$) in rats of Group 2 when compared with Group 1 (Table 1). There were statistically non-significant lower Ki67 levels ($p \geq 0.05$) in rats of Groups 3, 4 and 6 when compared with Group 2 (Table 1). In addition, results showed statistically significant lower Ki67 levels ($p \leq 0.05$) in rats of Group 11 when compared with Group 2 (Table 1).

Table 1: Ki67 concentrations (ng/mL) in liver tissues of rats.

Groups of rats	Doses of drug/extract administered	Ki67 (mean ± SEM) (ng/mL)	Group 2 versus Groups 1 and 3–12 ($p \leq 0.05$)
1	Physiological saline	4.37 ± 1.21	0.85
2	100 mg/kg bw LA	4.91 ± 0.11	
3	100 mg/kg bw LA (2 weeks) + 7.5 mg/kg bw ML (3 weeks)	2.65 ± 0.31	0.02*

(continued on next page)

Table 1: (*continued*)

Groups of rats	Doses of drug/extract administered	Ki67 (mean ± SEM) (ng/mL)	Group 2 versus Groups 1 and 3–12 ($p \leq 0.05$)
4	100 mg/kg bw LA (2 weeks) + 15 mg/kg bw ML (3 weeks)	2.12 ± 0.41	0.02*
5	100 mg/kg bw LA (2 weeks) + 7.5 mg/kg bw AM (3 weeks)	5.25 ± 1.82	0.87
6	100 mg/kg bw LA (2 weeks) + 10 mg/kg bw AM (3 weeks)	4.58 ± 1.15	0.93
7	7.5 mg/kg bw ML	9.10 ± 0.05	0.01*
8	15 mg/kg bw ML	2.84 ± 0.29	0.37
9	7.5 mg/kg bw AM	3.85 ± 0.89	0.96
10	10 mg/kg bw AM	2.28 ± 0.19	0.01*
11	Co-administration of 15 mg/kg bw ML + 100 mg/kg bw LA	2.31 ± 0.06	0.02*
12	Co-administration of 10 mg/kg bw AM + 100 mg/kg bw LA	2.69 ± 0.01	0.03*

Notes: bw = bodyweight; * Statistical significant difference

Multi-drug Resistance1/P-Glycoprotein Concentration in Rats

Results showed statistically significant higher MDR1 levels ($p \leq 0.05$) in rats of Group 2 when compared with Group 1 (Table 2). In addition, results showed statistically non-significant lower MDR1 levels ($p \geq 0.05$) in rats of Groups 3–10 when compared with Group 2 (Table 2). Furthermore, there were statistically significant lower MDR1 levels ($p \leq 0.05$) in rats of Groups 11, when compared with Group 2 (Table 2). However, there were statistically non-significant lower MDR1 levels ($p \geq 0.05$) in Group 12, when compared with Group 2 (Table 2).

Table 2: MDR1 concentrations (ng/mL) in liver tissues of rats.

Groups of rats	Doses of drug/extract administered	MDR1 (mean ± SEM) (ng/mL)	Group 2 versus Groups 1 and 3–12 ($p \leq 0.05$)
1	Physiological saline	17.44 ± 1.12	0.05*
2	100 mg/kg bw LA	29.09 ± 2.16	
3	100 mg/kg bw LA (2 weeks) + 7.5 mg/kg bw ML (3 weeks)	24.76 ± 7.77	0.79
4	100 mg/kg bw LA (2 weeks) + 15 mg/kg bw ML (3 weeks)	25.44 ± 1.22	0.79
5	100 mg/kg bw LA (2 weeks) + 7.5 mg/kg bw AM (3 weeks)	27.06 ± 1.72	0.88

Table 2: (*continued*)

Groups of rats	Doses of drug/extract administered	MDR1 (mean ± SEM) (ng/mL)	Group 2 versus Groups 1 and 3–12 ($p \leq 0.05$)
6	100 mg/kg bw LA (2 weeks) + 10 mg/kg bw AM (3 weeks)	25.61 ± 4.51	0.77
7	7.5 mg/kg bw ML	18.77 ± 3.32	0.10
8	15 mg/kg bw ML	18.59 ± 3.23	0.15
9	7.5 mg/kg bw AM	21.54 ± 1.30	0.60
10	10 mg/kg bw AM	15.37 ± 0.58	0.38
11	Co-administration of 15 mg/kg bw ML + 100 mg/kg bw LA	13.40 ± 4.24	0.04*
12	Co-administration of 10 mg/kg bw AM + 100 mg/kg bw LA	21.20 ± 2.45	0.25

Notes: bw = bodyweight; *Statistical significant difference

DISCUSSION

Histopathological evaluations showed normal histoarchitectures of the liver in rats of Groups 1–12 (Figures 1–12). These findings implied that administrations of doses of LA, ML and AM to rats did not result in evident histopathology of the liver after 5 weeks of exposure. This is possibly due to the fact that the cyto-toxicity of adverse chemical agents is exposure-dependent and drug-induced toxicity is usually first elicited on molecular markers, while further exposure will result in evident histopathology at tissue level.

Ki67 is not expressed by quiescent or resting cells in the G0phase, hence it is an excellent biomarker of cellular proliferation and cancer cells survival (Scholzen and Gerdes 2000; Luporsi *et al.* 2012; Xiao *et al.* 2013). The observed increased Ki67 levels in rats of Group 2, when compared with Group 1 (Table 1) implied LA-induction of abnormal proliferation.

Do ML and AM have anti-proliferation potentials against LA-induced hepatocytes hyperplasia in rats? Post-treatments of 100 mg/kg bodyweight of LA-induced hepatocytes hyperplasia with 7.5 mg/kg and 15 mg/kg bodyweight of ML, and 10 mg/kg bodyweight of AM resulted in decreased Ki67 levels in rats of Groups 3, 4 and 6 when compared with Group 2 (Table 1). These results implied that ML and AM possess anti-proliferation potentials.

Do ML and AM have cyto-protective potentials against LA-induced hepatocytes hyperplasia in rats? The co-administrations of 100 mg/kg bodyweight of LA with 15 mg/kg bodyweight of ML and 10 mg/kg bodyweight of AM resulted in significant downregulation of Ki67 levels in rats of Groups 11 and 12, when compared with Group 2 (Table 1). Therefore, ML and AM offered cyto-protective potentials against LA-induced abnormal proliferations in rats.

The MDR1 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 (Ming *et al.* 2010; Alfarouk *et al.* 2015). Hence, significant upregulation of MDR1 is characteristic of drug resistant tumours and has been associated with cancer cells survival. The significant upregulation of MDR1 levels in rats of Group 2, when compared with Group 1 (Table 2) implied LA-induction of drug resistance in rats of Group 2. The observed

LA-induction of increased Ki67 and MDR1 levels in this study are in agreement with those of Xu *et al.* (2008) which reported LA-induction of carcinogenesis via p53 upregulations and altered Bax/Bcl-2 ratio in mice.

Do ML and AM have anti-cancer potentials against LA-induced drug resistance in rats? Post-treatments of 100 mg/kg bodyweight of LA-induced drug resistance with 7.5 mg/kg and 15 mg/kg bodyweight of ML, 7.5 mg/kg and 10 mg/kg bodyweight of AM resulted in decreased MDR1 levels in rats of Groups 3–6, when compared with Group 2 (Table 2). These results implied that ML and AM ameliorated LA-induced upregulation of MDR1 and drug resistance.

Do ML and AM have cytoprotective potentials against LA-induced drug resistance in rats? Results showed decreased levels of MDR1 in rats of Groups 11 and 12, when compared with Group 2 (Table 2). Our findings implied that 15 mg/kg bodyweight of ML and 10 mg/kg bodyweight of AM offered cytoprotective potentials against LA-induced upregulation of MDR1 and drug resistance when co-administered with 100 mg/kg bodyweight of LA.

Do ML and AM have the capacity to target cancer stem cells (CSCs)? Ki67 and MDR1 are biomarkers of CSCs, hence our findings indicate that ML and AM possibly possesses anti-cancer compounds that can specifically target and eliminate CSCs.

Are the reported anti-cancer potentials of ML and AM in the present study in agreement or disagreement with those of previous studies? The observed anti-cancer potentials of ML in the present study are in agreements with those of Ohiagu *et al.* (2021), which reported anti-cancer potentials of ML against the viability and growth of cancer cells, and those of Appiah-Opong *et al.* (2016) which reported anti-cancer potentials of ML leaves against the growth of HL-60 leukemia cells. Similarly, the observed anti-cancer potentials of ML in the present study are in agreements with those of Akinlolu *et al.* (2021), which reported that post-treatments of LA-induced toxicity with ML resulted in melatonin-upregulation, decreased lipid peroxidation and downregulations of MDA, TNF-α and p53 in rats.

In addition, the observed anti-cancer potentials of AM in the present study are in agreements, which reported anti-cancer potentials of ML against the viability and growth of cancer cells via Bax-upregulation, Bcl2-downregulation, EGFR-downregulation (in breast cancer cases) and downregulations of oncogenes (Prasad *et al.* 2019); and those of Yajid *et al.* (2018) which reported anti-cancer potentials of AM against the growth and viability of breast cancer MCF-7 cells. Similarly, the observed anti-cancer potentials of AM in the present study are in agreements with those of Akinlolu *et al.* (2021), which reported that post-treatments of LA-induced toxicity with AM resulted in melatonin-upregulation, decreased lipid peroxidation and downregulations of MDA, TNF-α and p53 in rats.

CONCLUSION

The findings of this study implied that post-treatments of LA-induced hepatotoxicity with extract fractions of ML and AM leaves resulted in decreased Ki67 and MDR1 levels. These observations indicated that ML and AM leaves possibly contain chemical components that possess hepato-protective, anti-proliferation and anti-drug resistance potentials. In addition, ML showed better therapeutic potentials than AM in this study. Hence, we recommend that MLF1, which was fractionated from ML be further evaluated for the isolation and discovery of anti-cancer compounds.

ACKNOWLEDGEMENTS

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