Morinda lucida and Annona *muricata* reduced hepatic lipid peroxidation and promoted melatonin/TNFα/p53-mediated apoptosis in sodium arsenite-induced toxicity in rats

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SUMMARY

Arsenic-induced carcinogenesis can result in cancers of the liver in exposed organisms. This study evaluated anticancer potentials of MLF1 and AMF1 extracted from *Morinda lucida* and *Annona muicata* leaves respectively in Sodium arsenite (SA)-induced toxicity in rats.

Sixty adult female rats were randomly divided into 12 groups (n = 5). Group 1 was control. Group 2 received 5-weeks administrations of 10 mg/kg bodyweight of SA. Groups 3-6 received SA-dose for 2 weeks followed by 3-weeks post-treatments with MLF1-doses and AMF1-doses respectively. Groups 7-10 received only 5-weeks administrations of MLF1-doses and AMF1-doses respectively. Groups 11 and 12 received 5-weeks co-administrations of SA-dose with high-doses of MLF1 and AMF1 respectively. Drugs/extracts were administered orally. Liver histopathology (Heamatoxylin and Eosin) and ELISA concentrations of sera Melatonin and TNF-alpha were evaluated. Malondialdehyde (thiobarbituric-acid assay) and p53 (ELISA) levels were evaluated in liver homogenates. Data were statistically analysed.

Results showed normal liver histology in Groups 1-12. Post-treatments of SA-induced toxicity with MLF1 and AMF1 resulted in significant ($P \leq 0.05$) and non-significant decreased levels ($P \geq 0.05$) of Malondialdehyde, TNF-alpha and p53, but significant ($P \leq 0.05$) and non-significant increased Melatonin levels ($P \geq 0.05$) in Groups 3-12 compared with Group 2. MLF1 and AMF1 possess anticancer, antioxidant, pro-Melatonin, anti-inflammatory and hepato-protective potentials.

Key words: *Annona muricata* – Lipid peroxidation – Melatonin – *Morinda lucida* – p53 – TNF-alpha

INTRODUCTION

Arsenic compounds are ubiquitously present in nature and are dissipated into the environment

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Submitted: July 15, 2022. **Accepted:** July 28, 2022

https://doi.org/10.52083/GSIJ9555

through agricultural and industrial processes and medical applications (World Health Organization, 2001; International Agency for Research on Cancer, 2012; Aliyu et al., 2015). Arsenic-induced cytotoxicity is a via of increased generation of free radicals and further confinement of oxidative stress in body organs resulting in damages to DNA, proteins and lipids (Jomova et al., 2011), as well as increased micronuclei frequency and chromosomal aberrations. Arsenic is a carcinogen and can lead mainly to cancers of the skin, lung, bladder, liver and kidney in exposed organisms (Jomova et al., 2011; Singh et al., 2011). The resolution of carcinogenesis in experimental studies is assessed via activities of biomarkers of lipid peroxidation (Malondialdehyde) (Akinlolu et al., 2012; Lampiao and Du Plessis, 2013), oxidative stress/DNA damage (Melatonin) (Lampiao and Du Plessis, 2013; Zamfir et al., 2014; Mohammadi et al., 2016), inflammation/immune-response (TNF-alpha) (Liu et al., 2004, Zahr et al., 2010; Chu, 2013) and apoptosis (p53) (Chang et al., 2010; Toshinori and Akira, 2011; Xiao et al., 2013).

The ubiquitous environmental presence and pro-carcinogenic potentials of SA make the search for possible plant diets or anticancer compounds that may prevent or counteract SA-induced toxicity quite relevant. *Morinda lucida* (ML) is an ethno-medicinal plant often grown in Nigeria. The leaf extract of ML possesses trypanocidal, antimalarial activities, aortic vaso-relaxant effect, oral hypoglycemic property (Adejo et al., 2014; Adeleye et al., 2018) and antioxidant potentials (Akindele and Obi, 2020). *Annona muricata* (AM) is a member of the Annonaceae family with a long history of ethno-medicinal use (Moghadamtousi et al., 2015; Agu and Okolie, 2017). AM possesses anticancer, anticonvulsant, anti-arthritic, anti-parasitic, antimalarial, hepato-protective and anti-diabetic potentials (Moghadamtousi et al., 2015; Agu and Okolie, 2017). In addition, AM leaf extract was reported to have antioxidant potential, as well as capacity to reduce lipid peroxidation (Justino, 2018).

Rats, mice and humans share about one third of the genome. This includes the majority of coding regions and significant non-coding DNA regions under neutral selection, potentially containing regulatory regions. In addition, some hormones and genes associated with antioxidant defense mechanism and human cancers, such as Melatonin, TNFα and p53 seem to be highly conserved across mammalian evolution, and have counterparts in the rat genome (Simon, 2004; Akinlolu and Shokunbi, 2010). Therefore, this study evaluated the effects of fractionated and isolated compounds from *Morinda lucida* leaves (MLF1) and *Annona muricata* leaves (AMF1) on lipid peroxidation and immuno-modulations of Melatonin, TNF-alpha and p53 proteins in Sodium arsenite (SA)-induced oxidative stress, hepato-toxicity, inflammation and mutagenesis in rats.

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. Appropriate measures were observed to ensure minimal pain or discomfort of rats used in this study. The ethical approval number is UERC/ASN/2018/1161. Furthermore, 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), the Directive 2010/63/Eu of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, and the Guidelines of the U.S. Public Health Service and NIH regarding the care and use of animals for experimentation (NIH publication #85-23, revised in 985).

Collection, authentication and deposition of *Morinda lucida* and *Annona muricata* leaves

Freshly cut leaves of *Morinda lucida* (ML) and *Annona muricata* (AM) were obtained locally from forest reserves in Ilorin and samples identified, authenticated and deposited at the herbarium of the same Department of Botany, Faculty of Life Sciences of the study institution. ML and AM leaves were assigned Herbarium Identification Numbers UITH/004/1103 and UITH/003/1106 respectively.

Preparations and ethanolic extractions of *Morinda lucida* and *Annona muricata* leaves

ML and AM leaves were air-dried at the laboratory unit of the Department of Chemistry, Faculty of Physical Sciences of the study institution. The dried leaves of ML and AM were grround to powder form and extracted with ethanol. Thereafter, 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 *Morinda lucida* and *Annona muricata* leaves

The ethanol extracts of ML and AM leaves 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. Thirty-six eluents of 250 ml each of ML, and thirteen eluents of 250 ml each of AM. The resulting eluents were pooled based on the color of the solvents that elute them to give a total of 9 combined ML and 5 combined AM fractions**.** MLF1 and AMF1 fractions which had the best preliminary antioxidant potentials were used for testing anticancer potentials of plants' extracts in this study.

Chemicals and Reagents

Sodium arsenite 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.

Animal Care and Feeding

A total number of sixty (60) female 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 of the Faculty of Basic Medical Sciences of the study institution. The animals were fed daily with standard diet. The animals were grouped into twelve with five animals each in a wire gauzed cage. The total number of rats and the number of rats per group were as determined and approved by the policy guidelines of laboratory animal use and care of the University Ethical Review Committee of the study institution. The animals were kept under a normal room temperature of 37°C and double-crossed ventilation.

Experimental Procedures and Drugs Administration

Group 1 received physiological saline. Group 2 received 10 mg/kg bodyweight of Sodium arsenite (SA) for 5 weeks. Groups 3 and 4 received 10 mg/kg bodyweight SA for 2 weeks followed by treatments with 7.5 and 15 mg/kg bodyweight of MLF1 respectively for another 3 weeks. Groups 5 and 6 received 10 mg/kg bodyweight SA for 2 weeks followed by treatments with 7.5 and 10 mg/kg bodyweight of AMF1 respectively for another 3 weeks. Groups 7 and 8 received 7.5 and 15 mg/kg bodyweight of MLF1 respectively for 5 weeks. Groups 9 and 10 received 7.5 and 10 mg/kg bodyweight of AMF1 respectively for 5 weeks. Group 11 received co-administrations of 15 mg/kg bodyweight MLF1 and 10 mg/kg bodyweight SA for 5 weeks. Group 12 received co-administrations of 10 mg/kg bodyweight of AMF1 and 10 mg/kg bodyweight SA 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.

Histopathological evaluations of the Liver

At the end of the experimental procedures, following animal sacrifice, the liver of each rat was 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 by Akinlolu et al. (2017).

Evaluations of Lipid peroxidation

The thiobarbituric acid assay (TBARS assay) method was used to quantify Malondialdehyde

concentrations in liver homogenates of all rats of Control and Experimental Groups as previously described by Akinlolu et al. (2017).

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

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

Statistical Analyses

Statistical analyses were conducted using the 2019 Statistical Package for the Social Science software Version 23.0. Computed data of concentrations of biomarker was expressed as arithmetic means ± standard deviation and were subjected to statistical analyses using One-way Analysis of Variance to test for significant difference amongst Groups 1-12. Degree of freedom (df): (between groups and within groups) and F-values were computed. Scheffe Post-hoc analysis was used for separation of Mean values amongst Groups 1-12. Significant difference was confirmed at 95% confidence interval with $P \leq 0.05$.

RESULTS

Histopathological evaluations of the Liver

Histopathological evaluations showed normal histoarchitectures of the liver in rats of Groups 1-12 (Figs. 1-12). 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 vacuolations around them.

Table 1. Malondialdehyde (MDA) concentrations (mean±SD) (µmol/ml) in liver tissues of rats.

Groups of rats	Doses of drug/extract administered	MDA (Mean±SD) (µmol/ml)	*P≤0.05*: Group-2 versus Groups 1 and 3-12
1	Physiological saline (5 weeks)	2.22 ± 0.39	0.16
2	10 mg/Kg bodyweight Sodium arsenite (SA) (5 weeks)	2.99 ± 0.10	
3	10 mg/Kg bodyweight SA (2 weeks) + 7.5 mg/Kg bodyweight *Morinda lucida* (3 weeks)	2.43 ± 0.79	0.52
4	10 mg/Kg bodyweight SA (2 weeks) + 15 mg/Kg bodyweight *Morinda lucida* (3 weeks)	2.34 ± 0.79	0.39
5	10 mg/Kg SA (2 weeks) + 7.5 mg/Kg bodyweight *Annona muricata* (3 weeks)	2.95 ± 0.89	0.96
6	10 mg/Kg bodyweight SA (2 weeks) + 10 mg/Kg bodyweight *Annona muricata* (3 weeks)	2.31 ± 0.78	0.45
7	7.5 mg/Kg bodyweight *Morinda lucida* (5 weeks)	2.43 ± 0.38	0.20
8	15 mg/Kg bodyweight *Morinda lucida* (5 weeks)	2.34 ± 0.37	0.26
9	7.5 mg/Kg bodyweight *Annona muricata* (5 weeks)	2.35 ± 0.39	0.20
10	10 mg/Kg bodyweight *Annona muricata* (5 weeks)	2.44 ± 0.37	0.99
11	Co-administration of 15 mg/Kg bodyweight *Morinda lucida* + 10 mg/Kg bodyweight SA (5 weeks)	2.39 ± 0.61	0.38
12	Co-administration of 10 mg/Kg bodyweight *Annona muricata* + 10 mg/Kg bodyweight SA (5 weeks)	2.52 ± 0.63	0.49

Malondialdehyde (MDA) concentrations in Liver tissues of rats

Results showed statistically non-significant higher (*P≥0.05*) MDA levels in rats of Group 2 when compared with Group 1 (Table 1 and Fig. 13). In addition, results showed statistically non-significant lower (*P≥0.05*) MDA levels in rats of Groups 3-12 when compared with Group 2 (Table 1 and Fig. 13).

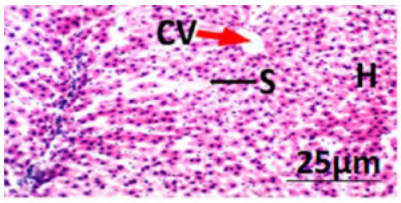
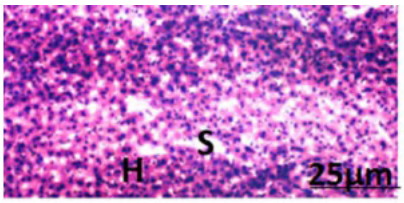


Fig. 1



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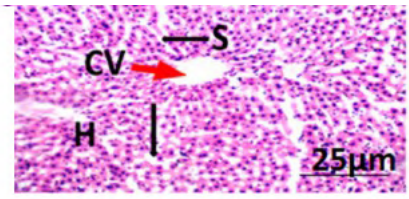

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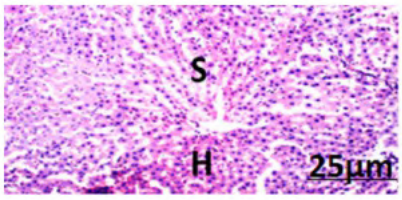

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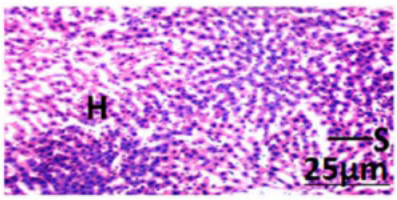

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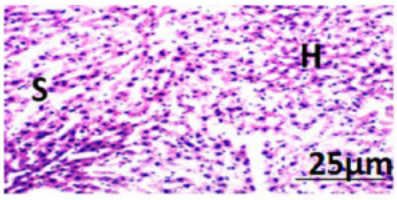

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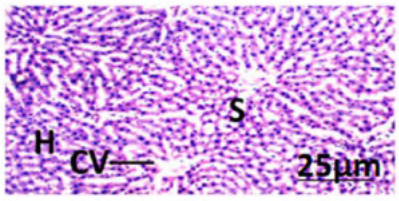


Fig. 7

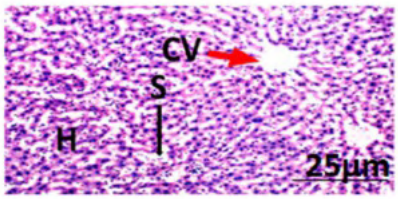


Fig. 8

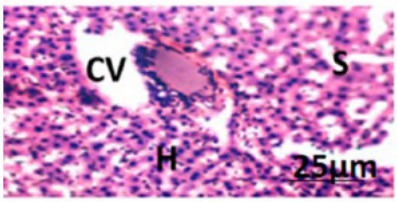
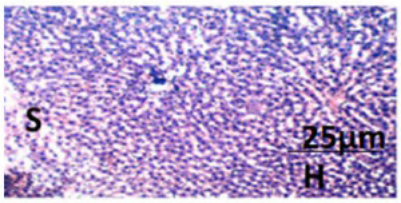


Fig. 9



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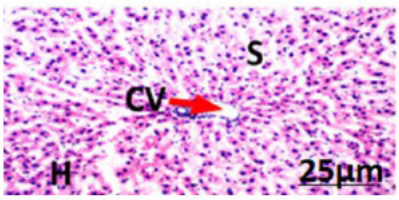

Fig. 11

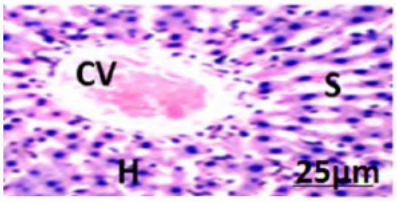

Fig. 12

Fig. 1.- Liver of rat of control group 1, which received normal saline. Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histopathological evaluations showed normal histoarchitecture of the liver components. **Fig. 2.**- Liver of rat of experimental group 2, which received 100 mg/kg bodyweight of lead acetate only. Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histo-pathological evaluations showed normal histoarchitecture of the liver components. **Figs. 3. and 4.**- Liver of rat of experimental groups 3 and 4, which received 100 mg/Kg bodyweight lead acetate (2 weeks) + 7.5 and 15mg/Kg bodyweight *Morinda lucida* (3 weeks) respectively. Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histopathological evaluations showed normal histoarchitecture of the liver components. **Figs. 5 and 6.**- Liver of rat of experimental groups 5 and 6, which received 100 mg/Kg bodyweight lead acetate (2 weeks) + 7.5 and 10mg/Kg bodyweight *Annona muricata* (3 weeks) respectively. Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histopathological evaluations showed normal histoarchitecture of the liver components. **Figs. 7 and 8.**- Liver of rat of experimental groups 7 and 8, which received only 7.5 and 15 mg/Kg bodyweight *Morinda lucida* (3 weeks) respectively. Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histopathological evaluations showed normal histoarchitecture of the liver components. **Figs. 9 and 10.**- Liver of rat of experimental groups 9 and 10, which received only 7.5 and 10 mg/Kg bodyweight *Annona muricata* (3 weeks) respectively. Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histopathological evaluations showed normal histoarchitecture of the liver components. **Fig. 11.**- Liver of rat of experimental group 11, which received co-administration of 100 mg/Kg bodyweight lead acetate + 15 mg/Kg bodyweight *Morinda lucida* (5 weeks). Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histopathological evaluations showed normal histoarchitecture of the liver components. **Fig. 12.**- Liver of rat of experimental group 12, which received co-administration of 100 mg/Kg bodyweight lead acetate + 10 mg/Kg bodyweight *Annona muricata* (5 weeks). Haematoxylin and Eosin (x 100). H = Hepatocytes, S = Blood sinusoids and CV = Central Vein. Histopathological evaluations showed normal histoarchitecture of the liver components.

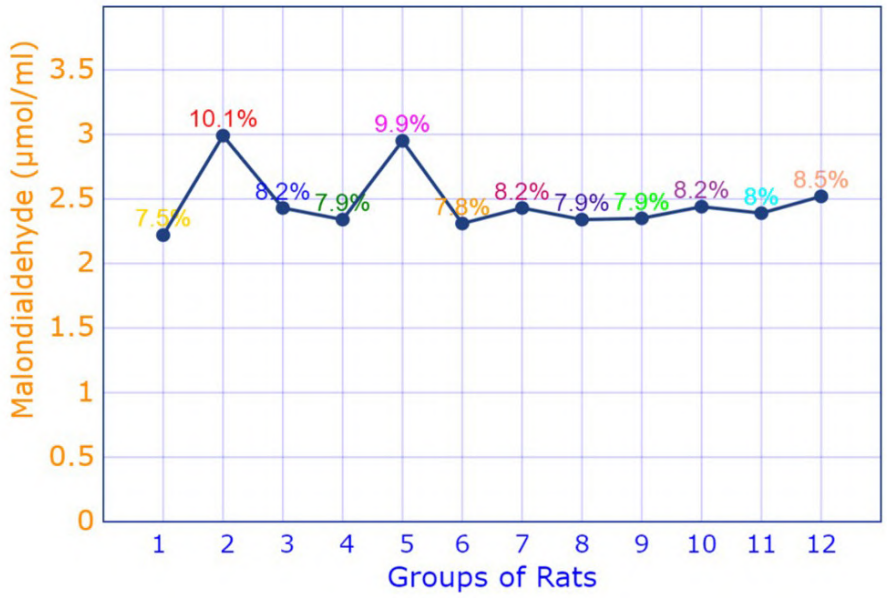

Fig. 13.- Concentrations of Malondialdehyde (µmol/nl) in liver homogenates of rats.

Sera Melatonin concentrations in rats

Results showed statistically significant lower (*P≤0.05*) levels of Melatonin in rats of Group 2 when compared with Group 1 (Table 2 and Fig. 14). In addition, results showed statistically significant higher (*P≤0.05*) levels of Melatonin in rats of Groups 4 - 10 when compared with Group 2 (Table 2 and Fig. 14). However, there were statistically non-significant higher (*P≥0.05*) levels of Melatonin in rats of Groups 3 and 12 when compared with Group 2 (Table 2 and Fig. 14).

Table 2. Melatonin concentrations (mean±SD) (ng/ml) in sera of rats.

Groups of rats	Doses of drug/extract administered	Melatonin (Mean±SD) (ng/ml)	*P*≤0.05: Group-2 versus Groups 1 and 3-12
1	Physiological saline (5 weeks)	0.29 ± 0.01	0.05*
2	10 mg/Kg bodyweight Sodium arsenite (SA) (5 weeks)	0.21 ± 0.08	
3	10 mg/Kg bodyweight SA (2 weeks) + 7.5 mg/Kg bodyweight Morinda lucida (3 weeks)	0.26 ± 0.03	0.94
4	10 mg/Kg bodyweight SA (2 weeks) + 15 mg/Kg bodyweight Morinda lucida (3 weeks)	0.31 ± 0.04	0.04*
5	10 mg/Kg SA (2 weeks) + 7.5 mg/Kg bodyweight Annona muricata (3 weeks)	0.39 ± 0.02	0.03*
6	10 mg/Kg bodyweight SA (2 weeks) + 10 mg/Kg bodyweight Annona muricata (3 weeks)	0.36 ± 0.03	0.03*
7	7.5 mg/Kg bodyweight Morinda lucida (5 weeks)	0.42 ± 0.04	0.02*
8	15 mg/Kg bodyweight Morinda lucida (5 weeks)	0.43 ± 0.07	0.02*
9	7.5 mg/Kg bodyweight Annona muricata (5 weeks)	0.33 ± 0.03	0.03*
10	10 mg/Kg bodyweight Annona muricata (5 weeks)	0.58 ± 0.07	0.02*
11	Co-administration of 15 mg/Kg bodyweight Morinda lucida + 10 mg/Kg bodyweight SA (5 weeks)	0.21 ± 0.08	0.99
12	Co-administration of 10 mg/Kg bodyweight Annona muricata + 10 mg/Kg bodyweight SA (5 weeks)	0.24 ± 0.07	0.95

* = Statistically significant difference

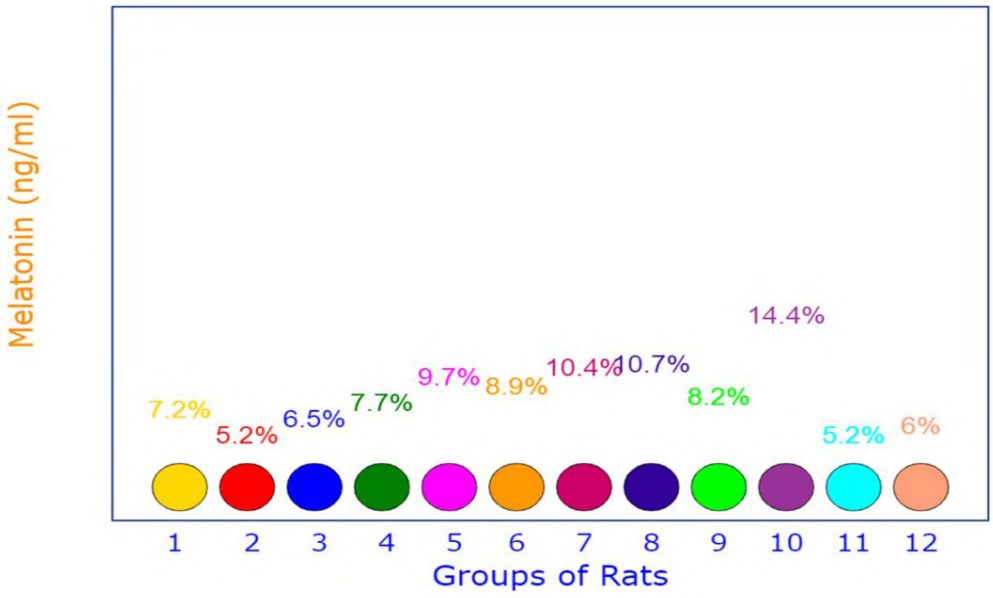

Fig. 14.- Melatonin concentrations (ng/ml) in sera of rats.

Sera TNFα concentrations in rats

Results showed statistically significant higher (P≤0.05) levels of TNFα in rats of Group 2 when compared with Group 1 (Table 3 and Fig. 15). In addition, results showed statistically significant lower (P≤0.05) levels of TNFα in rats of Group 11 when compared with Group 2 (Table 3 and Fig. 15). However, there were statistically non-significant lower (P≥0.05) levels of TNFα in rats of Groups 3 – 10 and 12 when compared with Group 2 (Table 3 and Fig. 15).

Table 3. Sera TNF-α concentrations (mean±SD) (ng/ml) in rats.

Groups of rats	Doses of drug/extract administered	TNFa (Mean±SD) (ng/ml)	*P≤0.05*: Group-2 versus Groups 1 and 3-12
1	Physiological saline (5 weeks)	5.73 ± 6.58	0.03*
2	10 mg/Kg bodyweight Sodium arsenite (SA) (5 weeks)	20.19 ± 4.40	
3	10 mg/Kg bodyweight SA (2 weeks) + 7.5 mg/Kg bodyweight *Morinda lucida* (3 weeks)	16.85 ± 4.34	0.46
4	10 mg/Kg bodyweight SA (2 weeks) + 15 mg/Kg bodyweight *Morinda lucida* (3 weeks)	13.35 ± 4.44	0.19
5	10 mg/Kg SA (2 weeks) + 7.5 mg/Kg bodyweight *Annona muricata* (3 weeks)	16.94 ± 4.69	0.34
6	10 mg/Kg bodyweight SA (2 weeks) + 10 mg/Kg bodyweight *Annona muricata* (3 weeks)	14.38 ± 4.23	0.22
7	7.5 mg/Kg bodyweight *Morinda lucida* (5 weeks)	16.94 ± 6.88	0.62
8	15 mg/Kg bodyweight *Morinda lucida* (5 weeks)	14.38 ± 6.57	0.86
9	7.5 mg/Kg bodyweight *Annona muricata* (5 weeks)	13.88 ± 6.58	0.92
10	10 mg/Kg bodyweight *Annona muricata* (5 weeks)	13.98 ± 6.56	0.91
11	Co-administration of 15 mg/Kg bodyweight *Morinda lucida* + 10 mg/Kg bodyweight SA (5 weeks)	4.74 ± 4.26	0.02*
12	Co-administration of 10 mg/Kg bodyweight *Annona muricata* + 10 mg/Kg bodyweight SA (5 weeks)	13.35 ± 4.44	0.24

* = Statistically significant difference

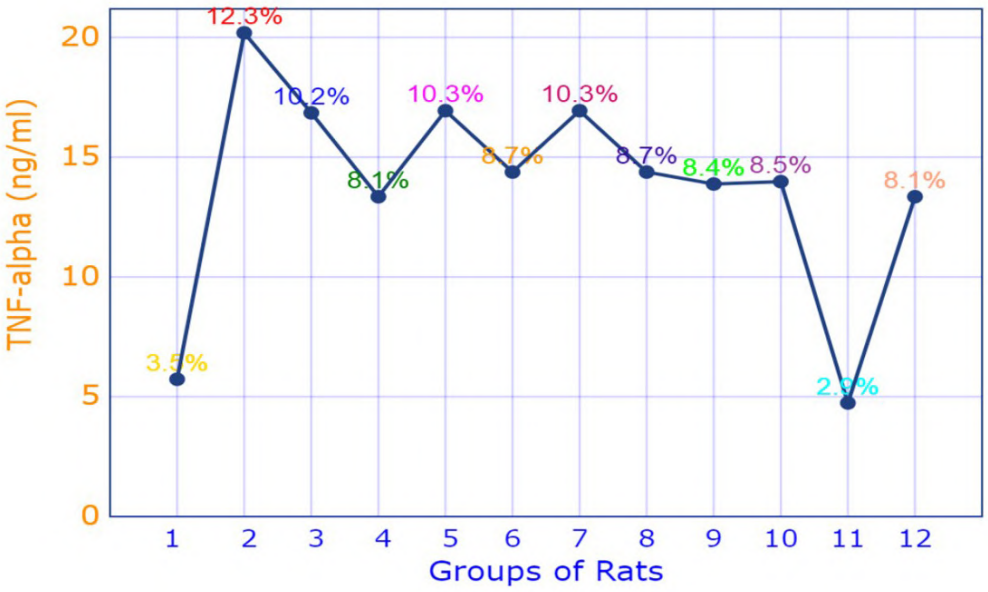

Fig. 15.- Sera concentrations (ng/ml) of TNF-alpha in rats.

p53 concentrations in Liver tissues of rats

Results showed statistically significant higher (*P≤0.05*) levels of p53 in rats of Group 2 when compared with Group 1 (Table 4 and Fig. 16). In addition, results showed statistically significant lower (*P≤0.05*) levels of p53 in rats of Groups 3-11 when compared with Group 2 (Table 4 and Fig. 16). However, there were statistically non-significant lower [*P≥0.05*] levels of p53 in rats of Group 12 when compared with Group 2 (Table 4 and Fig. 16).

Table 4. p53 concentrations (mean±SD) (ng/ml) in liver tissues of rats.

Groups of rats	Doses of drug/extract administered	p53 (Mean±SD) (ng/ml)	*P≤0.05*: Group 2 versus Groups 1 and 3-12
1	Physiological saline (5 weeks)	6.74 ± 1.92	0.02*
2	10 mg/Kg bodyweight Sodium arsenite (SA) (5 weeks)	45.75 ± 2.76	
3	10 mg/Kg bodyweight SA (2 weeks) + 7.5 mg/Kg bodyweight *Morinda lucida* (3 weeks)	24.40 ± 3.19	0.04*
4	10 mg/Kg bodyweight SA (2 weeks) + 15 mg/Kg bodyweight *Morinda lucida* (3 weeks)	16.5 ± 2.65	0.03*
5	10 mg/Kg SA (2 weeks) + 7.5 mg/Kg bodyweight *Annona muricata* (3 weeks)	26.20 ± 2.88	0.03*
6	10 mg/Kg bodyweight SA (2 weeks) + 10 mg/Kg bodyweight *Annona muricata* (3 weeks)	25.21 ± 2.73	0.03*
7	7.5 mg/Kg bodyweight *Morinda lucida* (5 weeks)	17.58 ± 0.38	0.04*
8	15 mg/Kg bodyweight *Morinda lucida* (5 weeks)	13.41 ± 1.80	0.03*
9	7.5 mg/Kg bodyweight *Annona muricata* (5 weeks)	22.1 ± 3.16	0.04*
10	10 mg/Kg bodyweight *Annona muricata* (5 weeks)	20.72 ± 4.45	0.04*
11	Co-administration of 15 mg/Kg bodyweight *Morinda lucida* + 10 mg/Kg bodyweight SA (5 weeks)	31.13 ± 2.89	0.05*
12	Co-administration of 10 mg/Kg bodyweight *Annona muricata* + 10 mg/Kg bodyweight SA (5 weeks)	39.28 ± 5.44	0.07

* = Statistically significant difference

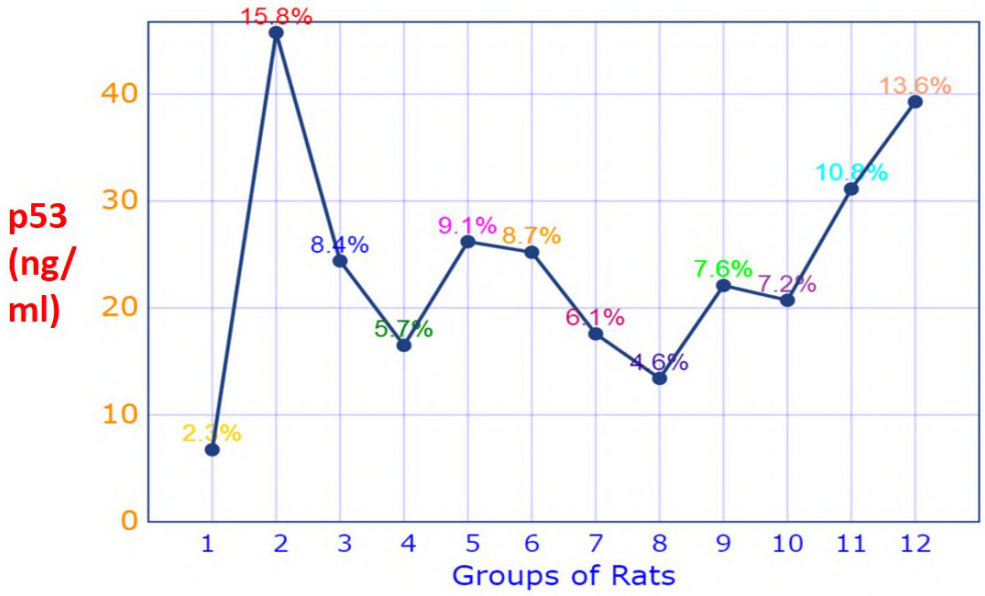

Fig. 16.- Concentrations of p53 (ng/ml) in liver homogenates of rats.

DISCUSSION

Histopathological evaluations showed normal histoarchitectures of the liver in rats of Groups 1-12 (Figs. 1-12). This implied that administrations of 10 mg/kg bodyweight of sodium arsenite (SA), 7.5 and 15 mg/kg bodyweight of MLF1 (extracted from Morinda lucida leaves) and 7.5 and 10 mg/kg bodyweight of AMF1 (extracted from Annona muricata leaves) to rats did not result in evident histopathology of the liver after 5 weeks of exposure.

Lipid peroxidation results in compromised cell membrane and cellular damage, and it is implicated in inflammation and carcinogenesis (Akinlolu et al., 2012). Malondialdehyde is a resultant mutagenic aldehyde product of lipid peroxidation (Akinlolu et al., 2012). Hence, increased Malondialdehyde levels imply increased oxidative stress. Results showed statistically non-significant higher ($P \geq 0.05$) Malondialdehyde levels in rats of Group 2 when compared with Group 1 (Table 1 and Fig. 13). This implied that the administration of 10 mg/kg bodyweight of SA resulted in increased oxidative stress in rats of Group 2.

Do MLF1 and AMF1 have cyto-protective and antioxidant potentials against SA-induced oxidative stress? Post-treatments of SA-induced oxidative stress with MLF1-doses and AMF1-doses resulted in decreased Malondialdehyde levels in rats of Groups 3-6 when compared with Group 2 (Table 1 and Fig. 13). Hence, MLF1 and AMF1 possess cyto-protective and antioxidant potentials.

Do MLF1 and AMF1 have chemo-preventive and antioxidant potentials against SA-induced oxidative stress? The co-administrations of SA-dose with 15 mg/kg bodyweight of MLF1 and 10 mg/kg bodyweight of AMF1 resulted in decreased Malondialdehyde levels in Groups 11 and 12 when compared with Group 2 (Table 1 and Fig. 13). Therefore, MLF1 and AMF1 possess chemo-preventive antioxidant potentials.

Melatonin is a mitochondria-targeted antioxidant (Russel et al., 2016), which inhibits lipid peroxidation by directly scavenging resulting alkoxyl radical and by maintaining cellular glutathione levels (which is otherwise reduced in oxidative stress) (Lampiao and Du Plessis, 2013; Zamfir et al., 2014; Mohammadi et al., 2016; Russel et al., 2016). Melatonin also inhibits the release of pro-inflammatory cytokines and downregulates cyclooxygenase-2, resulting in anti-inflammation and inhibition of cancer growth and metastasis (Lampiao and Du Plessis, 2013; Zamfir et al., 2014). Melatonin is mainly metabolized in the liv-

er (Lampiao and Du Plessis, 2013), hence its strong involvement in the resolutions of hepato-toxicity, hepatic oxidative stress and mutagenesis. Increased melatonin levels are thus associated with reduced DNA and cellular damage, decreased oxidative stress, inflammation and proliferation.

Results showed statistically significant lower ($P \leq 0.05$) levels of melatonin in rats of Group 2 when compared with Group 1 (Table 2 and Fig. 14). This implied that the administration of 10 mg/kg bodyweight of SA resulted in prominent reduction in the antioxidant system in rats of Group 2.

Do MLF1 and AMF1 have cyto-protective and antioxidant potentials against SA-induced prominent reduction in the antioxidant system? Post-treatments of SA-induced prominent reduction in the antioxidant system with doses of MLF1 and AMF1 resulted in increased Melatonin levels in rats of Groups 3-6 when compared with Group 2 (Table 2 and Fig. 14). Hence, MLF1 and AMF1 possess pro-melatonin, cyto-protective and prominent promotion of the antioxidant system potentials.

Do MLF1 and AMF1 have chemo-preventive and antioxidant potentials against SA-induced prominent reduction in the antioxidant system? The co-administration of SA-dose with 10 mg/kg bodyweight of AMF1 resulted in increased Melatonin levels in Groups 11 and 12 when compared with Group 2 (Table 2 and Fig. 14). Therefore, AMF1 possesses pro-Melatonin, chemo-preventive and prominent promotion of the antioxidant system potentials.

TNF-alpha (TNFα) is a pro-inflammatory cytokine and its upregulation resulted in induction of necrosis and consequent apoptosis (necroptosis) in animal models (Liu et al., 2004, Zahr et al., 2010; Chu, 2013). Hence, TNFα is a biomarker of interest in the evaluation of inflammation, tumor progression and cancer cells survival. Results showed statistically significant higher ($P \leq 0.05$) levels of TNFα in rats of Group 2 when compared with Group 1 (Table 3 and Fig. 15). Therefore, this implied that the administration of 10 mg/kg bodyweight of SA resulted in induction of cancer-associated inflammation and upregulation of TNFα in rats of Group 2.

Do MLF1 and AMF1 have cyto-protective and anti-inflammatory potentials against SA-induced cancer-associated inflammation? Post-treatments of SA-induced cancer-associated inflammation with doses of MLF1 and AMF1 resulted in the downregulation of TNFα levels in Groups 3-6 when compared with Group 2 (Table 3 and Fig. 15). Hence, MLF1 and AMF1 possess cyto-protective, anti-inflammatory and anticancer potentials.

Can MLF1 and AMF1 protect the organism and prevent SA-induced cancer-associated inflammation? The co-administrations of 10 mg/kg bodyweight of SA with 15 mg/kg bodyweight of MLF1 and 10mg/kg bodyweight of AMF1 resulted in decreased TNFα levels in Groups 11 and 12 when compared with Group 2 (Table 3 and Fig. 15), and thus protected the rats against SA-induced cancer-associated inflammation. This suggests that MLF1 and AMF1 possess chemo-preventive anti-inflammatory potentials.

p53 trans-activates several apoptotic and cell-cycle arrest-induction genes in response to inflammation, DNA damage, chromosomal aberrations, mutagenesis and carcinogenesis. p53 is usually expressed as a functionally latent form at a very low level in normal condition as mediated majorly by proteasomal degradation actions of RING-finger type E3 ubiquitin protein ligase MDM2. However, in response to DNA damage, there is upregulation, accumulation and activation of p53 in the cell nucleus via post-translational modifications such as phosphorylation and acetylation. Following successful repair of DNA and cellular damage, p53 level returns to normal low level (Chang et al., 2010; Toshinori and Akira, 2011; Xiao et al., 2013).

Results showed statistically significant higher ($P \leq 0.05$) levels of p53 in rats of Group 2 when compared with Group 1 (Table 4 and Fig. 16). This result implied that administration of 10 mg/kg bodyweight of SA resulted in mutagenesis with consequent upregulation of p53 in rats of Group 2. It must be noted that with increased upregulations of Malondialdehyde and TNFα levels, and reduced Melatonin levels, in only the rats of Group 2 (Tables 1-3 and Figs. 13-16), the characteristic expected immune response and DNA/cell-damaged repair mechanism is induction of apoptosis and

upregulation of p53 pro-apoptotic gene (Chang et al., 2010; Toshinori and Akira, 2011; Xiao et al., 2013). In the absence of cancer treatment, the sustained upregulation of p53 in rats of SA-only treated Group 2 at the end of the 5-week experimental procedure implied rapid and sustained SA-induced mutagenesis. This will gradually inhibit and prolong the DNA repair mechanism of p53 until its actions are spent out in-order to allow for un-inhibited mutagenesis. Consequently, cancer-cells survival and tumorigenesis will prevail.

Do MLF1 and AMF1 have cyto-protective and anticancer potentials against SA-induced mutagenesis? Post-treatments of 10 mg/kg bodyweight of SA-induced p53 upregulation with doses of MLF1 and AMF1 in rats of Groups 3-6 resulted in significant downregulations of p53 levels when compared with Group 2 (Table 4 and Fig. 16). This implied that MLF1 and AMF1 possess pro-apoptotic, tumor suppression and anticancer potentials that will bring p53 to normal levels after the resolution of mutagenesis.

Can MLF1 and AMF1 protect the organism and prevent SA-induced mutagenesis? The co-administrations of 10mg/kg bodyweight of SA with 15 mg/kg bodyweight of MLF1 and 10mg/kg bodyweight of AMF1 resulted in decreased p53 levels in Groups 11 and 12 when compared with Group 2 (Table 4 and Fig. 16), and thus protected the rats against SA-induced mutagenesis. This suggests that MLF1 and AMF1 possess chemo-preventive, pro-apoptotic and anticancer potentials.

Overall, the observations of this study suggest that post-treatments with doses of MLF1 (extracted from Morinda lucida leaves) and AMF1 (extracted from Annona muricata leaves) following exposure to sodium arsenite resulted in decreased malondialdehyde, TNF-alpha and p53 levels, and increased melatonin levels. This indicates that MLF1 and AMF1 conferred a degree of cyto-protective, antioxidant, pro-melatonin, hepatoprotective, anti-inflammatory and anticancer potentials against sodium-arsenite-induced toxicity, and are recommended for further evaluation as potential drug candidates for the treatment of cancers.

ACKNOWLEDGEMENTS

The authors acknowledge the technical support of Laboratory staff members of the Departments of Anatomy and Chemistry of the University of Ilorin, Nigeria, the Central Research Laboratory, Ilorin, Nigeria.

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