

April 2023

Anti-toxic principle from *Moringa oleifera* but not *Myristica fragrans* ameliorated Sodium Arsenite-induced toxicity on testes and sex hormones of rats

Adelaja Akinlolu

Mubarak Ameen

Muheen Biliaminu

Mukadam Abdulhamid

Olaolu Ajiboye

See next page for additional authors

Follow this and additional works at: <https://digital.car.chula.ac.th/tjps>

Recommended Citation

Akinlolu, Adelaja; Ameen, Mubarak; Biliaminu, Muheen; Abdulhamid, Mukadam; Ajiboye, Olaolu; Quadri, Tobilola; Jaji-Sulaimon, Rukayat; Oyewopo, Adeoye; Aiyedogbon, Aramide; Akanmode, Badejoko; Agbagba, Cephas; Biliaminu, Sikiru; and Akindele, Raheem (2023) "Anti-toxic principle from *Moringa oleifera* but not *Myristica fragrans* ameliorated Sodium Arsenite-induced toxicity on testes and sex hormones of rats," *The Thai Journal of Pharmaceutical Sciences*: Vol. 47: Iss. 2, Article 5.
Available at: <https://digital.car.chula.ac.th/tjps/vol47/iss2/5>

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Pharmaceutical Sciences by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.

Anti-toxic principle from *Moringa oleifera* but not *Myristica fragrans* ameliorated Sodium Arsenite-induced toxicity on testes and sex hormones of rats

Authors

Adelaja Akinlolu, Mubarak Ameen, Muheen Biliaminu, Mukadam Abdulhamid, Olaolu Ajiboye, Tobilola Quadri, Rukayat Jaji-Sulaimon, Adeoye Oyewopo, Aramide Aiyedogbon, Badejoko Akanmode, Cephas Agbagba, Sikiru Biliaminu, and Raheem Akindele



Anti-toxic principle from *Moringa oleifera* but not *Myristica fragrans* ameliorated Sodium Arsenite-induced toxicity on testes and sex hormones of rats

Adelaja Akinlolu¹, Mubarak Ameen², Muheen Biliaminu³, Mukadam Abdulhamid³, Olaolu Ajiboye³, Tobilola Quadri³, Rukayat Jaji-Sulaimon³, Adeoye Oyewopo³, Aramide Aiyedogbon³, Badejoko Akanmode³, Cephas Agbagba³, Sikiru Biliaminu⁴, Raheem Akindele⁵

¹Department of Anatomy, Faculty of Basic Medical Sciences, University of Medical Sciences Ondo, Ondo State, Nigeria, ²Department of Chemistry, Faculty of Physical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria, ³Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria, ⁴Department of Chemical Pathology and Immunology, University of Ilorin, Ilorin, Kwara State, Nigeria, ⁵Department of Physiology, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun State, Nigeria

Corresponding Author:

Dr. Adelaja Akinlolu,
Department of Anatomy,
Faculty of Basic Medical
Sciences, University of
Medical Sciences Ondo,
Ondo State, Nigeria.
Tel: +2348062765308.
E-mail: aadelaja@
unimed.edu.ng

Received: October 19, 2021

Accepted: March 08, 2022

Published: June 30, 2023

ABSTRACT

This study evaluated the repro-protective potentials of MOF₆ (isolated from *Moringa oleifera* leaves) and Trimyristin (TR) (isolated from *Myristica fragrans* seeds) in sodium arsenite (SA)-induced toxicity in rats. Sixty-five adult male rats were randomly divided into 13 groups ($n = 5$). Groups 1 and 3 received 9-week administrations of physiological saline and olive oil, respectively. Group 2 received 20 mg/kg bodyweight SA-only for 3 weeks and was untreated for 6 weeks. Groups 4–7 received a 3 week SA dose, followed by 6 weeks post-treatments with 5 mg and 7.5 mg/kg bodyweight MOF₆, 15 mg, and 30 mg/kg bodyweight TR, respectively. Groups 8–11 received only MOF₆ doses (6 weeks) or TR doses (9-weeks). Groups 12 and 13 received 6-week administrations of high-doses of MOF₆ and TR, respectively, followed by 3-week co-administrations of SA with extracts' high doses. Testicular histopathology, sperm cell count (SCC), and plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone were evaluated. Results showed testicular histopathology in Group 2 compared with normal testicular histology in Groups 4–7. FSH levels were normal in all groups. Statistical analyses showed significant ($P \leq 0.05$) decreased levels of SCC, LH, and testosterone in Groups 2, 6, and 7, compared with Groups 1, 4, and 5. Overall, MOF₆ but not TR ameliorated SA-induced toxicity on the testes and sex hormones of rats.

Keywords: Sodium arsenite, *Moringa oleifera*, *Myristica fragrans*, testes, sex hormones

INTRODUCTION

Moringa oleifera (MO) and *Myristica fragrans* (MF) are ethno-medicinal plants well grown in Nigeria. MO has been traditionally employed over centuries as a medicinal plant for the treatment of skin infections,

asthma, blood impurities, anxiety, bronchitis, and many other diseases.^[1,2] MO has also been used as nutritional supplement for centuries.^[1,2] MO leaves have been reported to possess antioxidant, anticancer, and neuroprotective potentials.^[1,2] MF is commonly known as nutmeg, and it is conventionally used to treat hypertension, diabetes, cancer, and many other

diseases.^[2] MF has been reported to possess antioxidant, hepatoprotective, antimicrobial, anti-diabetic, and aphrodisiac potentials.^[2]

Arsenic contamination is a major public health issue, according to the World Health Organization fact sheet of 1999.^[3] Human arsenic exposure is either via ingestion of contaminated food and water or through inhalation via exposures to agricultural pesticides, mining activities, non-ferrous ore smelting, semiconductor and glass manufacturing, and power generation by the burning of arsenic-contaminated coal.^[3] Sodium arsenite (SA) is a clastogenic metalloid that induces toxicity via increased generations of free radicals and the confinement of oxidative stress on multiple organs, including the testes.^[4,5] Hence, arsenic toxicity has been associated with male infertility and tumors of the body organs.^[3,6]

Ethno-medicinal plants have been conventionally employed for therapeutic purposes in many countries across the world, and plant resources have been previously employed in the development of drugs such as Artemisinin derived from *Artemisia annua* for the treatment of malaria.^[7] MO leaves and MF seeds are conventionally used to treat male infertility. We have previously established the anticancer,^[1] antioxidant,^[1,2,8] cytoprotective^[1,2,8] and neuro-regenerative^[1,2,8] potentials of fraction 6 of MO leaves (MOF₆), while the antioxidant^[9,10] and neuroprotective.^[2] Potentials of MF seeds have been reported. Although many studies reported the beneficial effects of MO leaves on experimentally induced male toxicity in animal studies,^[11-14] such studies employed aqueous extracts of MO leaves and not further fractionated extracts of MO leaves. Fractionation of the extract permits the separation of the plant's components into groups of compounds sharing similar physico-chemical properties. In addition, the isolation of active constituents as drug candidates is only obtainable from fractionated and purified extracts and not from aqueous extracts.

Therefore, in this study, MOF₆ and *Trimyristin* (TR) were purified and isolated from MO leaves and MF seeds, respectively, in order to further determine the specific fractions and chemical constituents that may be responsible for their possible pro-fertility potentials on the testes and sex hormones in SA-induced toxicity in adult male rats. Is SA-induced toxicity reversible without treatment? In addition, can prior consumption of MO leaves and MF seeds protect against or prevent the onset of SA-induced toxicity when the organism is later exposed to arsenic contamination? Hence, we developed appropriate study designs in order to answer these questions and eliminate confounding factors in the evaluation of the pro-fertility potentials of MOF₆ and TR in SA-induced toxicity in rats.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was sought and received from the University of Ilorin Ethical Review Committee. 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, Preparation, Purification, and Fractionation of Plant Extracts

The plant name MO was checked with <http://www.theplantlist.org> and accessed via <http://www.theplantlist.org/tpl1.1/record/tro-21400003>. Similarly, the plant name MF was checked with <http://www.theplantlist.org> and accessed via <http://www.theplantlist.org/tpl1.1/record/kew-2500629>. MO leaves and MF seeds were obtained locally from forest reserves in Ilorin during the rainy season (March–April, 2018), and samples were authenticated at the Forest Research Institute of Nigeria, Ibadan, and assigned Voucher Number 110648. MO leaves were air-dried at room temperature (25°C–30°C) for 2 weeks in the Department of Chemistry, University of Ilorin, Nigeria, in order to prevent thermal degradation of the phytochemical constituents of the plant material. The dried plant materials were pulverized, and 2 kg of the dried plant material was extracted with 70% ethanol for 72 h. 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; 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 fifty-five eluents of 200 mL each. The eluents were pooled based on the color of the eluents and the solvents that eluted them to give a total of seven combined fractions (MOF₁–MOF₇). The fraction MOF₆, which had the highest yield and whose antioxidant, cytoprotective, and neuroregenerative potentials we previously established,^[8] was used in the present study to evaluate the pro-fertility potentials of MO leaves.

Extraction of TR from MF seeds was carried out at the Research Laboratory of the Department of Anatomy, University of Ilorin, Nigeria, using the modified method described by Lugemwa (2012).^[15] Thirty grams (30g ± 0.05) of powdered MF seeds were weighed onto weighing paper and transferred to the microscale round-bottomed flask, using the plastic funnel to assist in the transfer. 90 mL of tert-butyl methyl ether and three boiling chips were added to the flask, and, using the black plastic connector, the distillation column/air condenser was connected to the flask. The position of the flask was carefully adjusted so that the mixture boiled very gently. The finely divided seeds caused the mixture to bump (boil violently). Most of the liquid was allowed to filter by gravity, then, when it became slower, a pipet bulb was attached and squeezed gently to apply pressure to complete the filtration. After most of the solvent had evaporated, a sticky, yellowish solid remained as TR.

Chemicals and Reagents

SA was a product of Sigma–Aldrich Japan Co. (Tokyo, Japan). Normal saline and olive oil were obtained from the MOMROTA pharmaceutical company in Ilorin, Kwara State, Nigeria.

Animal Care, Feeding, Experimental Procedures, and Drug Administration

Sixty-five adult male Wistar rats (average weight of 200 g) were acclimatized in the animal house of the Faculty of Basic Medical Sciences of the University of Ilorin, Nigeria. The rats were randomly divided into 13 groups of 5 rats each and fed with a standard rat diet bought from Ogo-Oluwa Livestock Feed Enterprise, Ilorin, Nigeria. All rats were provided with water ad libitum. MOF₆ and SA were dissolved in normal saline, while TR was dissolved in olive oil. Normal saline, doses of SA, and doses of plant extracts were orally administered to rats, and the doses were determined from a previous study by Akinlolu *et al.*, 2020,^[2] which evaluated the neuroprotective potentials of MOF₆ and TR against SA-induced neurotoxicity.

The rats were grouped as follows:

Vehicles- and SA-Treated Control Groups (1–3):

- Group 1: 1ml/kg bodyweight of normal saline for nine weeks
- Group 2: 20 mg/kg bodyweight of SA for 3 weeks and left untreated for another 6 weeks
- Group 3: 1 mL/kg bodyweight of olive oil for 9 weeks.

Extracts Ant-fertility Treatment Groups (4–7): All rats in these groups received 20 mg/kg bodyweight of SA for 3 weeks, followed by treatment with specific extract doses for another 6 weeks.

- Group 4: 20 mg/kg bodyweight of SA + 5 mg/kg bodyweight of MOF₆
- Group 5: 20 mg/kg bodyweight of SA + 7.5 mg/kg/bodyweight of MOF₆
- Group 6: 20 mg/kg bodyweight of SA + 15 mg/kg/bodyweight of TR
- Group 7: 20 mg/kg bodyweight of SA + 30 mg/kg/bodyweight of TR

Toxicological Profiling of Extracts, i.e., Extracts Only Treated Groups (8–11).

- Group 8: 5 mg/kg/bodyweight of MOF₆ for 6 weeks
- Group 9: 7.5 mg/kg/bodyweight of MOF₆ for 6 weeks
- Group 10: 15 mg/kg/bodyweight of TR for 9 weeks
- Group 11: 30 mg/kg/bodyweight of TR for 9 weeks

Extracts from Anti-fertility Prevention Groups (12–13).

- Group 12: Rats in this group received 7.5 mg/kg bodyweight of MOF₆ for 6 weeks, followed by co-administration of 20 mg/kg bodyweight of SA and 7.5 mg/kg bodyweight of MOF₆ for 3 weeks.
- Group 13: Rats in this group received 30 mg/kg bodyweight of TR for 6 weeks, followed by co-administration of 20 mg/kg bodyweight of SA and 30 mg/kg bodyweight of TR for 3 weeks.

Animal Sacrifice

Rats of different groups were sacrificed by cervical dislocation at the end of the three phases of the experimental procedure, for example, the 3rd week (Group 2), the 6th week (Groups 8 and 9), and the 9th week (Groups 1, 2–7 and 10–13). No form of anesthesia was used to avoid further introduction of foreign materials into the rat system and consequent enzymatic actions on the testes, and to avoid confounding factors on

sex hormones, which may be due to differential interactions between anesthetic agents and SA or plants' extracts.

Weight and Relative Weight of the Testes

The scrotal sac of each rat was opened, and the whole testis was removed, collected, and weighed to the nearest 0.1 g. The relative weight of the testis was calculated using (Weight of Testis/Final Body Weight of Rat × 100).

Histo-pathological Evaluation

The testis was fixed in 10% formal saline for at least 5 times of its volume. Tissue preparation and staining of the sections were carried out via the hematoxylin and eosin methods, as previously described by Akinlolu *et al.* (2013).^[16]

Enzyme-Linked Immunosorbent Assays (ELISA) Analyses of Sex Hormones

Serum was collected from the sacrificed rats with a 5 mL syringe via cardiac puncture. The serum was then poured into a 5 mL plain specimen bottle and placed in the centrifuging tube. It was centrifuged for 15 min at 3000 revolutions per minute. The supernatant of the serum was then decanted into another plain specimen bottle, stored in a freezer at -4°C, and thereafter assayed for concentrations of Luteinizing Hormone (LH) (ng/mL), Follicle Stimulating Hormone (FSH) (ng/mL), and Testosterone (ng/mL) in rats of the control and experimental groups using the ELISA technique as described by Akinlolu *et al.* (2021).^[1]

This ELISA assay uses the quantitative sandwich enzyme immunoassay method. Antibody specific for each of LH, FSH, and testosterone was pre-coated onto a microplate. Samples and standards were pipetted into the wells while presenting LH, FSH, or testosterone was bound by the immobilized antibody. A biotin-conjugated antibody specific for each of LH, FSH, and testosterone was added to the wells following the removal of unbound substances. Thereafter, Avidin-conjugated horseradish peroxidase was added to the wells after washing. A substrate solution was added to the wells, and color developed in proportion to the amount of each of LH, FSH, and testosterone bound in the initial step following the removal of any unbound avidin-enzyme reagent via washing. Finally, color intensity was measured, and the color development was stopped.

Evaluation of Sperm Cell Count (SCC)

Epididymal sperm was obtained by mincing the epididymis in normal saline, and filtering through a nylon mesh (80 pt. pore size). The sperm cells were counted using a hemocytometer. The number of sperm cells in five squares was counted and averaged using the modified Johnsen's Score methodology of 1970, as described by Akinlolu *et al.*, (2013).^[10]

Statistical Analyses

Statistical analyses were conducted using the 2016 Statistical Package for the Social Science software, Version 23.0. The computed data of concentrations of each biomarker were expressed as arithmetic means ± standard error of mean and

were subjected to statistical analyses using one-way analysis of variance to test for significant differences amongst Groups 1–13. A significant difference was confirmed at a 95% confidence interval with an associated P -value of < 0.05 ($P \leq 0.05$). In addition, the Tukey *post hoc* test was used for the separation of mean values amongst Groups 1–13. The statistical comparison of the concentration of each biomarker between two groups was considered significant only at $P \leq 0.05$.

RESULTS

Death

All rats in Group 14 died during the 8th–9th week of experimental procedures, while rats in Groups 1–13 survived throughout the experimental procedure.

Morphology and Relative Weight of the Testes

Morphological observations via physical examinations of the rats, scrota, and testes of all groups were normal. Similarly, the relative weights of the testes in rats from Control and Experimental Groups 1–13 at the end of the experimental procedure [Table 1] were within normal values.

Histo-pathological Evaluations

Histo-pathological evaluations of the testes showed exposure-dependent degenerative changes such as increased interstitial spaces with irregular tubular outlines, distorted seminiferous tubules, hemorrhage between the seminiferous tubules, gradual obliteration of the lumen, and luminal sloughing of cells with loss of spermatogenic cells in Group 2 [Figure 1b and c] compared with normal testicular histology in Groups 4–7 [Figure 1a and 1d-g].

SCC in Rats (Million Cells per mL)

Results showed statistically significant ($P \leq 0.05$) decreased SCC (million cells per mL) in Group 2 compared with normal

SCC in Groups 1, 4, and 5 [Table 2]. Similarly, there was a statistically significant ($P \leq 0.05$) decrease in SCC in Groups 6 and 7 compared with Groups 1, 4, and 5 [Table 2].

Sera Concentrations of Sex Hormones in Rats

Results showed statistically significant ($P \leq 0.05$) decreased levels of LH (ng/mL) and testosterone (ng/mL) in Group 2 compared with Groups 1, 4, and 5 [Table 3]. Similarly, there were statistically significant ($P \leq 0.05$) decreased levels of LH (ng/mL) and testosterone (ng/mL) in Groups 6 and 7 compared with Groups 1, 4, and 5 [Table 3]. In addition, results showed normal FSH (ng/mL) levels in Groups 1–13 [Table 3]. However, there were significantly higher ($P \geq 0.05$) FSH levels in Groups 6–8 and 11 compared with Group 1.

DISCUSSION

The results of this study showed that 3 weeks of SA-induced testicular toxicity was not ameliorated by further treatments for 6 weeks with 15 or 30 mg/kg bodyweight of TR in rats of Groups 6 and 7 [Table 3]. Therefore, the poor anti-clastogenic and low pro-fertility potentials of TR against SA-induced toxicity could have been responsible for the deaths of all the rats of Group 13, which received 30 mg/kg bodyweight of TR for 6 weeks, followed by co-administration of 20 mg/kg bodyweight of SA and 30 mg/kg bodyweight of TR during the experimental procedure.

Morphological observations of the gross anatomy of rats, scrota, and testes, as well as the relative weights of the testes in Groups 1–12, were normal or within normal values. This implied that administrations of SA, MOF₆, and TR did not result in adverse effects on the morphology of the gross anatomy of rats, scrota, testes, or weight of the testes in the examined rats.

Histo-pathological evaluations of the testes showed exposure-dependent increased interstitial spaces with irregular tubular outlines, distorted seminiferous

Table 1: Weight and relative weight (g) of the testes in rats

Groups	Drugs/extracts administration	Final bodyweight (g)	Weight of testes (g)	Relative weight of testes
1	1 mg/kg bw Normal Saline for 9 weeks	275.31±17.27	11.01	4
2	20 mg/kg bw SA for 3 weeks and untreated for another 6 weeks	216.80±6.47	7.37	3.4
3	1 mg/kg bw Olive Oil for 9 weeks	268.74±3.65	13.37	4.6
4	20 mg/kg bw SA (3 weeks)+5 mg/kg bw MOF ₆ (6 weeks)	247.60±7.11	13.37	5.4
5	20 mg/kg bw SA (3 weeks) +7.5 mg/kg bw MOF ₆ (6 weeks)	254.68±2.63	16.06	4.7
6	20 mg/kg bw SA (3 weeks)+15 mg/kg bw TR (6 weeks)	207.93±3.71	12.89	6.2
7	20 mg/kg bw SA (3 weeks)+30 mg/kg bw TR (6 weeks)	221.67±8.59	10.86	4.9
8	5 mg/kg bw MOF ₆ for 6 weeks	202.24±2.73	6.88	3.4
9	7.5 mg/kg bw MOF ₆ for 6 weeks	250.54±2.96	11.52	4.6
10	15 mg/kg bw TR for 9 weeks	269.43±7.84	10.24	3.8
11	30 mg/kg bw TR for 9 weeks	234.28±9.44	11.01	4.7
12	7.5 mg/kg bw MOF ₆ for 6 weeks, followed by 20 mg/kg/bw SA+7.5 mg/kg bw MOF ₆ for 3 weeks	255.53±2.18	13.54	5.3

bw: Body weight, TR: *Trimyrustin*, SA: Sodium arsenite

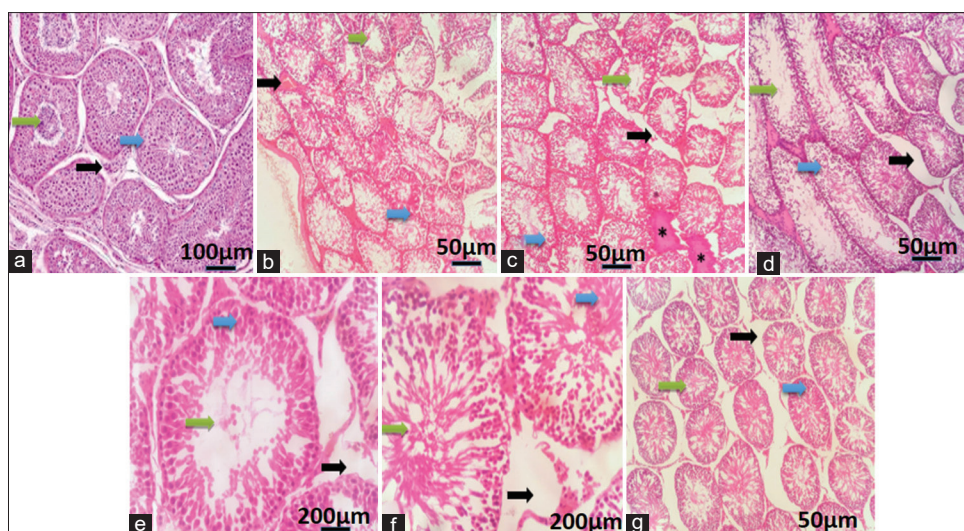


Figure 1: Photomicrograph samples of testes of rats of Control and Experimental Groups. Haematoxylin and Eosin. (a $\times 100$; Scale Bar: 100 μm). (b-d and g $\times 100$; Scale Bar: 50 μm). (e and f $\times 400$; Scale Bar: 200 μm). Green Arrow (Seminiferous Tubules), Black Arrow (Interstitial Space) and Blue Arrow (Spermatogenic Cells). (a) Control Group 1 which received physiological saline showed normal histoarchitectural components of the testes: Seminiferous tubules with abundant spermatozoa in different spermatogenic cell stages and regular interstitial tissue spaces can be observed. (b and c) Group 3 which received 20 mg/kg bodyweight of sodium arsenite (SA) only for 3 weeks and left untreated for another 6 weeks showed increased interstitial spaces, distorted seminiferous tubules, haemorrhage (asterisk) between the seminiferous tubules, gradual obliteration of the lumen and luminal sloughing of cells with loss of spermatogenic cells. (d) Group 6 which received 20 mg/kg bodyweight of SA followed by treatment with 7.5 mg/kg bodyweight of MOF_6 showed normal histoarchitectural components of the testes. (e) Group 8 which received 20 mg/kg bodyweight of SA followed by treatment with 30 mg/kg bodyweight of *Trimyristin* showed normal histoarchitectural components of the testes with minimal distortions (f) Group 5 which received 20 mg/kg bodyweight of SA followed by treatment with 5 mg/kg bodyweight of MOF_6 showed normal histoarchitectural components of the testes. (g) Group 13 which received co-administrations of 20 mg/kg bodyweight of SA and 7.5 mg/kg bodyweight of MOF_6 showed normal histoarchitectural components of the testes

Table 2: Sperm cells counts in rats (million cells per mL)

Groups of rats	Doses of drugs/extract	Mean $\pm$ SD (million cells per mL)
1	1 mg/kg bw normal saline for 9 weeks	80.0 $\pm$ 0.500
2	20 mg/kg bw SA for 3 weeks and untreated for another 6 weeks	70.0 $\pm$ 0.577 ^{a*}
3	1 mg/kg bw Olive Oil for 9 weeks	70.0 $\pm$ 0.340 ^{a+b}
4	20 mg/kg bw SA (3 weeks) + 5 mg/kg bw MOF_6 (6 weeks)	80.0 $\pm$ 0.50 ^{a+b*}
5	20 mg/kg bw SA (3 weeks) + 7.5 mg/kg bw MOF_6 (6 weeks)	80.0 $\pm$ 0.34 ^{a+b*}
6	20 mg/kg bw SA (3 weeks) + 15 mg/kg bw TR (6 weeks)	70.0 $\pm$ 0.50 ^{a+b}
7	20 mg/kg bw SA (3 weeks) + 30 mg/kg bw TR (6 weeks)	70.0 $\pm$ 0.34 ^{a+b}
8	5 mg/kg bw MOF_6 for 6 weeks	90.0 $\pm$ 0.710 ^{a+b*}
9	7.5 mg/kg bw MOF_6 for 6 weeks	80.0 $\pm$ 0.34 ^{a+b*}
10	15 mg/kg bw TR for 9 weeks	70.0 $\pm$ 0.50 ^{a+b}
11	30 mg/kg bw TR for 9 weeks	70.0 $\pm$ 0.34 ^{a+b}
12	7.5 mg/kg bw MOF_6 for 6 weeks, followed by 20 mg/kg/bw SA + 7.5 mg/kg bw MOF_6 for 3 weeks	70.0 $\pm$ 0.340 ^{a+b}

bw: Bodyweight, SA: Sodium arsenite, TR: *Trimyristin*, SEM: Standard error of mean. ^aComparison between rats of Control Group 1 and Experimental Groups 2–12. ^bComparison between rats of Experimental Group 2 and Experimental Groups 3–12. *Statistical significant difference at $P \leq 0.05$. -Statistical non-significant difference at $P \leq 0.05$

tubules, hemorrhage between the seminiferous tubules, gradual obliteration of the lumen, and luminal sloughing of cells with loss of spermatogenic cells in Group 2 [Figure 1b and c] compared with normal testicular histology in Groups 4–7 [Figure 1a and d-g].

The observed histo-architectural pathologies of the testes in Group 2 [Figure 1b and c] are in conformity with previously reported SA-induced testicular histopathology and

adverse effects of SA on male fertility in animal models.^[17,18] However, post-treatments with 5 mg bodyweight of MOF_6 [Figure 1f], 7.5 mg/kg bodyweight of MOF_6 [Figure 1d], co-administration of 20 mg/kg bodyweight of SA and 7.5 mg/kg bodyweight of MOF_6 [Figure 1g], and post-treatment with 30 mg/kg bodyweight TR [Figure 1e] prevented SA-induced histopathological distortions resulting in normal testicular histology [Figure 1a and d-g].

Table 3: Concentrations (ng/mL) of sex hormones in rats

Groups of rats	Doses of drugs/extracts	LH (ng/mL) (Mean±SEM)	FSH (ng/mL) (Mean±SEM)	Testosterone (ng/mL) (Mean±SEM)
1	1 mg/kg bw Normal Saline for 9 weeks	5.8±1.453	0.5±0.009	4.1±1.815
2	20 mg/kg bw SA for 3 weeks and untreated for another 6 weeks	1.8±0.415 ^{a*}	0.4±0.041 ^a	1.0±0.378 ^{a*}
3	1 mg/kg bw Olive Oil for 9 weeks	3.4±1.011 ^{a*b*}	0.4±0.041 ^{a-b}	1.3±0.240 ^{a*b*}
4	20 mg/kg bw SA (3 weeks)+5 mg/kg bw MOF ₆ (6 weeks)	4.5±1.842 ^{a-b*}	0.4±0.041 ^{a-b}	4.7±1.842 ^{a-b*}
5	20 mg/kg bw SA (3 weeks)+7.5 mg/kg bw MOF ₆ (6 weeks)	4.0±0.071 ^{a-b*}	0.5±0.063 ^{a-b}	4.1±0.299 ^{a-b*}
6	20 mg/kg bw SA (3 weeks)+15 mg/kg bw TR (6 weeks)	1.5±0.119 ^{a*b}	1.3±0.145 ^{a*b*}	0.5±0.046 ^{a*b*}
7	20 mg/kg bw SA (3 weeks)+30 mg/kg bw TR (6 weeks)	1.1±0.100 ^{a*b}	1.1±0.85 ^{a*b*}	0.4±0.571 ^{a*b*}
8	5 mg/kg bw MOF ₆ for 6 weeks	4.5±1.842 ^{a-b*}	0.8±0.009 ^{a-b*}	4.5±0.289 ^{a-b*}
9	7.5 mg/kg bw MOF ₆ for 6 weeks	4.0±0.071 ^{a-b*}	0.7±0.002 ^{a-b}	4.5±0.058 ^{a-b*}
10	15 mg/kg bw TR for 9 weeks	1.6±0.252 ^{a*b*}	0.7±0.087 ^{a-b}	0.7±0.104 ^{a*b}
11	30 mg/kg bw TR for 9 weeks	1.4±0.130 ^{a*b*}	0.8±0.042 ^{a-b*}	0.8±0.042 ^{a*b}
12	7.5 mg/kg bw MOF ₆ for 6 weeks, followed by 20 mg/kg/bw SA+7.5 mg/kg bw MOF ₆ for 3 weeks	2.5±0.900 ^{a*b*}	0.5±0.073 ^{a-b}	1.9±0.522 ^{a*b}

bw: Bodyweight, SA: Sodium arsenite, TR: *Trimyristin*, SEM: Standard error of mean, LH: Luteinizing hormone, FSH: Follicle stimulating hormone. ^aComparison between rats of Control Group 1 and Experimental Groups 2–13. ^bComparison between rats of Experimental Group 2 and Experimental Groups 3–12. *Statistical significant difference at $P \leq 0.05$. -Statistical non-significant difference at $P \leq 0.05$

In addition, the observed significant decrease in SCC in Groups 2, 6, and 7 compared with normal SCC in Groups 1, 4, and 5 [Table 2] implied that post-treatments of SA-induced testicular toxicity with 5 and 7.5 mg/kg bodyweight of MOF₆ prevented a decrease in SCC and improved testicular functions. Contrariwise, post-treatments of SA-induced testicular toxicity with 15 and 30 mg/kg bodyweight of TR did not prevent decreased SCC in rats of Groups 6 and 7 [Table 2].

Spermatogenesis is regulated via the release of LH and FSH by the anterior pituitary gland for the initiation and maintenance of spermatogenesis. LH stimulates Leydig cells in the testes to produce testosterone, and it is responsible for the maintenance of normal plasma testosterone concentrations.^[19] Hence, low LH levels will result in low testosterone levels. The findings of this study showed significantly decreased LH levels in rats in Groups 2, 6, and 7 compared with Groups 1, 4, and 5 [Table 3]. These observations implied that post-treatments of SA-induced testicular toxicity with 5 and 7.5 mg/kg bodyweight of MOF₆ prevented a significant reduction of LH levels in rats in Groups 4 and 5 [Table 3] and improved testicular functions. This is in concordance with the observed pro-fertility potentials of MO in animal models, which reported that MO promoted increased testosterone levels, sperm cell count, and testicular functions.^[11-14,20]

In addition, the Leydig cells are located in the interstitial spaces between seminiferous tubules.^[19] Testosterone promotes the growth and division of testicular germinal cells and also promotes the attachment of different generations of germ cells in seminiferous tubules during spermatogenesis.^[19] Low levels of intra-testicular testosterone may result in the detachment of germ cells from the seminiferous epithelium, thereby initiating apoptosis.^[19] Hence, the increased interstitial spaces and other observed histopathologies of the testes in rats of Group 2 [Figure 1b and c] were probably due to impaired function of Leydig cells and significant decreased levels of LH and testosterone [Table 3]. However, post-treatments of SA-induced testicular toxicity with 5 and 7.5 mg/kg bodyweight of MOF₆

prevented a significant reduction of testosterone levels in rats in Groups 4 and 5 [Table 3] and restored normal testosterone levels, testicular histoarchitecture, and functions.

FSH levels in rats in Control and Experimental Groups 1–13 were within normal ranges ($P \geq 0.05$) [Table 3]. These results implied that exposures of rats to 20 mg/kg bodyweight of SA only (Group 2) for 3 weeks, though they resulted in testicular histopathologies, did not result in decreased FSH levels. This is probably because decreased SCC stimulates increased FSH levels and its further binding to Sertoli cells (which envelop and nourish spermatogenic cells) to aid spermatogenesis.^[19] Hence, the upregulated levels of FSH in rats in Groups 5–12 compared with SA-only treated Group 2 were probably due to ameliorative mechanisms developed against SA-induced testicular histopathologies and decreased SCC [Figure 1a-g and Table 3].

In contrast to the pro-fertility potentials of 5 and 7.5 mg/kg bodyweight of MOF₆, post-treatments with 15 and 30 mg/kg bodyweight TR did not prevent a significant reduction of plasma levels of LH and testosterone in SA-induced testicular toxicity in rats of Groups 6 and 7 [Table 3]. This implied that the minimal distortions of the testes observed in rats treated with 30 mg/kg bodyweight of TR [Figure 1e] would have possibly retrogressed to histopathologies of the testes beyond the 9 weeks of the experimental procedure.

In addition, can prior consumption of MO leaves and MF seeds prevent the onset of arsenic-induced toxicity following exposure? Therefore, in order to answer this question, 7.5 mg/kg bodyweight of MOF₆ and 30 mg/kg bodyweight of TR were administered to rats of Groups 12 and 13, respectively, for 6 weeks, followed by co-administrations of 20 mg/kg/bodyweight of SA with 7.5 mg/kg bodyweight of MOF₆ and 30 mg/kg bodyweight of TR, respectively, for 3 weeks. Results showed a significant decrease in LH and testosterone levels in rats in Group 12 compared with Groups 1, 4, and 5 [Table 3]. These results implied that prior administration of

MOF₆ did not prevent the onset of SA-induced toxicity on the testes and sex hormones.

Is SA-induced toxicity reversible without treatment? This study attempted to examine the reversibility of SA-induced toxicity by administering 20 mg/kg bodyweight of SA to rats in Group 2 for 3 weeks, and the rats were left untreated for another 6 weeks. This becomes important because if the adverse effects of SA-induced toxicity on male fertility were naturally reversible by the body's immune and defense mechanisms, then any observed repro-protective effects of MOF₆ or TR could have been confounded by such mechanisms of reversal. However, SA-induced toxicity resulted in evident histopathology of the testes in rats in Group 2 [Figure 1b and c]. In addition, there was a significant reduction in SCC, plasma levels of LH, and testosterone in rats in Group 2 compared with Control Group 1 [Tables 2 and 3]. These results implied that the adverse effects of SA-induced toxicity on male fertility after 3 weeks of administration were not reversed, even after the rats were left for another 6 weeks without further exposure to SA. Hence, the observed repro-protective and ameliorative potentials of 5 and 7.5 mg/kg bodyweight of MOF₆ against SA-induced toxicity on the testes and sex hormones are due to the active pro-fertility constituents of MOF₆.

CONCLUSION

This study opined that the antifertility effects of SA on the testis should be of major concern to the public. In addition, post-treatments of SA-induced toxicity in rats with extract doses of MO (MOF₆) resulted in normal testicular histoarchitecture and levels of sex hormones. However, post-treatments with extract doses of TR did not ameliorate SA-induced toxicity on the testes and sex hormones. Therefore, MOF₆ possesses pro-fertility potentials, while TR does not possess pro-fertility potentials against SA-induced toxicity on the testes and sex hormones in rats.

CONFLICT OF INTEREST

The authors wish to confirm that there are no known conflicts of interest associated with this study.

FUNDING

This research did not receive any grants from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Akinlolu AA, Oyewopo AO, Kadir RE, Lawal A, Ademiloye J, Jubril A, et al. *Moringa oleifera* and *Musa sapientum* ameliorated 7,12-Dimethylbenz[a]anthracene-induced upregulations of Ki67 and Multidrug resistance1 genes in rats. *Intern J Health Sci (Qassim)* 2021;15:26-33.
2. Akinlolu AA, Ameen M, Quadri T, Odubela O, Omotoso G, Yahya R, et al. Extraction, isolation and evaluation of anti-toxic principles from *Moringa oleifera* (MOF₆) and *Myristica fragrans* (Trimyristin) upregulated Acetylcholinesterase concentrations in Sodium arsenite-induced neurotoxicity in rats. *J Phytomed Therap* 2020;19:503-19.
3. Singh AP, Goel RK, Kaur T. Mechanisms pertaining to Arsenic toxicity. *Toxicol Intern* 2011;18:87-93.
4. Hughes ME, Beck BD, Yu C, Lewis AS, Thomas DJ. Arsenic exposure and toxicology: A historical perspective. *Toxicol Sci* 2011;123:305-32.
5. Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, et al. Arsenic: toxicity, oxidative stress and human disease. *J Appl Toxicol* 2011;31:95-107.
6. Asadi N, Bahmani M, Kheradmand A, Rafieian-Kopaei M. The impact of oxidative stress on testicular function and the role of antioxidants in improving it: A review. *J Clin Diagn Res* 2017;11:IE01-5.
7. Akinlolu AA, Kasim LS, Shokunbi MT. Neurotoxic effects of the administration of artemisinin combination therapy (Artemether and Quinine) and Ascorbic acid on the cytoarchitecture of the cerebellum and trapezoid nuclei of adult male wistar rats. *Afr J Med Med Sci* 2012;41:149-55.
8. Omotoso GO, Kadir ER, Lewu SE, Gbadamosi IT, Akinlolu AA, Adunmo GO, et al. *Moringa oleifera* ameliorates cuprizone-induced cerebellar damage in adult female rats. *Res J Health Sci* 2018;6:13-25.
9. Gupta AD, Maithil N. Chemistry, antioxidant and antimicrobial potential of nutmeg (*Mristica fragrans* Houtt). *J Genet Eng Biotechnol* 2013;11:25-31.
10. Abourashed EA, El-Alfy AT. Chemical diversity and pharmacological significance of the secondary metabolites of nutmeg (*Mristica fragrans* Houtt). *Phytochem Rev* 2016;15:1035-56.
11. Abdull Razis AF, Ibrahim MD, Kntayya SB. Health benefits of *Moringa oleifera*. *Asian Pac J Cancer Prev* 2014;15:8571-6.
12. Sadek KM, Abouzed TK, Abouelkhair R, Nasr S. The chemoprophylactic efficacy of an ethanol *Moringa oleifera* leaf extract against hepatocellular carcinoma in rats. *Pharm Biol* 2017;55:1458-66.
13. Elblehi SS, El Euony OI, El-Nahas AE. Partial ameliorative effect of *Moringa* leaf ethanolic extract on the reproductive toxicity and the expression of steroidogenic genes induced by subchronic cadmium in male rats. *Environ Sci Pollut Res Int* 2019;26:23306-18.
14. Zeng B, Luo J, Wang P, Yang L, Chen T, Sun J, et al. The beneficial effects of *Moringa oleifera* leaf on reproductive performance in mice. *Food Sci Nutr* 2019;7:738-46.
15. Lugemwa FN. Extraction of betulin, trimyristin, eugenol and carnosic acid using water-organic solvent mixtures. *Molecules* 2012;17:9274-82.
16. Akinlolu AA, Ghazali OK, Lewu K, Babatope F, Uthman I, Adefule AK. Effects of amlodipine besylate on testicular histology, sperm cells count, morphology and motility in adult male wistar rats. *J Exp Clin Anat* 2013;12:75-81.
17. Baltaci BB, Uygur R, Caglar V, Aktas C, Aydin M, Ozen OA. Protective effects of quercetin against arsenic-induced damaged in rats. *Andrologia* 2016;48:1202-13.
18. Bashandy SA, El Awdan SA, Ebaid H, Alhazza IM. Antioxidant potential of *Spirulina platensis* mitigates oxidative stress and reprotoxicity induced by sodium arsenite in male rats. *Oxid Med Cell Longev* 2016;2016:7174351.
19. Guyton A, Hall J. Guyton and Hall Textbook of Medical Physiology. 11th ed. New York: Saunders; 2011. p. 974-85.
20. Stohs SJ, Hartman MJ. Review of the safety and efficacy of *Moringa oleifera*. *Phytother Res* 2015;29:796-804.