Toxicological and phytoprotective effect of *Keayodendron bridelioides* and *Monodora myristica* extracts in Wister rats

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ABSTRACT

Objectives: The potential toxicity of *Keayodendron bridelioides* (KB), *Monodora myristica* (MM) were examined, and phytoprotection of MM and KB stemming from their phytochemical contents against sodium arsenite (SA) induced clastogenicity in Wister's rat. **Materials and Methods:** Dose range studies of KB in rats, genotoxicity of MM and KB by SOS-inductive respomse were investigated using *E. coli* PQ37. Male rats were exposed to varying concentrations of MM, KB over a five week period to evaluate MM and KB phytoprotectives properties were also evaluated against sodium arsenite induced micronucleated erythrocytes, hepatotoxicity and sperm quality and morphology. **Results:** In contrast to KB, MM induced micronuclei formation in rat erythrocytes, MM and KB were however not genotoxic. MM, SA alone and in combination were hepatotoxic, characterized by elevated hepatic transaminases. Hepatoxicity were ameliorated by co-administration of KB ($P < 0.05$). MM and KB did not induce changes in semen morphology ($P > 0.05$); but decreased sperm count and motility ($P < 0.05$). Extracts exhibited anti-clastogenic (KB > MM), hepatoprotective (KB > MM) activities and maintained semen viability against SA treatment. **Conclusion:** Finding applications as herbal medicinal and food components KB and MM may be useful in mitigating the effect of toxicants in biological systems susceptible to oxidative damage.

Key words: *Keayodendron bridelioides*, *Monodora myristica* genotoxicity, micronuclei and phytoprotection

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INTRODUCTION

Diverse variety of Nigerian plants and their by-products finds wide use in Nigerian ethnobotany as food, dietary, and herbal medicinal components. Their bioactive phytochemicals and biomolecules constituents, e.g. polyphenols, carbohydrates, proteins, minerals and oils confers such utilitarian values.[1] In turn, thousands of these plant products are used daily for herbal medicinal and dietary purposes. Health management in Nigeria is presently suboptimal as a result medicinal plants find wide application in herbal remedies alongside orthodox medical practice. Unfettered herbal medical practice is prevalent and considered safe by tradition passed down over several 100 of years, without orthodox scientifical toxicological assessment and standardized procedures. Nigerian indigenous flora till date continues to contribute vastly in the delivery of health care to its populace,[2-4] irrespective of the limited knowledge on the pharmacological efficacies of most of these herbs,[5] their spectrum of activity and conceivably associated toxicities. Amongst others, *Keayodendron bridelioides* (KB) and *Monodora myristica* (MM) are commonly found in Nigerian Ethnobotany and used as herbal medicinal constituents[6] and food additives to spice up local cuisine. Both KB and MM when used as a spice in food are believed to exhibit curative effects on a wide range of ailments in addition to exhibiting aphrodisiac effect in males according to folklores.

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Although there is limited information on KB in the literature, MM had been reported to exhibit antineoplastic properties,[7] show promise as an anti-diabetic compound[8] and exhibits anti-plasmodia activity.[9] The presence of phytochemical antioxidants in KB and MM suggests that they may also be useful as chemoprotective agents in biological systems subject to oxidative stress-induced cellular damage.[10,11] Such damages induced by oxidative stress have been implicated in the etiology of a plethora of disease conditions.[12-14] Arsenic compounds are ubiquitous in nature and released into the environment by anthropogenic activities.[15] Consumption of arsenicals through contaminated water is prevalent in many areas of the world.[16] Arsenicals, like sodium arsenite (SA), are clastogen that trigger chromosomal breakage.[17] SA has been reported to compromises the integrity of the liver of mouse, rat, fish, and goat.[18-20] This study investigates the potential toxic effect of KB and MM *in rodents*. In addition, we also examined the potential chemoprotective effect of KB and MM on SA-induced toxicities using male Wister's rat as a model organism. Observation from this experiments on MM and KB, will contribute to existing knowledge in the areas of functional foods research, by identifying novel roles for existing plants products, application of their biomolecules in therapeutics and advancing health promotion as necessary dietary supplements.

MATERIALS AND METHODS

Chemicals

Sodium arsenite (98% pure, BDH, Poole, England) 2.5 mg/kg body weight (corresponding to 1/10th of the oral LD_{50} of SA was administered to the rats.[21,22] Other chemicals were of analytical grade. SOS-chromo tests kits (EBPI, Ontario, Canada).

Animals

All animals used for this study were purchased from the primate colony Department of Biochemistry, University of Ibadan, Nigeria. Animals had access to food and water *ad libitum* or as otherwise stated. The animals were humanely treated according to the guidelines in the Guideline for the Care and use of Laboratory Animals.

Plants extract

Monodora myristica and KB seeds were purchased locally and correctly identified (January, 2010) at the Department of Botany, University of Ibadan, Nigeria. Certificate of analysis was collected and stored. The seeds were air-dried at 50°C until the moisture content was low and were milled into powder for extraction: AL (650 g/L); MM (500 g/L) and KB (500 g/L) in distilled water based on their relative solubility's and left to stand for 3 days. The extracts were sterilized by filtration (Whatman No. 1 filter paper), and the insoluble materials were removed. The extracted solvent was distilled until almost dry and was weighed.

Reagent-based chemical analysis of extract

Generally, the standard protocols to identify the constituents as described[23,24] were carried out. Test for alkaloids was conducted based on the reaction with hydrogen chloride, Dragendorff's reagent, Mayer's and Wagner's reagent. Test for carbohydrate was conducted based on the reaction with H_2SO_4 and sulfonated alpha-naphthol. Tannin was determined based on the reaction with gelatin salt. Saponins were conducted based on complete hemolysis of the blood around the extract using methanol as a negative control. Flavonoid was detected based on the reaction with hydrogen chloride, methanol, and few magnesium turnings. Terpene was evaluated based on the reaction with sulfuric acid and chloroform. While Resins was conducted based on the reaction with sulfuric acid and acetic anhydride. The evaluation of Cardiac glycosides was based on the reaction with sulfuric acid, potassium hydroxide, and Fehling's solution while anthraquinones was analyzed based on the ether-chloroform maceration treatment with sodium hydroxide.

SOS-chromotest

The SOS-chromotest allows for the detection of genotoxic materials. The test was performed without metabolic activation as previously described[25] with modifications.[26,27] A vial of lyophilized *Escherichia coli* PQ-37 bacterium was activated by mixing with the SOS-chromotest growth media and incubated overnight (16 h) at 37°C. The resulting bacteria solution was visually examined for turbidity (viability) and further diluted with growth media to obtain a suspension with a final absorbance of 0.05 at OD600. MM, KB and 4-nitroquinoline oxide (4-NQO (20 μL each) was dispensed into different well in a 96-well microplate already containing 10 μL of saline. The sample was serially diluted (2 fold dilutions) to obtain five different concentrations. Subsequently, 100 μL of activated *E. coli* PQ-37 was added to each well containing e-waste samples and 4-NQO (positive control) and incubated for 2 h at 37°C. Alkaline phosphatase-(ALP) blue chromogenic substrate (100 μL) was then added to all well and incubation for another 90 min until a green color was produced followed by the addition of 50 μL of a stop solution to terminate the overall reaction. Viability of *E. coli* PQ37 and KB and MM genotoxicity was obtained spectrophotometrically at (405 nm) and (615 nm) respectively. KB and MM were classified as genotoxic only when they fulfill the following criteria: (a) Dose-response relationship of the test sample was observed; and (b) SOS Induction Factor (SOSIF), based on the number of times

the test sample O.D was higher that the negative control. A SOSIF response is considered significantly genotoxic for a test sample when (SOSIF > 1.5). SOSIF < 1.5 is classified as probably genotoxic, and SOSIF < 1 is considered nongenotoxic. The SOS induction potency (SOSIP) from the test was also used to indicate the degree of genotoxicity of a sample. The SOSIP was obtained from the linear portion of the dose-response curve of the SOS-chromotest $(OD_1 - OD_{3\,(615\,nm)})/(C_1 - C_3)$ reflecting the SOS inducing ability of KB and MM.

Keayodendron bridelioides dose range-finding studies

There was no known reference for KB-administration in the literature, necessitating a dose range-finding for KB: Male albino Wistar rats (200–250 g)-acclimatized for 1-week-were randomly divided into six groups of three rats each. Each group was administered KB (0, 50, 75, 100, 250, or 500 mg/kg) daily by gavage for 1-week. Daily observations for signs of toxicity/mortality and change in body weight were recorded. The rats were sacrificed 24-h after the last dosage of KB extract. Whole blood was collected via cardiac puncture, centrifuged at 3,000 ×g for 30 min and the serum collected for the quantification of hepatic transaminases. Harvested liver was processed for further histopathological assessment of toxicity.

Experimental grouping

Thirty male albino rats weighing 200–250 g were distributed into six groups of five each. Groups I, II, III, IV, V and VI were treated with distilled water, SA (2.5 mg/kg) only, MM (100 mg/kg) only, KB (100 mg/kg) only, SA + MM and SA + KB, respectively. SA was administered once weekly while all the extracts were administered every 3rd day via oral intubation for 5 weeks. All experimental animals had access to standard rat pellet and water *ad libitum*. The animals were injected (i.p.) with 0.04% colchicin (1 mL/kg) body weight 2 h prior to sacrifice. Twenty-four hours after the last treatment, the rats were bled by retro-orbital bleeding and sacrificed by cervical dislocation. The liver, femur, and caudal epididymis were harvested from each rat and processed for various assays.

Preparation of samples for assays

Two hours prior to sacrifice, the animals were injected (i.p.) with 0.04% colchicine (1 ml. 100 g^{-1} body weight). The rats were bled by retro-orbital bleeding and sacrificed via cervical dislocation 24 h after the last dose of $NaAsO_2$ on the 4th week. The liver, bone marrow, and epididymis were harvested for biochemical and histopathology for analysis. Serum activities of gamma glutamyl transferase (GGT), ALP, alanine amino transferase (ALT), aspartate aminotransferase (AST) were determined as guided by the procedures in the reagent's kit (Randox).

Micronucleus assay

Clastogenic effects were assessed in the rat bone marrow using the micronucleus assay as described by[28] as modified.[29] Bone marrow cells from both femurs were smeared on slides. The slides were fixed in methanol, air-dried and treated with May-Gruenwald solution and stained with Giemsa solution. The slides were scored for the presence of micronucleated polychromatic erythrocytes (mPCEs) using Nikon Epi-fluorescent microscope model E 200 (Nikon, NY, USA).

Sperm motility assay

The caudal epididymis was minced in warmed normal saline (37°C). One drop of sperm suspension was placed on a slide glass to analyze 200 motile sperm in four different fields. The motility of epididymal sperm was evaluated microscopically within 2–4 min of their isolation from the epididymis and data were expressed as percentages.

Sperm count

Epididymal sperm was obtained by mincing the epididymis in normal saline and filtering through a nylon mesh (80 pt pore size). The sperm was counted using a hemocytometer, the number of sperm in five squares were counted and an average taken following the method of Freund and Carol.[30]

Morphological abnormalities

A portion of the sperm suspension was placed on a glass slide and smeared out, fixed in 95% ethanol and stained with eosin. A total of about 400 sperm from each rat was examined for abnormalities in different regions of spermatozoa, according to the method described by Wyrobek and Bruce.[31]

Data analysis

Results are expressed as mean ± standard deviation. Differences between groups were analyzed by one-way analysis of variance with the aid of Statistical Package

Table 1: The qualitative phytochemical analysis of KB and MM

Class of natural products	MM	KB
Alkaloids	Present	Absent
Cardenolides	Present	Present
Anthraquinones	Present	Absent
Saponins	Present	Present
Tannins	Present	Present
Flavonoids	Present	Present
Carbohydrates	Present	Present
Lipids	Absent	Absent

MM=*Monodora myristica*; KB=*Keayodendron bridelioides*

for Social Sciences (SPSS) software, SPSS Inc., Chicago, USA, standard version 10.0.1. $P < 0.05$ was considered as statistically significant.

RESULTS

Phytochemicals

The phytochemical profile of water extracts of KB and MM is presented in Table 1. Indicated qualitative evidence of pharmacologically active compounds: Tannins, saponins, reducing sugars, anthraquinones, flavonoids and alkaloids present in both extracts.

Keayodendron bridelioides range-finding study

All animals treated with KB survived at the different doses they were exposed to [Table 2]. Administration of KB at different doses resulted in a decrease in overall final body weight compared to control animals. This loss of loss in weight was significant ($P < 0.05$) at the 50, 100 and 500 mg/kg body weight groups. There was an increase in final body weight at 250 mg/kg and an insignificant reduction at 75 mg/kg. However, there was no significant difference in the relative liver weight. In addition, Table 2 shows the indices of hepatic damage induced by KB. Serum markers of hepatic transaminases (ALP, GGT, ALT and AST) indicate that at very high doses KB appears to confer protection on hepatocytes as depicted by ALT and AST levels in serum. A dose-dependent increase in ALP and GGT levels up to (250 mg/kg) was observed, followed by a significant drop in the serum levels of both transaminases at doses of 500 mg/kg. We settled for 100 mg/kg KB for all other experiments.

Genotoxicity

Genetic toxicity of MM and Keayodendron bridelioides on Escherichia coli PQ37 genomic integrity

Effect of MM and KB on *E. coli* PQ37 genomic integrity is presented in Figure 1a and b. Compared to the 4-NQO (positive control) MM and KB are not genotoxic. At increasing concentration, both appear to exhibit a dose-dependent increase in SOS induction (SOSIF) approaching the threshold of 1.5. Values beyond this level (1.5) test substance are considered genotoxic.

Body, organ weights and biochemical indices of toxicity in experimental groups

An increase in final body weight of rats was observed in all the experimental groups [Table 3]. These increases in weight were most significant ($P < 0.05$) in KB only treated groups. A decrease ($P < 0.05$) in relative liver weight in the MM and MM + SA treated groups was observed when compared to control.

As expected, SA treatment significantly increased serum indicators of hepatic damage compared with the control (ALP > GGT > ALT > AST) Table 3. MM and KB (100 mg/kg) when co-administered with SA (2.5 mg/kg) for 5wk did not significantly change this trend. Notably there was a decrease in ALP levels that was more pronounced in KB + SA group. There was an

Table 2: Effect of KB treatment on Wister's rat organs and hepatic transaminases in serum in a dose range-finding

Treatment	IBW (g)	FBW (g)	RLW (g)	ALP (IU/L)	GGT (IU/L)	ALT (IU/L)	AST (IU/L)
Distilled water	225.00±5.00	233.00±1.14	2.58±0.00	195.96±0.00	2.32±0.00	8.50±0.00	49.00±0.00
KB (50 mg/kg)	241.67±4.43	200.00±0.00*	2.72±0.60	537.28±5.20*	3.86±2.91	24.50±5.29*	50.67±5.03
KB (75 mg/kg)	216.67±3.43	210.00±2.58	3.09±1.01	298.08±5.60*	3.86±3.72	20.61±3.51*	49.00±17.69
KB (100 mg/kg)	241.67±4.43	200.00±2.00*	3.14±0.69	534.52±5.57*	15.83±2.24*	13.17±4.91*	42.67±11.85*
KB (250 mg/kg)	208.33±5.43	213.37±1.14	2.96±0.51	486.68±6.67*	15.05±2.09*	10.17±6.17	46.67±8.74
KB (500 mg/kg)	225.00±0.00	207.22±2.10*	2.73±0.20	335.80±8.41*	6.17±0.22*	6.50±1.80	47.83±10.91

*Statistical significant (P<0.05) as compared with control. KB=*Keayodendron bridelioides*; IBW=Initial body weight; FBW=Final body weight; RLW=Relative liver weight; ALP=Alkaline phosphatase; GGT=Gamma glutamyl transferase; ALT=Alanine aminotransferase; AST=Aspartate aminotransferase

Table 3: Effect of extracts and SA treatment on body, organ weight and hepatic transaminases level in serum of wister rats

Treatment	IBW (g)	FBW (g)	RLW (g)	ALP (IU/L)	GGT (IU/L)	ALT (IU/L)	AST (IU/L)
Distilled water	201.00±2.81	210.00±4.50	3.02±0.53	236.81±15.18	2.55±1.90	23.12±5.90	50.00±2.97
SA (2.5 mg/kg)	205.00±3.18	215.00±5.69	2.38±0.21	731.40±5.07*	7.97±9.48*	34.40±2.38*	42.50±1.64*
MM (100 mg/kg)	235.00±5.22	240.00±2.50*	2.26±0.21*	622.38±1.97*	6.37±1.45*	33.32±5.59*	61.2±1.52*
KB (100 mg/kg)	220.00±5.18	255.00±2.92*	2.48±0.17	210.02±8.75*	3.71±2.64	20.50±1.87	31.80±3.83*
MM+SA	250.00±2.41	260.00±3.52*	2.17±0.32*	653.43±1.27*	7.72±1.73*	35.62±2.15*	70.8±2.77*
KB+SA	230.00±2.09	235.00±2.86*	2.51±0.14	402.41±1.65*	9.73±7.49*	22.20±10.22	42.80±1.33*

*Statistical significant (P<0.05) as compared with control. SA=Sodium arsenite; MM=*Monodora myristica*; KB=*Keayodendron bridelioides*; IBW=Initial body weight; FBW=Final body weight; RLW=Relative liver weight. Body weight; ALP=Alkaline phosphatase; GGT=Gamma glutamyl transferase; ALT=Alanine aminotransferase; AST=Aspartate aminotransferase

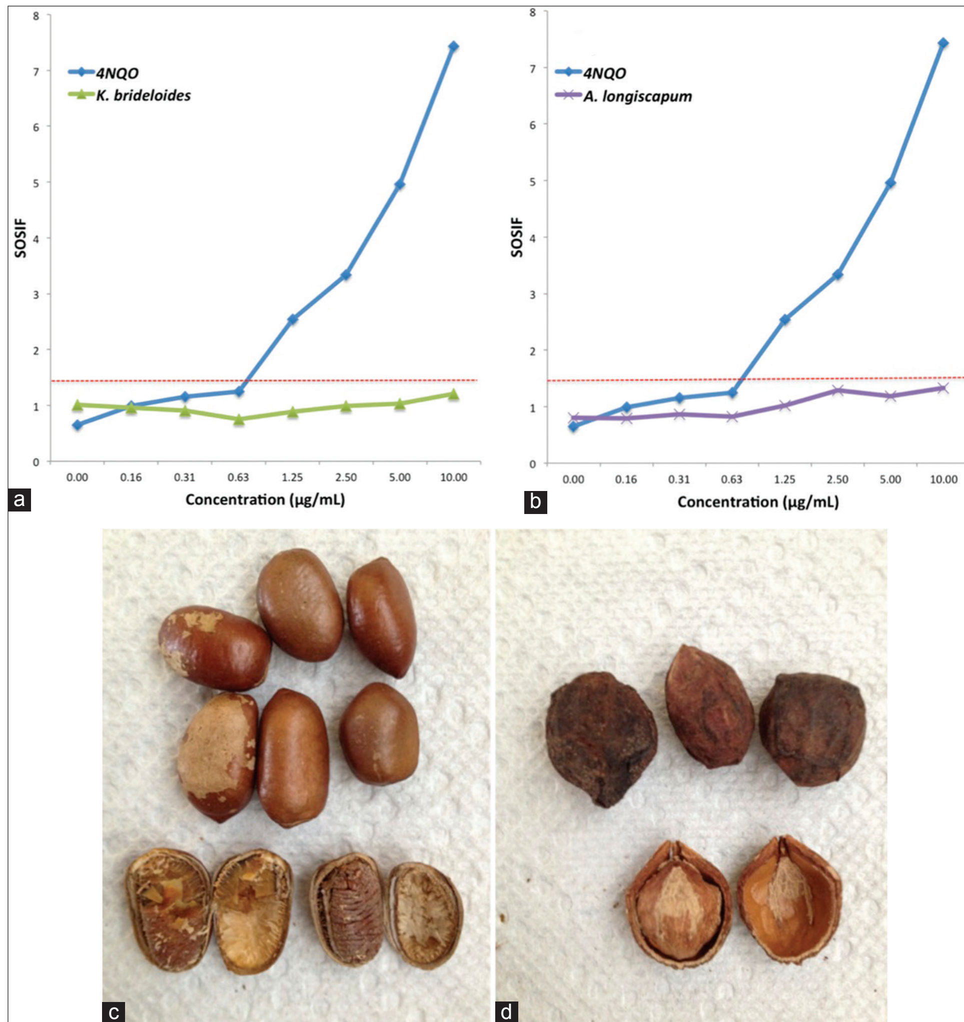

Figure 1: SOS induction factor of *Keayodendron bridelioides* (KB) and *Monodora myristica* (MM) extracts on *Escherichia coli* PQ37 bacteria-SOS-chromotest (a and b). The dotted line indicate a threshold ($T > 1.5$) beyond which sample are considered genotoxic. $T < 1.5$ are probably genotoxic and $T > 1$ are nongenotoxic. 4-nitro quinoloneoxide served as positive control. Photomicrograph of KB and MM (c and d) showing intact and open pods

Table 4: Induction of mPCEs in rat bone marrow cells after exposure to extracts and sodium arsenite

Treatment	mPCEs/1000 PCEs
Distilled water	6.20±4.32
SA	35.00±7.58*
MM	21.00±6.60*
KB	6.60±2.30
MM+SA	23.8±5.90*
KB+SA	9.60±6.02

*Statistical significant (P<0.05) as compared with control. PCEs=Polychromatic erythrocytes; mPCEs=Micronucleated polychromatic erythrocytes; SA=Sodium arsenite; MM=*Monodora myristica*; KB=*Keayodendron bridelioides*; mPCEs=Micronucleated polychromatic erythrocytes; PCEs=Polychromatic erythrocytes

approximately 3-fold increase in GGT and ALP levels in the groups treated with MM only ($P < 0.05$). KB alone significantly decreased ($P < 0.05$) the level of ALP, ALT and AST when compared with the control [Table 3].

Effect of treatment on micronucleated polychromatic erythrocyte formation

Table 4 shows mPCEs induction by different treatment. A 7-fold increase ($P < 0.05$) in mPCEs formation in the bone marrow of animals treated with SA was observed when compared with control. Similarly, MM induced a 4-fold increase in mPCEs. Co-administration of MM + SA failed to broaden the formation of mPCEs in the rat bone marrow signifying that SA and MM may not have a synergistic effect. On the other hand, KB did not induce mPCEs formation in the bone marrow of the rats ($P < 0.05$), but rather suppresses it in the presence of SA significantly ($P < 0.05$).

As depicted in Table 5, there was a reduction in the sperm count and motility in the group treated with MM when compared to the control. KB and SA when administered both alone and simultaneously reduced sperm count and motility as compared to the control. When compared with

the control, all other groups significantly ($P < 0.05$) induce morphological abnormalities in spermatozoa.

DISCUSSION

Keayodendron bridelioides and MM are a major constituent of a recreational broth "pepper soup" extensively consumed in Nigeria. KB and MM also find broad base herbal medicinal application, from malaria to a plethora of other ailments. Pepper soup is alleged to have all manner of healing properties in addition to its implied aphrodisiac potentials. Phytochemical identified in KB and MM [Table 1] is known to play important roles in medicinal plants bioactivity. Consequently, their relevance as food and herbal essences that produces physiological action can be validated for wellbeing. However, there is a need for provenance, standardization and cataloging of myriad claims attributed to such herbs and spices, both for current users and posterity.

Flavonoids exhibit antioxidant activity by scavenging or chelating process[32-34] on free radicals and metals ions, e.g. copper and iron. This can impair metal absorption from the diet useful in metallo-enzyme function. The flavonoids content of the extracts are thus health promoting[35] at least by reducing lipid peroxidation and chelating excess metals in the diet that may be toxic and deleterious. Overall, phenolic compounds are radical's terminators[36] and can help maintain a reduced cellular environment. The presence of Tannins in the extract may interfere with iron absorption and decreases the cellular iron pool, in excess free iron is implicated in the Fenton process sequel to oxidative stress. In addition, the presence of saponin in the extract has correlations in the practice of herbal medicine where saponins are used as an anti-inflammatory agent.[37] It can be surmised that both KB and MM may act as antioxidants *in vivo*.

Table 5: Effect of the extracts and sodium arsenite on the reproductive toxicity indices

Treatment	Sperm motility (%)	Sperm count	Morphological abnormalities (%)
Distilled water	90.00±0.01	126.00±8.48	9.95±0.43
SA	64.00±8.94*	90.60±1.28*	12.14±0.62*
MM	76.00±8.94*	97.60±7.91*	14.28±1.22*
KB	70.00±2.12*	98.60±3.78*	13.09±1.81*
MM+SA	76.00±1.14*	106.80±8.04*	15.22±3.34*
KB+SA	34.00±1.32*	81.00±9.11*	13.40±1.41*

*Statistical significant ($P<0.05$) as compared with control. MM+SA=*Monodora myristica*+sodium arsenite; KB+SA=*Keayodendron bridelioides*+sodium arsenite

We established a nontoxic dose from KB dose-response study due to paucity of data on KB. While there was significant body weight loss ($P < 0.05$), all rats treated with different doses of KB survived. In addition, changes in relative liver, weights were not significant compared with control [Table 2].

Keayodendron bridelioides treatment increased serum activities of hepatic transaminases-GGT, ALP, AST and ALT-dose dependently. ALP increased ($P < 0.05$) across the treated groups (from 50 to 500 mg/kg) compared with control. Increases ($P < 0.05$) in GGT activity was observed at higher doses of 250 and 500 mg/kg. ALT and AST activities showed little differences compared with the control across the treated groups. Increase in the serum amount of hepatic transaminases maybe attributed to KB hepatotoxicity at very high doses. This can occur in part due to enzyme-induction and metabolism of the extract. Histopathological examination showed no lesions in the liver of the control group [Figure 2]. However, KB treated groups with doses of 250 and 500 mg/kg presented with excessive periportal hepatic necrosis and cellular infiltration by mononuclear cells. KB (100 mg/kg) showed no toxic effect on hepatocytes when compared with the preceding groups. In addition, no visible lesions were observed with the appearance of mild sinusoidal congestion. Groups treated with (50 and 75 mg/kg) showed slight liver toxicity

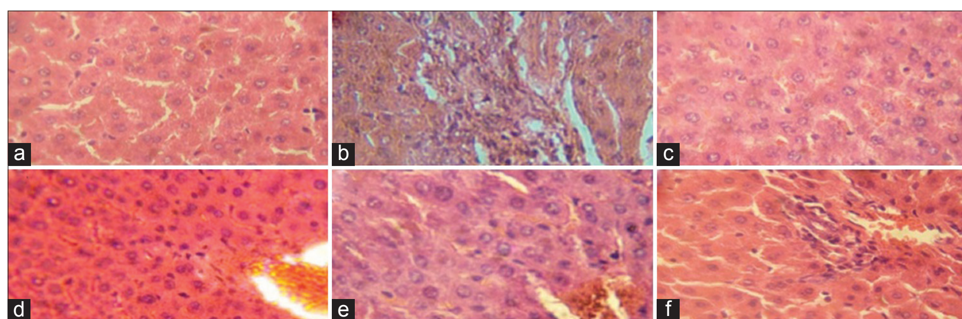

Figure 2: Liver sections of rats treated with *Monodora myristica* (MM), *Keayodendron bridelioides* (KB) and SA. (a) Control no visible lesions observed, (b) NaAsO2 (2.5 mg/kg) showing diffused degeneration of hepatocytes, portal congestion, fibroplasia and periportal cellular infiltration by mononuclear cells, (c) KB only (100 mg/kg), showing no visible lesions and (d) KB + SA (100 mg/kg and 2.5 mg/kg) showing congestion of vacuole and hepatocytes cellularity. (e) MM only (100 mg/kg) with diffuse vacuolar hepatocyte degeneration. (f) MM + SA (100 mg/kg and 2.5 mg/kg) presented with severe portal congestion and cellular infiltration of portal vessels

with high cellularity and multiple foci of hepatic necrosis observed. High cellularity can be as a result of increased cell proliferation. Consequently, a dose of 100 mg/kg body weight was chosen for all another experiment. This dose is justified as it showed the lowest form of toxicity, damage and increased cellularity in hepatocyte.

The SOS-chromotest for genotoxicity, qualitatively detects the presence of genotoxic materials in compounds or mixtures of varying type. Our findings indicate that the extract of MM and KB are not genotoxic [Figure 1a and b], providing a clue that they may confer protection from DNA damage by their phenolic components.

There was a general increase in the body weight of animals treated with KB and MM; the observed increase was highest in KB only, least in KB + SA [Table 3]. Relative liver weights were all reduced in treated groups compared to control the group. The increases in body weight could be due to androgenic modulation by the extracts since androgens possess anabolic activity.[38] Consumption of arsenicals through contaminated water is prevalent in many areas of the world. Exposure to SA can be toxic, and our results [Table 3] corroborate earlier findings in existing literature. The extracts (100 mg/kg) appear to be hepatoprotective as they decreased hepatic transaminases activities in serum to levels comparable to control rats. KB + SA treatment however increased GGT level in serum to levels higher than SA only treatment. Co-treatment with MM did not alleviate SA-induced toxicity as revealed by GGT and ALT; AST was unexplainably almost two fold higher though ALP was reduced. Increases in serum AST and ALT has been associated with hepatocellular injury and necrotic hepatocytes death[39] respectively. To buttress this observation, MM treatment alone increased ($P < 0.05$) hepatic transaminase in serum to levels higher than KB induction. Comparatively, it can be taken that to a greater extent MM is more toxic to hepatocytes that KB.

Clastogen induces chromosomal breakage simply described as the microscopically visible changes to chromosomes[40] may results in deletion, addition or rearrangement of sections of chromosomes. These changes may cause mutations that precede the onset of certain diseases. SA can induce chromosomal breakage as such can act as co-mutagen by inhibiting thiol containing enzymes,[41] e.g. DNA ligase consequential in defective DNA replication/repair and recombination.[42] SA-induced micronuclei formation [Table 4] was significantly reduced by MM and KB co-treatment. Alone, MM induced micronuclei formation significantly ($P < 0.05$) but inhibited SA-induce micronucleus. KB exhibited higher chemoprotective potency alone and against SA micronuclei induction. Making them potentially useful candidates as chemoprotective and medicinal substances in reducing radiation-induced damages worthy of future mechanistic exploration.

Compared with control treatment with extracts reduced sperm count, motility and increased morphological abnormalities in experimental animals. This is suggestive of a modulatory role on the reproductive system. In addition, treatment with SA reflected the same effect [Table 5] albeit to a greater extent decreased sperm motility but increases in count and morphological abnormalities. The volume and ratio of live/dead semen remain unchanged in all groups. MM + SA treatment significantly increased ($P < 0.05$) sperm cells abnormal morphological characteristics. In line with our finding, impairment of male rat reproductive system treated with SA and sperm count reduction[43] have been reported. MM in folk medicine is believed to be an effective antidote against "sexual impotency" amongst another ailment. This belief is conflicting with our finding and demands further investigation.

CONCLUSION

Taken together, aqueous extract of (KB and MM) mitigate the effect of SA-induced clastogenicity and are not genotoxic. To a greater extent, KB was more potent in this regard. The extracts appear to be chemoprotective with limited hepatoprotective potentials with selective elevation of hepatic transaminases. Reduction in sperm count, motility and increased sperm abnormalities raising the question as to whether KB and MM are selectively toxic to the reproductive system that warrant further investigations.

REFERENCES

1. Hill AF. Economic Botany. A Textbook of Useful Plants and Plant Products. 2nd ed. New York: McGarw-Hill Book Company Inc.; 1952.
2. Abila B, Richens A, Davies JA. Anticonvulsant effects of extracts of the west African black pepper, Piper guineense. J Ethnopharmacol 1993;39:113-7.
3. Akah PA, Nwambie AI. Evaluation of Nigerian traditional medicines: 1. Plants used for rheumatic (inflammatory) disorders. J Ethnopharmacol 1994;42:179-82.
4. Albano E. Alcohol, oxidative stress and free radical damage. Proc Nutr Soc 2006;65:278-90.
5. Gill LS. Ethnomedical Uses of Plants in Nigeria. 1st ed. Benin City, Nigeria: University of Benin Press; 1992. p. 2-15.
6. Quiroz D, Towns A, Legba SI, Swier J, Brière S, Sosef M, *et al.* Quantifying the domestic market in herbal medicine in Benin, West Africa. J Ethnopharmacol 2014;151:1100-8.
7. Bakarnga-Via I, Hzounda JB, Fokou PV, Tchokouaha LR, Gary-Bobo M, Gallud A, *et al.* Composition and cytotoxic activity of essential oils from *Xylopia aethiopica* (Dunal) A. Rich, *Xylopia parviflora* (A. Rich) Benth.) and *Monodora myristica* (Gaertn) growing in Chad and Cameroon. BMC Complement Altern Med 2014;14:125.

8. Adefegha SA, Oboh G. Inhibition of key enzymes linked to type 2 diabetes and sodium nitroprusside-induced lipid peroxidation in rat pancreas by water extractable phytochemicals from some tropical spices. Pharm Biol 2012;50:857-65.
9. Lekana-Douki JB, Bongui JB, Oyegue Liabagui SL, Zang Edou SE, Zatra R, Bisvigou U, *et al. In vitro* antiplasmodial activity and cytotoxicity of nine plants traditionally used in Gabon. J Ethnopharmacol 2011;133:1103-8.
10. Martindale JL, Holbrook NJ. Cellular response to oxidative stress: Signaling for suicide and survival. J Cell Physiol 2002;192:1-15.
11. Filomeni G, De Zio D, Cecconi F. Oxidative stress and autophagy: The clash between damage and metabolic needs. Cell Death Differ 2014;Sep 26. doi: 10.1038/cdd.2014.150. [Epub ahead of print].
12. Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: The therapeutic potential of Nrf2 activation. Mol Aspects Med 2011;32:234-46.
13. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006;52:601-23.
14. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc 1998;75:199-212.
15. Chan PC, Huff J. Arsenic carcinogenesis in animals and in humans: Mechanistic, experimental, and epidemiological evidence. Environ Carcinog Ecotoxicol Rev 1997;15:83-122.
16. Chatterjee A, Das D, Chakraborti D. A study of ground water contamination by arsenic in the residential area of Behala, Calcutta due to industrial pollution. Environ Pollut 1993;80:57-65.
17. Owumi SE, Odunola OA, Aliyu M. Co-administration of sodium arsenite and ethanol: Protection by aqueous extract of Aframomum longiscapum seeds. Pharmacognosy Res 2012;4:154-60.
18. Roy S, Roy M, Pandey PK, Tiwari SP. Effects of tissue trace minerals status and histophathological changes in chronic. Arsenicosis in goats. Vet World 2009;2:8-9.
19. Vutukuru SS, Prabhath NA, Raghavender M, Yerramilli A. Effect of arsenic and chromium on the serum amino-transferases activity in Indian major carp, *Labeo rohita*. Int J Environ Res Public Health 2007;4:224-7.
20. Sharma A, Sharma MK, Kumar M. Modulatory role of Emblica officinalis fruit extract against arsenic induced oxidative stress in Swiss albino mice. Chem Biol Interact 2009;180:20-30.
21. Escribano A, Revilla M, Hernández ER, Seco C, González-Riola J, Villa LF, *et al.* Effect of lead on bone development and bone mass: A morphometric, densitometric, and histomorphometric study in growing rats. Calcif Tissue Int 1997;60:200-3.
22. Preston RJ, Dean BJ, Galloway S, Holden H, McFee AF, Shelby M. Mammalian *in vivo* cytogenetic assays. Analysis of chromosome aberrations in bone marrow cells. Mutat Res 1987;189:157-65.
23. Trease GE, Evans WC. Trease and Evans Pharmacognosy. 16th ed. Elsevier: Saunders Ltd., U.K.; 2009. p. 616.
24. Harbourne JB. Phytochemical Methods: A Guide to Modern Technique of Plants Analysis. 2nd ed. London: Chapman and Hall; 1998. p. 302.
25. Quillardet P, Hofnung M. The SOS chromotest, a colorimetric bacterial assay for genotoxins: Procedures. Mutat Res 1985;147:65-78.
26. Kevekordes S, Mersch-Sundermann V, Burghaus CM, Spielberger J, Schmeiser HH, Arlt VM, *et al.* SOS induction of selected naturally occurring substances in *Escherichia coli* (SOS chromotest). Mutat Res 1999;445:81-91.
27. Mersch-Sundermann V, Kevekordes S, Mochayedi S. Sources of variability of the *Escherichia coli* PQ37 genotoxicity assay (SOS chromotest). Mutat Res 1991;252:51-60.
28. Heddle JA, Salmone MF. The micronucleus assay I: *In vivo*. Topics in environmental physiology and medicine. In: Stitch HF, San RHC, editors. Topics in environmental physiology and medicine. Short Test for Chemical Carcinogens. New York, Springer Verlag; 1981. p. 243-9.
29. Heddle JA, Suhharsan RA, AB. The micronucleus assay II; *In vitro*. Topics in environmental physiology and medicine. In: Stitch HF, San RHC, editors. Short Test for Chemical Carcinogens. New York, Heidelberg, Berlin: Springer-Verlag; 1981. p. 4.
30. Freund M, Carol B. Factors affecting haemocytometer counts of sperm concentration in human semen. J Reprod Fertil 1964;8:149-55.
31. Wyrobek AJ, Bruce WR. Chemical induction of sperm abnormalities in mice. Proc Natl Acad Sci U S A 1975;72:4425-9.
32. Blois MS. Antioxidant determination by the use of a stable free radical. Nature 1958;181:1199-200.
33. Cook MN, Olshan AF, Guess HA, Savitz DA, Poole C, Blatt J, *et al.* Maternal medication use and neuroblastoma in offspring. Am J Epidemiol 2004;159:721-31.
34. Kessler M, Ubeaud G, Jung L. Anti-and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol 2003;55:131-42.
35. Ferguson LR. Role of plant polyphenols in genomic stability. Mutat Res 2001;475:89-111.
36. Shahidi F, Wanasundara PK. Phenolic antioxidants. Crit Rev Food Sci Nutr 1992;32:67-103.
37. Xu R, Zhao W, Xu J, Shao B, Qin G. Studies on bioactive saponins from Chinese medicinal plants. Adv Exp Med Biol 1996;404:371-82.
38. Johnson MH, Everitt BJ. Essential Reproduction. Great Britain: Blackwell Scientific Publication; 1988.
39. Navarro VJ, Senior JR. Drug-related hepatotoxicity. N Engl J Med 2006;354:731-9.
40. Testoni MI, Bolzán AD, Bianchi MS, Bianchi NO. Effects of antioxidants on streptonigrin-induced DNA damage and clastogenesis in CHO cells. Mutat Res 1997;373:201-6.
41. Li JH, Rossman TG. Inhibition of DNA ligase activity by arsenite: A possible mechanism of its comutagenesis. Mol Toxicol 1989;2:1-9.
42. Lasko DD, Tomkinson AE, Lindahl T. Eukaryotic DNA ligases. Mutat Res 1990;236:277-87.
43. Jana K, Jana S, Samanta PK. Effects of chronic exposure to sodium arsenite on hypothalamo-pituitary-testicular activities in adult rats: Possible an estrogenic mode of action. Reprod Biol Endocrinol 2006;4:9.

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