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A preliminary study on the toxicity and novelty-induced behavioral effects of herbal medicine (*Mama Decoction*®) in rats

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Mama decoction (MD) is a commonly used formulated herbal product in Nigeria for the management of malaria; no study on its central and possible toxicological effects have been investigated, hence this study. MD was administered orally to rats at 143, 286, and 572 mg/kg daily for 30 days. Novelty-induced behavior was observed and recorded on both day 1 and day 30 of administration. Furthermore, mortality, biochemical and histopathological tests were evaluated appropriately. The animals were sacrificed on day 30 after the behavioral scoring and blood samples obtained for biochemical assays. Histopathological examinations of the liver, kidney, brain, spleen, testes and lungs were carried out. The results showed that acute oral administrations of MD had no significant effect on locomotion at all dose levels used on Day 1 while during the subchronic administration of MD, only the dose of 286 mg/kg had significant effect on locomotion. Furthermore, the grooming behavior was significantly ($p<0.01$) decreased dose-dependently. Biochemical analysis showed that sub-chronic administration of MD caused significant decrease in both triglyceride and cholesterol levels but caused a significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in the plasma. In the liver, triglyceride, cholesterol and ALT were significantly decreased while AST was significantly increased but it had no effect on the ALP. The histopathological analysis revealed that most of the organs were essentially normal. In conclusion, the study showed that oral administration of MD is relatively safe when used within the recommended maximum dose of 286 mg/kg, however, there is need for caution in using it for a long period.

Key words: *Azadirachta indica*, *Alstonia boonei*, *Morinda lucida*, *Mangifera indica,* biochemical, sub-chronic toxicity.

INTRODUCTION

Mama decoction (MD) (prepared from the mixture of leaves of *Mangifera indica, Azadirachta indica, Morinda lucida* and *Alstonia boonei)* is a herbal medicine that has been used to treat malaria traditionally in Nigeria. Since there has been no study on its central behavioral and possible toxicological effects, this study was necessary.

M. indica Linn. (Anacardiaceae, Mango tree) is a large evergreen tree, with a heavy, dome-shaped crown. Its leaf, which is used as antimicrobial in the treatment of burns, scalds, sores, wounds, abscesses and other infections in humans and animals, had been reported to contain saponins, glycosides, unsaturated sterols,

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polyphenols, euxanthin acid, mangiferine, mangin and gallo-tannins (Ngo, 2001). Mangiferin possessed antiviral (Zheng and Lu, 1990), antitumor, immunomodulatory and antiHIV (Guha et al., 1996), antidiabetic (Ichiki et al., 1998), anti-inflammatory (Garrido et la., 2004) and antioxidative activities (Martnez et al., 2000). *M. lucida* Benth (Rubiaceae) leaves are used as an ingredient of "fever teas", as antimalarial as well as febrifuge, analgesic and laxative remedies. The antiplasmodial and antibacterial activities of the plant had been associated with the anthraquinones (Sittie et al., 1999; Omar et al., 2003). *A. indica* A. Juss. (Meliaceae; Neem) is widely distributed in the tropics especially in Africa and Asia. Extracts are used traditionally to treat malaria and other illnesses in several malaria endemic countries (Soh and Benoit-Vical, 2007). The efficacy of its extracts against *Plasmodium falciparum and Plasmodium berghei* microgamete exflagellation *in vitro* is attributed to limonoids, a class of highly oxygenated terpenoids which possess insecticidal, anti-microbial, anti-inflammatory and immuno-modulatory activities (Biswas et al., 2002; Roy and Saraf, 2006; Jones et al., 1994). Several studies demonstrated that *A. indica* leaf, seed and stem bark extracts possessed *in vitro* inhibitory activity on *P. falciparum* asexual stages (Udeinya et al., 2008). Its leaves combined with those of *M. indica* is used for the treatment of malaria in Uganda (Tabuti, 2007) while in Togo, they are added to those of *Picralima nitida* and *A. boonei* for malaria therapy (Gbeassor et al., 1996). *A. boonei* De Wild (Apocynaceae) is widely distributed in Africa and used in folklore medicine as antimalaria, anti-inflammatory, analgesic and antipyretic among other uses (Olajide et al., 2000; Betti, 2004). The extract of the stem bark have also been known to possess potent neuroleptic and anxiolytic properties in behavioral studies using mice, probably due to alstonine content, its major chemical constituent (Elisabetsky and Costa-Campos, 2006). Therefore, the present study investigated the toxicity potential and behavioural effect of MD in rats.

MATERIALS AND METHODS

Reagents and materials

Aspartate, alanine transferase, triglyceride and cholesterol assay kits (RANDOX®) were purchased from Randox Laboratories Limited, Autrium, U.K. All other chemicals were of analytical grade, formalin (BDH), diethylether (BDH) and 5% alcohol (BDH).

Animals

Twelve female and twelve male albino rats, weighing between 111-121 g (3 months old) were used. The animals were procured from the animal house of Pharmacology Department, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The animals, divided into four groups (6 rats per group) of both sexes, were kept in plastic cages in the animal house with free access to both food and water. All animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication 85-23, revised 1996).

Plant collection

The leaves of *M. lucida* Benth (Rubiaceae), *A. boonei* De Wild (Apocynaceae), *M. indica* Linn (Anacardiaceae) and *A. indica* A. Juss (Meliaceae) were collected from the Obafemi Awolowo University campus and authenticated at the Botany Department herbarium, Obafemi Awolowo University, Ile-Ife where their specimens were deposited. The leaves were oven-dried at 40°C and powdered.

Preparation of Mama decoction (MD)

Each powdered leaf (25 g) was weighed respectively into a round bottom flask and the mixture boiled together with 1,750 ml of distilled water on a heating mantle for 1 h. After cooling, the decoction was filtered in order to remove the plant residues, followed by the addition of 0.2% sodium benzoate as preservative and 5% Simple Syrup B.P. as a sweetner (following the preparation method used for the commercial production of MD by the Village Chemist, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria).

Administration of Mama decoction

The doses used for this work were calculated based on the adult human dose of the decoction (300 ml of the decoction containing 17.14 g of the leaf mixture of the four plants equivalent to 286 mg/kg, taken twice daily where the average adult weight was taken as 60 kg). Thus, three dose levels (143 mg/kg (low dose), 286 mg/kg (medium dose) and 572 mg/kg (high dose) were chosen for oral administration in rats. Equivalent volume for the dose selected in each case was administered to the animals based on the body weight. The LD_{50} value of MD was earlier obtained as 3,807 mg/kg per oral in a preliminary study by using Lorke (1983) method. The first three animal groups were assigned to 143 mg/kg (low), 286 mg/kg (medium) and 572 mg/kg (high) doses, respectively while the fourth group served as the control. Freshly prepared MD was administered orally twice daily at those three different doses (143, 286 and 572 mg/kg) for 30 days, using an oral syringe, based on the animal body weights. The dose volumes were adjusted every week to compensate for changes in body weights of the animals. The control group was similarly dosed orally with distilled water twice daily. The dosing procedures continued on the four animal groups for thirty days while each animal was daily assessed for any sign of toxicity or death.

Novelty-induced behavior

Each of the 24 animals was separately put in the open field box and was scored for locomotion, rearing and grooming. The locomotion (number of floor units entered; crossed with all the paws), rearing (number of times the animal stood on its hind limbs or with the forearm against the wall of the observation box or in free air or frequency of standing on hind limbs) and grooming (the number of body cleaning with paws, picking of the body and pubis with mouth and the face washing actions). The behavioral activities of each rat were thus scored and recorded (Suarez et al., 1996; Ajayi and Ukponmwan, 1994). After an animal has been studied for its novelty-induced behavior, the box was cleaned with cotton wool, soaked with ethanol to prevent interference of any odor with the subsequent animal to be studied. The behavioral tests were

commenced on each animal after 1 h of administration of the MD on the day 1 of the experiment while the same behavioral tests were carried out on the control animals which were administered with distilled water. The behavioral testing was repeated for the entire groups on day-30 of the experiment.

Sacrificing the animals

After the novelty-induced behavior (NIB) testing on day 30, the animals were each anaesthetized with diethylether. When the animals were confirmed completely anaesthetized, they were carefully dissected. The blood for biochemical analysis was obtained using cardiac puncture technique and transferred into the pre-labeled ethylenediaminetetraacetic acid (EDTA) sample bottles which were gently rolled between the palms to allow the blood to mix thoroughly with EDTA anticoagulant. The brain, kidney, lungs, liver, spleen and testes were removed, stored in sample bottles containing formalin solution and were all preserved for histopathological analysis.

Biochemical analysis of blood samples

Collection of plasma

Heparinised blood samples were centrifuged at 5000 revolution per min for 5 mins. The supernatant layer (containing the plasma) was aspirated using a 1000 µL micropipette and transferred into a well labeled plastic container with a stopper. The entire plasma samples were kept in a freezer until ready for use. The following biochemical parameters were determined: triglyceride (TAG), total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) using a commercially available assay kits made by RANDOX Laboratory Ltd, Antrim, UK in accordance with the standard procedures (Kuete et al., 2010).

Preparation of the liver homogenates

Each liver sample was taken out of the freezer, blotted out of any blood in it and weighed using electronic balance. It was homogenized using an electronic homogenizer (Stir-R) at 1600 rpm and a 10% homogenate was prepared in 0.25 M sucrose.

Histopathology

The histopatholigical examination was carried following the standard procedures (Abdel Salam and Sleem, 2010; Soujanya et al., 2013). Tissue biopsies from the brain, kidney, liver, lungs and the testes were fixed in formalin solution and were processed with automated tissue processor (Handon citadel 2000). Sections were cut at 4 microns thick with the rotary microtome and stained with haematoxylin and eosin while additional thin sections of the kidney were cut at 3 microns and stained with periodic acid (Schiff). Histological sections were examined using Leica light microscope.

Statistical analysis

All behavioral data were analyzed by ANOVA (SAA Institute Cary, NC), post hoc tests (Student-Newman-Keuls test) were carried out to determine the source of a significant mean effect. Results are expressed as mean ± S.E.M. while p values < 0.05 were taken to indicate statistically significant differences.

RESULTS

The body weight

Sub-chronic administration of MD did not cause any significant [F(3,23)=1.525, p=0.2387; n=6 per group] change in the body weight of rats used in this study. (Control: 26.85 ± 5.59; 143 mg/kg: 22.56 ± 4.33; 286 mg/kg: 26.65 ± 3.70; 572 mg/kg:12.41 ± 7.5).

Novelty-induced behavior (open field)

The results obtained showed that there was no significant effects of MD administration on locomotion [F (3,20)=2.46, p=0.098], rearing [F(3,20)=0.70, p=0.563] and grooming [F(3,20)=0.57, p=0.640] on day 1 when compared with the control group (Table 1). However, at Day 30, after the sub-chronic administration of MD, there was a significant [F(3,20)=4.60, p=0.015] increase in locomotor activity at the medium dose level of 286 mg/kg when compared with the control whereas the other two doses did not exhibit any significant effects on locomotion. In rearing, the results showed that there was no significant difference [F(3,20)=1.24, p=0.326] due to treatment. In grooming, it was observed that there was a significant [F(3,20)=14.98, p=0.0001] dose-dependent decrease in this behavior when compared with the control (Table 2).

Biochemical analysis

Plasma

Biochemical analysis of the plasma revealed that there was significant difference in both triglyceride [F (3, 23) = 6.09, p=0.004; n=6] and cholesterol [F (3, 23) =12.94, p=0.0001; n=6] levels of treated animals only at the low dose of 143 mg/kg only when compared with the vehicle-treated rats (control). Furthermore, the results showed that other biochemical parameters such as ALT [F (3, 23) = 25.38, p=0.0001; n=6], AST [F (3, 23) = 19.58, p=0.0001; n=6] and ALT [F (3, 23) = 42.83, p=0.0001; n=6] were significantly increased in all treatment groups when compared with the control group (Table 3).

Liver

In the assay of triglyceride, one-way ANOVA indicated that a significant [F(3,23)=11.19, p=0002; n=6] decrease was produced by both 143 and 572 mg/kg dose levels with a dose-dependent significant decrease in cholesterol level that was significant [F(3,23)=140.66, p=0.0001; n=6] when compared with the control. Similarly, ALT was significantly [F(3,23)=297.69, p=0.0001; n=6] decreased in a dose-dependent manner in the treatment groups that

Table 1. Novelty-induced behavior (Day 1).

Dose (mg/kg, p.o.)	Locomotion(30 min)	Rearing(30 min)	Grooming(30 min)
Control	44.2 ± 14.0	24.5 ± 6.4	16.3 ± 5.5
143	42.5 ± 9.2	30.3 ± 13.3	15.3 ± 2.5
286	88.8 ± 17.1	36.7 ± 7.5	15.2 ± 1.7
572	60.8 ± 10.2	22.2 ± 5.9	10.4 ± 0.5

Data presented as mean ± SEM, n=4-6.

Table 2. Novelty-induced behavior (Day 30).

Dose (mg/kg, p.o.)	Locomotion(30 min)	Rearing(30 min)	Grooming(30 min)
Control	36.2 ± 7.0	25.5 ± 2.3	33.3 ± 3.1
143 mg/kg	36.8 ± 4.8	18.3 ± 4.7	17.3 ± 3.2*
286 mg/kg	97.5 ± 16.6*	40.2 ± 12.8	12.0 ± 2.4*
572 mg/kg	59.2 ± 19.0	24 .0 ± 7.1	10.4 ± 2.6*

Data arranged as mean ± SEM, n=4-6; * p<0.05 statistically significant compared to the control animals (ANOVA followed by Newman-Keuls' test).

Table 3. Effects of MD (143, 286, 572 mg/kg, p.o.) after the 30 days subchronic administration on biochemical parameters in plasma.

Group	TRG (mg/dl)	CHOL (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	153.37 ± 2.83	165.95 ± 6.23	38.37 ± 1.05	120.09 ± 1.00	122.87 ± 0.91
143 mg/kg	125.17 ± 1.92*	128.83 ± 5.73*	58.83 ± 1.85*	141.17 ± 2.20*	147.17 ± 3.42*
286 mg/kg	149.67 ± 9.41	165.83 ± 3.36	54.00 ± 1.69*	136.33 ± 3.12*	148.83 ± 1.92*
572 mg/kg	137.17 ± 2.76	156.00 ± 3.45	57.00 ± 2.52*	148.50 ± 3.75*	165.00 ± 3.46*

TRG, Triglyceride; CHOL, cholesterol, ALT, alanine aminotransferase; **AST,** aspartate transaminase and ALP, alkaline phosphatase. Values are mean ± SEM (n=6 per group), *p<0.05 compared to control animals (ANOVA followed by Newman-Keuls' test).

were administered with MD sub-chronically when compared with the vehicle-treated rats. However, the results showed that AST was significantly [F(3,23)=28.04, p=0.0001; n=6] increased in all the MD treated rats. In the assay of ALP, the significant [F(3,23)=3.47, p=0.035; n=6] difference was only noted among the treated groups but not with vehicle-treated rats (Table 4).

Histopathology

The result of the histopathology of the organs studied revealed that there was a moderately interstitial expansion of the lungs with infiltration by lymphocytes in one out of six rats [control and MD: 143, 286 and 582 mg/kg]. Similarly, there was significant expansion of the white pulp with reactive germinal center in the spleen of one out of six rats [control and MD: 143, 286 and 582 mg/kg]; while the other organs (liver, kidney, heart, brain and testes) were essentially normal in all the treated animals with MD and control.

DISCUSSION

In the present study, investigations on the potential toxicity and central nervous system effects of both acute and sub-chronic administration of MD were carried out. In our study, biomarkers were selected to include a wide range of behavioral, biochemical and histological parameters. Both acute and sub-chronic oral administration of MD in rats revealed neither mortality nor any sign of physical toxicity. The novelty–induced behavioral studies on days 1 and 30 showed that locomotion was significantly increased at the dose of 286 mg/kg, p.o. Generally, the rearing behavior was unaffected, although it was non-significantly increased at 286 mg/kg. The grooming behavior was not significantly affected during the acute administration (Day 1) but was significantly decreased dose-dependently during the sub-chronic administration. Thus, the overall results showed that MD significantly possessed central effects suggesting the need for further investigation to unravel its possible central mechanism (s) of action.

Table 4. Effects of MD (143, 286, 572 mg/kg, p.o.) after the 30 days subchronic administration on biochemical parameters in liver of rats.

Group	TRG (mg/dl)	CHOL (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	4.82 ± 0.32	11.85 ± 0.47	25.54 ± 1.07	15.65 ± 0.83	46.13 ± 2.03
143 mg/kg	3.56 ± 0.15*	4.11 ± 0.41*	4.79 ± 0.26*	28.53 ± 0.73*	41.06 ± 2.78
286 mg/kg	4.23 ± 0.30	4.41 ± 0.26*	5.15 ± 0.38*	29.80 ± 2.35*	47.65 ± 0.96
572 mg/kg	2.94 ± 0.15*	3.00 ± 0.10*	5.88 ± 0.16*	30.24 ± 0.44*	49.33 ± 1.39

TRG, Triglyceride; CHOL, cholesterol; ALT, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase. Values are mean ± SEM (n=6 per group), *p<0.05 compared to control animals (ANOVA followed by Newman-Keuls' test).

The biochemical analysis showed that all the biochemical parameters were significantly affected by the sub-chronic administration of MD as revealed in the results obtained. Both triglyceride and cholesterol levels were significantly decreased in both the plasma and liver only at 143 mg/kg (low dose level). At 572 mg/kg dose level, the two biochemical parameters were decreased in the liver, whereas at 286 mg/kg (medium dose level) no significant effect on these biochemical parameters were observed. This clearly suggests that sub-chronic administration of MD at the medium (human therapeutic) dose has no significant effect on these biochemical parameters. High cholesterol production is known to occur in the liver; other sites of high as well as in other body organs such as intestines, adrenal glands and reproductive organs (Kumar and Clark, 2005). It occurs free or as an ester which is formed with the help of an enzyme called lecithin cholesterol acyltransferase (LCAT). In a severe liver damage, the level of this enzyme reduction can lead to an increase in the concentration of cholesterol in the plasma. The implication of this is the accumulation of these lipids in the arterial walls leading to atherosclerosis due to obstruction and distortion of the arteries with serious implications related to cardiac disorders such as angina, myocardial infarction, strokes, peripheral vascular disease and hypertension (Brunzell et al., 2008). Conversely, abnormally low levels of cholesterol, known as hypocholesterolemia, have been reported to be associated with depression, cancer and cerebral hemorrhage (Lewington et al., 2007). From the results obtained in this study, the low levels of both triglyceride and cholesterol obtained are still within the normal range. Thus, MD can not cause any cardiovascular disease that may be attributed to lipid concentrations. The ALT, AST and ALP results showed that all these biochemical parameters were significantly increased dose-dependently in serum while in the liver only ALT was significantly decreased dose-dependently. There was no significant effect on ALP but AST was significantly increased in the liver. The significant increase in ALT, AST and ALP levels in plasma, following the administration of MD clearly suggested the possibility of liver or bone damage since these are diagnostic markers for the detection of possible liver or bone damage as a diagnostic tool (Attiah et al., 2013). However, further biochemical analysis of the liver did not show this pattern completely except for the increase in AST similarly observed in the liver. Slight-to-moderate elevations of ALT (usually with higher increases in AST levels) may appear in any condition that produces acute hepatocellular injury. Confirmatory procedures to ascertain the liver status were performed and it was observed that there were no significant histopathological effects on the liver. In fact, it was very clear that most of the organs were essentially normal. However, one of the major differences observed was that there was a moderately interstitial expansion with infiltration by lymphocytes in the lungs of one out of six rats [(control and MD: 143, 286 and 572 mg/kg) during the subchronic administration of MD. Similarly, there was significant expansion of the white pulp with reactive germinal center in the spleen of one out of six rats (control and MD: 143, 286 and 582 mg/kg). The remaining organs namely liver, kidney, heart, brain and testes were essentially normal in all the animals treated with MD and the control. Thus, the observed effects on the lungs and spleen could not have been due to the treatment with MD since one of the control animals also manifested those effects in its lungs and spleen. Indeed, it had earlier been reported that infiltration of lymphocytic cells into any organ usually occurred as a result of some hypersensitivity reaction, possibly due to an antigen-immune response (Cotran et al., 1999). In conclusion, therefore, MD possessed a central effect as well as with relative safety at the recommended doses but there is need for caution when used at high doses or on prolonged periods. In humans, it is normally taken twice daily at a dose of 286 mg/kg for only 2-4 days to treat malaria.

ABBREVIATIONS

NIB, Novelty-induced behavior; **TAG,** triglyceride; **AST,** aspartate aminotransferase; **ALT,** alanine aminotransferase; **ALP,** alkaline phosphatase.

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