

Mondia whitei, an African Spice Inhibits Mitochondrial Permeability Transition in Rat Liver

Olanlokun Oludele, Bakare Idris, Ofoegbu Benard, Uleh Pius, and Olorunsogo Olufunso

Laboratories for Biomembrane Research and Biotechnology, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo State 21540, Nigeria

ABSTRACT: *Mondia whitei* is an African traditional spice with aphrodisiac properties. Inhibition of mitochondrial permeability transition (mPT) pore is an important cytoprotective process essential for cell survival. In this study, the effects of methanol extract (ME), dichloromethane (DCMF), ethyl acetate (EF), and methanol fractions (MF) of *Mondia whitei* on mPT, mitochondrial ATPase, lipid peroxidation, testosterone hormone (TH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) were investigated; sperm analyses were also carried out. Male experimental rats were treated intraperitoneally with 25, 50, and 100 mg/kg bw of ME, DCMF, EF, and MF of *Mondia whitei* for two weeks. The positive and the negative controls received sildenafil citrate and the vehicle, respectively. The results showed that mPT was inhibited by MF at the highest dose. The ME, DCMF, and MF did not enhance ATPase activity. The levels of TH, FSH, and LH varied linearly with the drug dose only in EF. Malondialdehyde levels in the treated groups were significantly higher than the normal control. There were no significant defects in sperm produced by the animals in all the treated groups relative to the control. This study showed that the extract and fractions of *Mondia whitei* have cytoprotective effects and may prevent mitochondrial-mediated cell death.

Keywords: aphrodisiac, phytomedicine, cytoprotection, mitochondrial ATPase, sex hormones

INTRODUCTION

Chemoprevention and treatment of diseases by dietary supplements of plant origin have been discovered long ago. In addition to this, the use of plants and plant products as seasoning agents has long been discovered before the advent of synthetic flavours and taste enhancers. *Mondia whitei* (Hook) skeels, a perennial plant of the *Periplocaceae* family, is a multipurpose plant that is believed to serve as an appetizer, treatment of indigestion and erectile dysfunction (1). In addition to this, the androgenic effects of the aqueous and hexane extracts of this plant in male rats have been documented (2,3) while its use as spice in African dishes has also been reported (4). Dry or fresh root of *Mondia whitei*, when chewed raw, tastes bitter-sweet at first but modifies the taste buds to taste sweet or sugary overtime. It is used to season meats or stews to enhance their flavours and improve preservation (1). Mitochondria are critical to the survival of cells and the synthesis of steroid hormones (5). Because of these

reasons, mitochondrial integrity must be preserved in order to perform its synthetic and metabolic functions. Mitochondrial dysfunction can occur via the opening of the mitochondrial permeability transition (mPT) pore leading to loss of metabolic roles and ultimately leading to cell death. In spite of the widespread use of *Mondia whitei* for seasoning and aphrodisiac purposes, there is a lack of information on its cytoprotective or cytotoxic effects; therefore, the inductive or inhibitory effects of the extracts and fractions of *Mondia whitei* on mitochondrial permeability transition are the major drive of this study. Also, there is limited information on the effects of *Mondia whitei* on mitochondrial adenosine triphosphatase (mATPase) activity and its possible consequential effects on mPT induction. In this regard, we investigated in addition to basic parameters (sperm motility and androgenic effects), the induction of mPT, mATPase activity, and the generation of malondialdehyde (MDA) in adult male experimental rats treated with extracts and fractions of the African popular spice, *Mondia whitei*.

Received 14 May 2018; Accepted 12 July 2018; Published online 30 September 2018

Correspondence to Olanlokun Oludele, Tel: +234-803-801-4201, E-mail: jodel72000@yahoo.com

Copyright © 2018 by The Korean Society of Food Science and Nutrition. All rights Reserved.

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

MATERIALS AND METHODS

Materials

Luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone hormone (TH) test kits were obtained from RANDOX kits (Randox Laboratories Limited, Crumlin, Antrim, UK). Mannitol, sucrose, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), potassium hydroxide (KOH), ethylene-bis(oxyethylenetriolo)tetraacetic acid (EGTA), ethylenediaminetetraacetic acid, bovine serum albumin (BSA), calcium chloride dihydrate, sodium succinate, spermine, rotenone, Folin-Ciocalteu reagent, thiobarbituric acid, butan-1-ol, sodium dodecyl sulphate, acetic acid, Trizma[®] hydrochloride, and adenosine triphosphate (ATP) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Sodium hydroxide, sodium carbonate, copper sulphate, sodium potassium tartrate, disodium hydrogen phosphate, and tetratoxosulphate (VI) acid were purchased from BDH Chemicals Ltd. (Poole, UK). Potassium chloride, L-ascorbic acid, ammonium molybdate, and trichloroacetic acid were obtained from William Hopkins Ltd. (Birmingham, UK). Sildenafil citrate (SD) was obtained from Pfizer (Porto Salvo, Portugal). Other chemicals and reagents used were of analytical grade.

Plant source, identification, extraction, and partitioning

Mondia whitei roots were obtained from uncultivated forests in Ibadan and were identified at the Ekiti State University, Ado-Ekiti, Nigeria by Mr. F.O. Omotayo. A specimen was donated to the herbarium and a specimen voucher number (UHAE 2017/063) was assigned to the specimen. The air-dried roots of *Mondia whitei* (1.5 kg) were milled and soaked in methanol for 96 h with intermittent shaking, decanted, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator (Stuart RE300, Stuart, Stone, Staffordshire, UK) and thereafter concentrated to dryness in a water bath at room temperature. The methanol extract (ME, 750 mg) was defatted using *n*-hexane and partitioned successively with dichloromethane, ethyl acetate, and methanol using vacuum liquid chromatography, and the solvent fractions were concentrated to dryness to give dichloromethane fraction (DCMF), ethyl acetate fraction (EF), and methanol fraction (MF).

Experimental animals, grouping, treatments, and ethical considerations

All experimental procedures in this study, that require the use of experimental animals, conformed to the guidelines for research involving animals as recommended by the Declaration of Helsinki and the National Institute of Health. In addition, this study was approved by the University of Ibadan, Animal Care and Use Research Ethics

Committee and an Approval Number UI-ACUREC/17/0089 was assigned to this study. Eighty male albino rats (100±10 g) were obtained from the Animal House, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria and were acclimatized for 2 weeks before the commencement of the experiment. The animals were fed with normal rat chow (Ladokun Feeds Ltd., Ibadan, Nigeria) and clean water *ad libitum* and were assigned into 16 groups of 5 animals each. Graded doses of SD [0.035, 0.07, and 0.14 mg/kg body weight (bw)], extracts and fractions of *Mondia whitei* whole root (25, 50, and 100 mg/kg bw) were administered intraperitoneally to the animals while the negative control group received the vehicle (5% v/v dimethyl sulfoxide) once daily for 14 days after which the animals were fasted overnight before sacrifice.

Mitochondria isolation and measurement of mPT

Mitochondria were isolated according to the method of Johnson and Lardy (6). The animals were sacrificed using cervical dislocation, opened up, and the livers were excised, rinsed with isolation buffer (210 mM mannitol, 70 mM sucrose, 5 mM HEPES-KOH, and 1 mM EGTA), weighed, chopped, and homogenized in a 9% suspension of the buffer. The homogenate was loaded into the centrifuge and spanned twice at 2,300 rpm for 5 min each to sediment unbroken cells and cell debris. Mitochondria were pelleted when the supernatant was centrifuged at 13,000 rpm for 10 min. Pelleted mitochondria were washed twice with washing buffer (210 mM mannitol, 70 mM sucrose, 5 mM HEPES-KOH, and 5% BSA) at 12,000 rpm for 10 min each time. Mitochondria were thereafter re-suspended in suspension buffer (210 mM mannitol, 70 mM sucrose, and 5 mM HEPES-KOH), dispensed into Eppendorf tubes and kept on ice before use. The isolation procedure was carried out at 4°C.

The suitability of the isolated mitochondria for the permeability transition study was assessed by incubating mitochondria (1 mg/mL) in suspension buffer containing 0.8 µM rotenone for 3.5 min before the addition of 5 mM succinate and the change in absorbance was monitored for 12 min at 30 s intervals (7). Large amplitude swelling in the presence of calcium ions, was demonstrated when 12 mM calcium chloride was added 30 s prior to the addition of succinate. Mitochondria were pre-incubated in 5 mM spermine to reverse calcium induced swelling. Mitochondria isolated from the test groups having corresponding mitochondrial protein with the normal control were subjected to the mPT assay. The absorbance in each case was measured at 540 nm in 752 N UV-visible spectrophotometer.

mATPase assay

mATPase activity was assayed as described by Lardy and

Wellman (8). Mitochondria were isolated according to the procedure described for the mPT assay except that 0.25 M sucrose buffer was used for isolation. Each assay test tube contained 0.25 mM sucrose, 5 mM KCl, and 0.1 M tris-hydrochloride buffer, and the total volume was made up to 2 mL with distilled water as required. The ATP (0.01 M) was added to the designated tubes and the test tube rack was transferred into a shaker water bath. Mitochondria (0.5 mg/mL protein) were added to the zero time tubes, and the reaction was stopped immediately by adding 1 mL of 10% sodium dodecyl sulfate (SDS). A set of tubes contained only ATP without mitochondria to assess ATP hydrolysis in the absence of the enzyme while the control tubes contained mitochondria from the normal (untreated) animal incubated with ATP. The standard (uncoupler) control tubes contained other reagents and ATP. Mitochondria from the normal (untreated) animal were added to the uncoupler tubes and 2,4-dinitrophenol (25 μ M) was immediately added. Mitochondria from animals treated with the drug and the treated groups (including the negative control) were incubated with ATP. The reaction tubes were incubated for 30 min at 27°C after which the reaction was stopped by the addition of 1 mL 10% SDS sequentially, the way mitochondria were added. Ammonium molybdate (1 mL of 1.25% in 6.5% H₂SO₄ preparation) was added to 1 mL of the incubated mixture, ascorbate (1 mL of 9% preparation) was added, and the set up was left at room temperature for 30 min after which the absorbance of the blue complex was read at 660 nm. The inorganic phosphate concentration was estimated from the phosphate standard curve.

Mitochondrial lipid peroxidation

Quantitation of MDA as a product of lipid peroxidation using mitochondria as lipid-rich media was conducted according to the method of Varshney and Kale (9). Mitochondria (0.4 mL) isolated from the treated animals were mixed with 1.6 mL of 0.15 M Tris-KCl and 0.5 mL of 30% trichloroacetic acid. The test tubes were placed in a water bath and heated for 45 min at 80°C. This was then cooled to room temperature and centrifuged at 3,000 rpm for 10 min. The clear supernatant was aspirated and the absorbance was read against a reference blank of distilled water at 532 nm. MDA levels were calculated using an extinction coefficient of 0.156 μ M⁻¹·cm⁻¹ Adám-Vizi and Seregi (10).

$$\text{Lipid peroxidation (nmol MDA/mg protein)} = \frac{\text{Absorbance} \times \text{Volume of mixture}}{E_{532\text{nm}} \times \text{Volume of sample} \times \text{mg protein/mL}}$$

Hormonal analysis

Assay test kits obtained from RANDOX kits were used

for LH, TH, and FSH using standard kits reference methods and values were estimated using ROBONIK 11-2000 ELISA reader (ROBONIK, Mumbai, India).

Sperm analyses

A cell suspension was made from the excised caudal epididymis by macerating the cauda in a saline solution. The cell suspension was maintained at 37°C for 10 min before motility assessment. A 20 μ L sample of semen preparation was placed on the counting chamber, and the motile sperm was counted under a light microscope (\times 400).

Statistical analysis

Data were expressed as mean \pm standard deviation except for permeability transition assessment in which the representative curves were used out of a minimum of 4 determinations. All groups were compared by one way analysis of variance (ANOVA) and the significance of mean difference between the different groups was determined by Tukey's post hoc test and $P < 0.05$ was considered statistically significant.

RESULTS

The integrity of mitochondria isolated from animals treated with graded doses of SD, extracts and fractions of *Mondia whitei* are as presented in Fig. 1. In Fig. 1A, the data presented show that there was no permeability transition pore opening, evident by insignificant change in absorbance of mitochondria isolated from the livers of animals treated with SD, respiring on succinate in the presence of rotenone. This shows that mitochondrial swelling of the treated animals, measured as a decrease in absorbance, did not differ from the changes in the absorbance of the mitochondria isolated from normal animal using similar mitochondrial proteins. Similar results were obtained for ME, DCMF, and MF of *Mondia whitei* (Fig. 1B, 1C, and 1E).

Fig. 1B~1E show the effects of different doses of ME, DCMF, EF, and MF of *Mondia whitei* on permeability transition pore opening respectively on isolated rat liver mitochondria after the animals were treated intraperitoneally for 14 days. The results show that ME, DCMF, and MF have similar effects on mitochondrial swelling because they inhibited mitochondrial permeability transition in a dose dependent manner. In order of potency, the MF had the highest inhibitory potential at the highest dose.

The EF of *Mondia whitei* induced opening of the mitochondrial pore in a dose-dependent fashion because there was large amplitude swelling by this fraction at the highest dose (Fig. 1D).

The androgenic effects of SD, ME, DCMF, EF, and MF of *Mondia whitei* are presented in Fig. 2. The results ob-

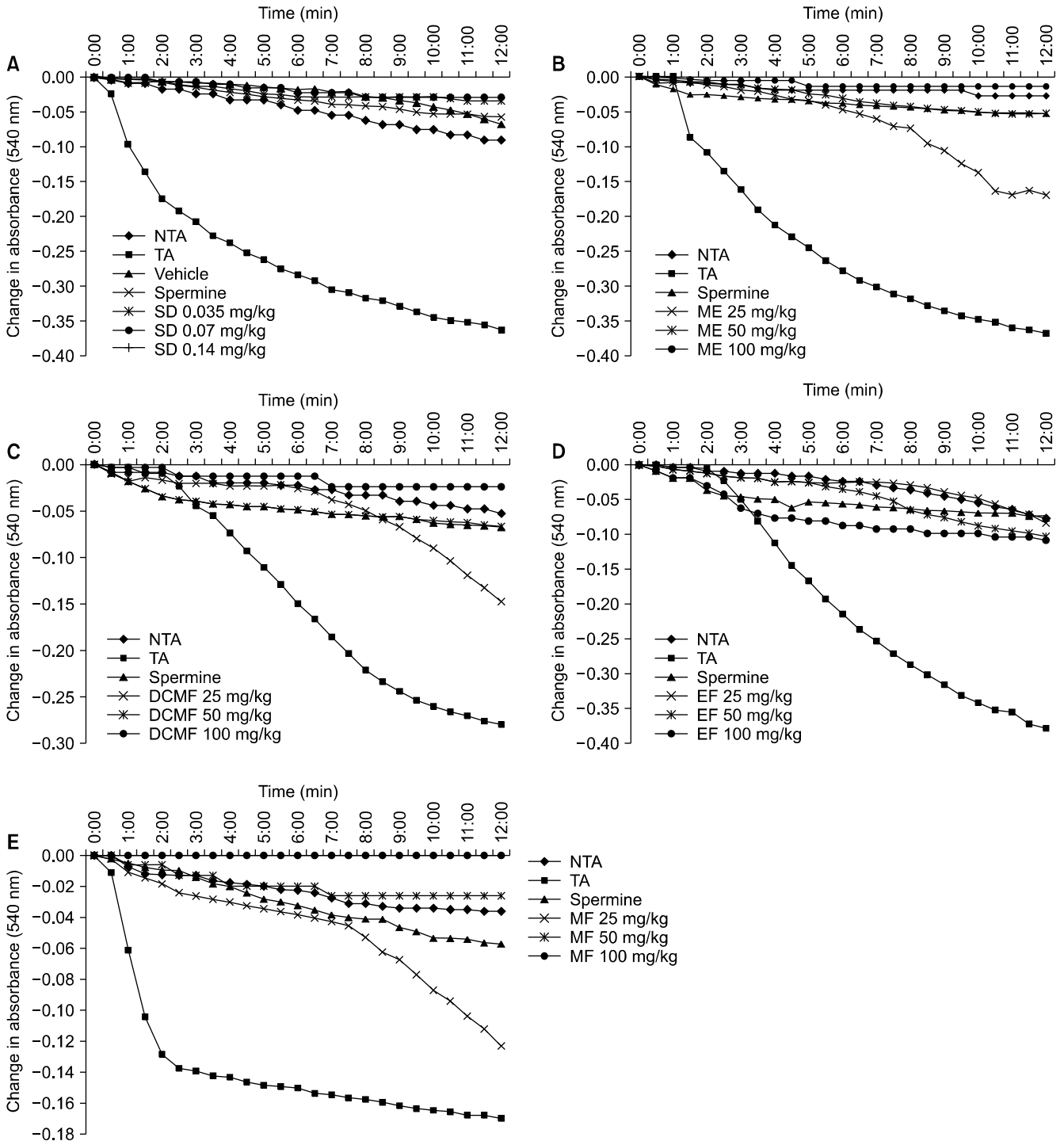


Fig. 1. Effects of varying doses of sildenafil citrate administration on mitochondrial swelling. Representative recordings of mitochondrial pore opening inhibitory effects of (A) sildenafil citrate (SD), (B) methanol extract (ME), (C) dichloromethane fraction (DCMF), (D) methanol fraction (MF), and (E) ethyl acetate fraction (EF) of *Mondia whitei* upon intraperitoneal administration of the extract for 14 days. Non-triggering agent tracing (NTA) indicates the intact mitochondria isolated from the control rat without calcium, TA (triggering agent) indicates the inductive effect of 120 μ M $CaCl_2$ on mitochondria from the control rat which was reversed with 4 mM spermine.

tained show that the intermediate dose (0.07 mg/kg bw) of SD, ME, DCMF, and MF of *Mondia whitei* had the least effect on LH. However, the effect of EF of *Mondia whitei* was dose dependent, and the highest dose (100 mg/kg bw) had the highest effect. There was no significant difference ($P > 0.05$) in the level of LH in normal animals

and the group that received vehicle only, showed that the androgenic effects of the SD, ME, DCMF, EF, and MF of *Mondia whitei* were not as a result of the vehicle administration. Similar results were obtained for FSH (Fig. 2B). However, the intermediate dose had the highest value for TH (Fig. 2C). This shows that the androgenic ef-

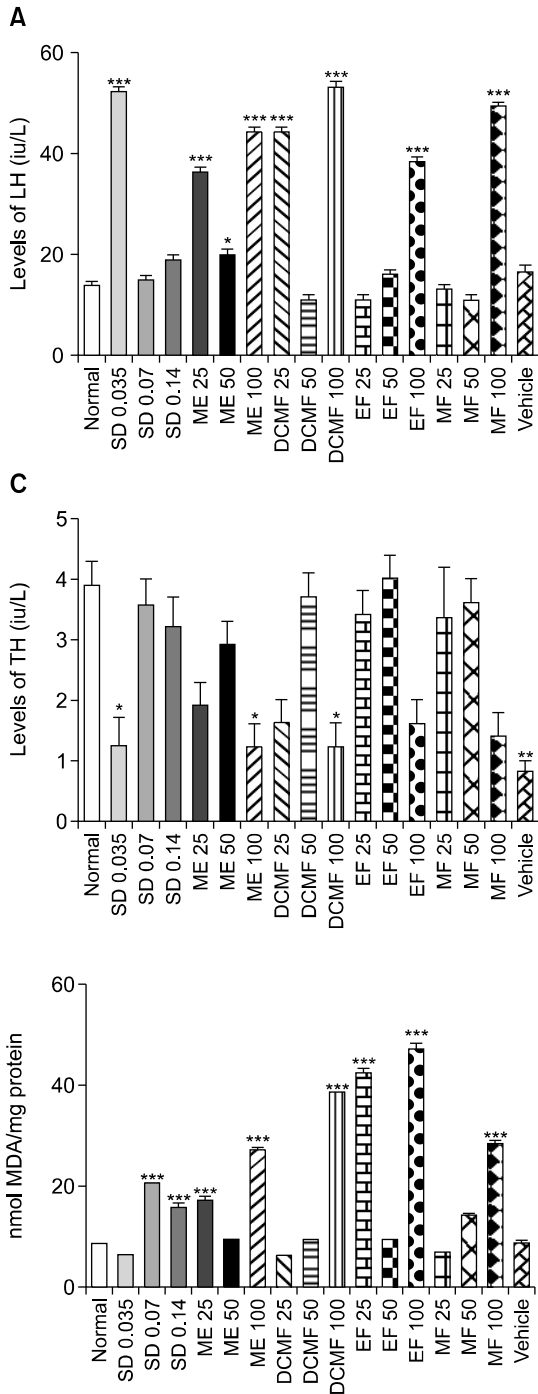


Fig. 3. Effects of extracts and fractions of *Mondia whitei* on malondialdehyde (MDA) levels as an index of lipid peroxidation using mitochondria as lipid rich media. Values are mean±standard deviation of triplicate determinations (***) P <0.01 vs normal control group). SD, sildenafil citrate; ME, methanol extract; DCMF, dichloromethane fraction; EF, ethyl acetate fraction; MF, methanol fraction.

ffects of SD, ME, DCMF, EF, and MF of *Mondia whitei* did not vary linearly with the dose administered because a further increase in the dose did not have a linear effect in increasing TH levels. The results obtained in mitochondrial lipid peroxidation are presented in Fig. 3. The levels of MDA in 100 mg/kg bw of the EF administered had the highest value of all the test samples compared

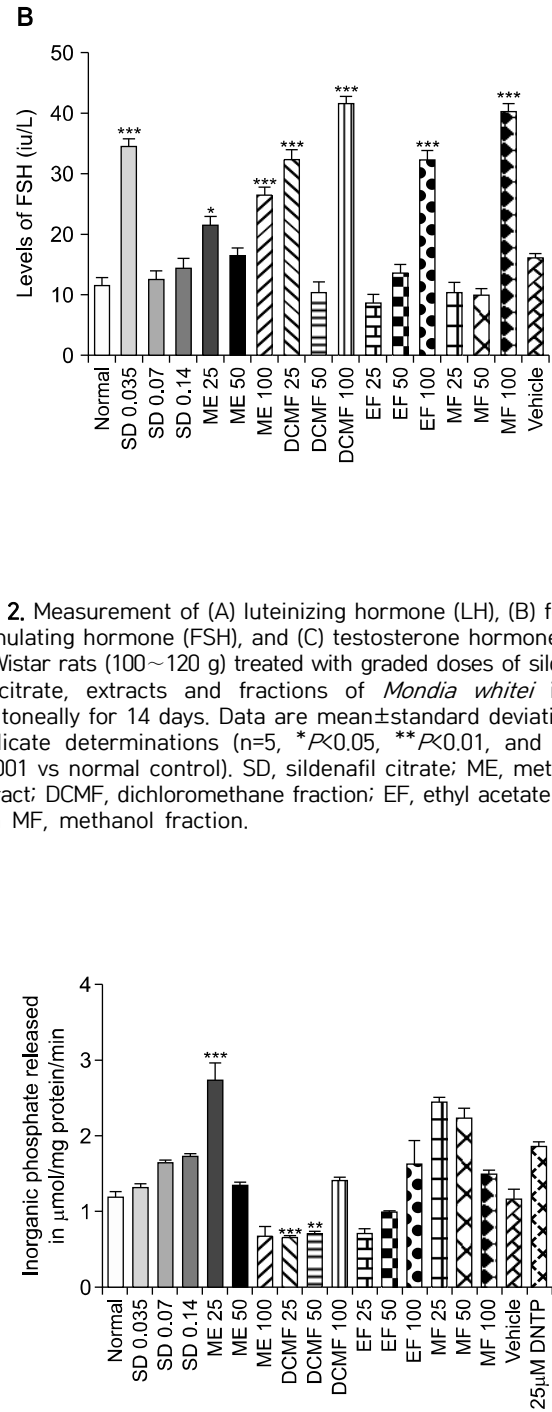


Fig. 2. Measurement of (A) luteinizing hormone (LH), (B) follicle stimulating hormone (FSH), and (C) testosterone hormone (TH) in Wistar rats (100~120 g) treated with graded doses of sildenafil citrate, extracts and fractions of *Mondia whitei* intraperitoneally for 14 days. Data are mean±standard deviation of triplicate determinations (n=5, * P <0.05, ** P <0.01, and *** P <0.001 vs normal control). SD, sildenafil citrate; ME, methanol extract; DCMF, dichloromethane fraction; EF, ethyl acetate fraction MF, methanol fraction.

Fig. 4. Effects of extracts and fractions of *Mondia whitei* on mitochondrial ATPase activity at physiological pH (7.4). Data are mean±standard deviation of triplicate determinations (n=5). The mitochondrial ATPase activities in dichloromethane fraction (DCMF) 25 and 50 mg/kg treated groups were significantly lower compared with the normal control group while in methanol extract (ME) 25 mg/kg treated group, mitochondrial ATPase activity was significantly higher compared with the normal control group. The 2,4-dinitrophenol (DNTP) is a standard uncoupler. SD, sildenafil citrate; EF, ethyl acetate fraction; MF, methanol fraction (** P <0.01 and *** P <0.001 compared with the normal control group).

with the normal control while SD generated the least MDA when compared with the other groups.

The effects of extracts and fractions of *Mondia whitei* on

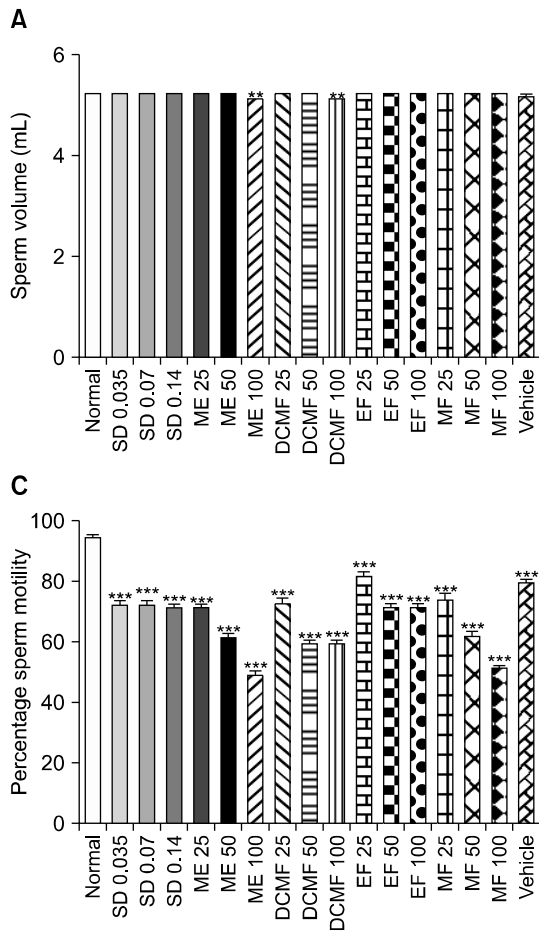


Fig. 5. Sperm analysis of animals treated with varying doses of extracts and fractions of *Mondia whitei*. There are only two groups [100 mg/kg bw of dichloromethane fraction (DCMF) and methanol extract (ME) groups] that have significant reduction in sperm volume compared with the normal control (A). Sperm count (B) and sperm motility (C) decreased significantly in dose-dependent manner when compared with the normal control. Values are mean±standard deviation of triplicate determinations. SD, sildenafil citrate; EF, ethyl acetate fraction; MF, methanol fraction (** $P < 0.01$ and *** $P < 0.001$ compared with the normal control group).

mitochondrial ATPase are presented in Fig. 4. The results obtained show that at physiological pH (7.4), the extracts and fractions have different effects; SD, DCMF, and EF of *Mondia whitei* did not enhance mitochondrial ATPase activity whereas at the lowest dose, ME enhanced ATPase activity.

The effects of treatment of rats with varying doses of extract and fractions of *Mondia whitei* on sperm analysis is presented in Fig. 5. The results obtained show that there was no significant difference in the volume of semen produced by the test groups compared with the control although they were treated with different fractions at different doses. It was the ME and DCMF (100 mg/kg bw in each case) of *Mondia whitei* that had a significant reduction ($P < 0.05$) in sperm volume compared with the control (Fig. 5A). Our results further show that sperm count (Fig. 5B) and sperm motility (Fig. 5C) decreased as the dose increased and this effect was noticed in all the treated groups.

DISCUSSION

The search for increase in venereal desire, sexual pleasure, and performance has correspondingly increased the search for orthodox and herbal aphrodisiac without side

effects. However, most drugs come with direct or secondary side effects that may cause their eventual withdrawal. Quite a number of natural substances had been known as aphrodisiac; substances such as horny goat weed (11), potency wood (12), maca (13), ginseng (14), yohimbine from yohim tree (15), and ‘Spanish fly’ (16) are all used for sexual gratification. In addition to the energy producing role of mitochondrion, an important additional function of this organelle is the control of cell death via permeability transition pathway.

The stabilisation of mitochondrial permeability transition pore when the animals were treated with ME, dichloromethane, and methanol fractions of *Mondia whitei* is suggestive of the cytoprotective effects of the plant’s solvent extracts and fractions from cell death via mitochondrial-mediated apoptosis. Triggering mitochondrial membrane permeability leads to the release of mitochondrial protein cell death promoters and loss of mitochondrial functions necessary for cell survival (17). This means that extract and fractions of *Mondia whitei* were able to cause the upregulation of antiapoptotic proteins and downregulate proapoptotic ones by preventing irreversible mitochondrial permeability. Interestingly, SD, a patented aphrodisiac drug, is found to inhibit mitochondrial permeability transition pore opening. This agrees with previous reports (18) although its mechanism of

action is not known. These results supported previous reports that prevention of mitochondrial permeability is critical to cell survival and maintenance of overall cellular function in eukaryotic cells. Our observation that the EF of *Mondia whitei* had inductive effects on mitochondrial membrane permeability showed that there are different phytochemicals in the plant that are extractable by different solvents, and these phytochemicals exert biochemical responses when administered into biological systems. This implies that while some natural compounds in *Mondia whitei* can activate mitochondrial permeability transition, some that can inhibit the opening of the permeability transition pore complex are also present in the same plant.

The mechanism by which the permeability transition pore opening can be stimulated includes response to mitochondrial calcium overload, increase in the generation of reactive oxygen species, and increase in the cytosolic inorganic phosphate (Pi) level through the activation of mATPase. mATPase is the same mitochondrial ATP synthase that performs the bifunctional role of ATP synthesis under physiological conditions and hydrolysis of ATP in its headpiece (catalytic) region in pathological conditions (19). In this study, levels of Pi generated via ATPase activity at physiological pH were found to be low. This means that there is a reduction in the level of Pi of the treated animals which is one of the inducers of mitochondrial pore opening. Hydrolysis of ATP by mATPase is one of the underlining factors that can lead to a concomitant increase in Pi concentration and eventual increase in lipid peroxidation and mPT opening (20).

Previous studies have established that in addition to TH, FSH has inhibitory effects on programmed cell death. This shows that an increase in the levels of these androgenic hormones as observed in this study is an indication of the cytoprotective effects of *Mondia whitei*. The cytoprotective effects of progesterone especially in neuronal cell death may also occur via an indirect pathway in which the hormone is converted to estradiol (21,22). Estrogen treatment increases the mitochondrial capacity for oxidative phosphorylation while simultaneously decreasing reactive oxygen species production. Specifically, progesterone inhibits mitochondrial complex I in a similar fashion to rotenone (23,24). This finding suggests that although these hormones may not be supplemented in the diet, administration of the extracts and fractions of *Mondia whitei* can elicit the upregulation of the *in vivo* biosynthesis of these hormones.

Although there was slight increase in the levels of cellular MDA, as an index of lipid peroxidation, this increase was not sufficient to cause cell death via oxidative damage. Again, the cellular increase in the levels of progesterone and TH might have mitigated the damaging potentials of peroxidative products and in turn, might have

enhanced the tolerance of mitochondria to reactive oxygen species. Interestingly, the reactive oxygen species generated as a result of the administration of these drug candidates may not have been sufficient to elicit oxidative damage.

The increase in the count of viable sperm, its motility, and volume are critical parameters for fertilization to take place (25). With reference values of ejaculate volume (>1.5 mL) and motility (>40%) required for fertilization in humans, the data presented for the treated groups is adjudged sufficient. The decrease in sperm count and motility observed in this study may be as a result of changes in the expression of cyclic guanosine monophosphate (cGMP) receptors with a concomitant changes in cGMP breakdown thus producing changes in nitric oxide (NO) production. The NO may thereafter affect the neurotransmitter activity across the brain system, which may ultimately result in a decrease in total sperm output (26). As part of its cytoprotective effect, previous works have shown that extracts of *Mondia whitei* have antioxidant properties (27,28).

Conclusively, the results obtained in this study provide useful information on the cytoprotective effects of *Mondia whitei* and comparative measures on its effect on mitochondrial ATPase activity relative to SD. The biological activities of *Mondia whitei* might be traceable to the phytochemicals it contains which may have aphrodisiac value and can also inhibit the irreversible permeability of the mitochondrial membrane. *Mondia whitei* also prevents hydrolysis of ATP thus decreasing the Pi levels that could complement the inductive effects of mitochondrial calcium level to trigger mitochondrial permeability transition. It is interesting to note that the EF caused permeability of the mitochondrial membrane and caused oxidative damage because of the high levels of MDA it generated. This means that purification of the active compounds from the plant will be of immense benefit rather than the administration of the whole extract.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Oketch-Rabah HA. 2012. *Mondia whitei*, a medicinal plant from Africa with aphrodisiac and antidepressant properties: a review. *J Diet Suppl* 9: 272-284.
2. Watcho P, Kamtchouing P, Sokeng SD, Moundipa PF, Tantchou J, Essame JL, Koueta N. 2004. Androgenic effect of *Mondia whitei* roots in male rats. *Asian J Androl* 6: 269-272.
3. Watcho P, Donfack MM, Zelefack F, Nguielefack TB, Wansi S, Nguola F, Kamtchouing P, Tsamo E, Kamanyi A. 2005. Effects of the hexane extract of *Mondia whitei* on the reproduc-

- tive organs of male rat. *Afr J Tradit Complementary Altern Med* 2: 302-311.
4. Kuete V, Krusche B, Youns M, Voukeng I, Fankam AG, Tankeo S, Lacmata S, Efferth T. 2011. Cytotoxicity of some Cameroonian spices and selected medicinal plant extracts. *J Ethnopharmacol* 134: 803-812.
 5. Miller WL. 2013. Steroid hormone synthesis in mitochondria. *Mol Cell Endocrinol* 379: 62-73.
 6. Johnson D, Lardy H. 1967. Isolation of liver or kidney mitochondria. *Methods Enzymol* 10: 94-96.
 7. Lapidus RG, Sokolove PM. 1993. Spermine inhibition of the permeability transition of isolated rat liver mitochondria: an investigation of mechanism. *Arch Biochem Biophys* 64: 246-253.
 8. Lardy HA, Wellman H. 1953. The catalytic effect of 2,4-dinitrophenol on adenosinetriphosphate hydrolysis by cell particles and soluble enzymes. *J Biol Chem* 201: 357-370.
 9. Varshney R, Kale RK. 1990. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int J Radiat Biol* 58: 733-743.
 10. Adám-Vizi V, Seregi A. 1982. Receptor independent stimulatory effect of noradrenaline on Na,K-ATPase in rat brain homogenate. Role of lipid peroxidation. *Biochem Pharmacol* 34: 2231-2236.
 11. Gauthaman K, Adaikan PG, Prasad RN. 2002. Aphrodisiac properties of *Tribulus terrestris* extract (protodioscin) in normal and castrated rats. *Life Sci* 71: 1385-1396.
 12. Waynberg J, Brewer S. 2000. Effects of Herbal vX on libido and sexual activity in premenopausal and postmenopausal women. *Adv Ther* 17: 255-262.
 13. Zheng BL, He K, Kim CH, Rogers L, Shao Y, Huang ZY, Lu Y, Yan SJ, Qien LC, Zheng QY. 2000. Effect of a lipidic extract from *Lepidium meyenii* on sexual behavior in mice and rats. *Urology* 55: 598-602.
 14. Nocerino E, Amato M, Izzo AA. 2000. The aphrodisiac and adaptogenic properties of ginseng. *Fitoterapia* 71: S1-S5.
 15. Morales A, Condra M, Owen JA, SurrIDGE DH, Fenemore J, Harris C. 1987. Is yohimbine effective in the treatment of organic impotence? Results of a controlled trial. *J Urol* 137: 1168-1172.
 16. Kotta S, Ansari SH, Ali J. 2013. Exploring scientifically proven herbal aphrodisiacs. *Pharmacogn Rev* 7: 1-10.
 17. Galluzzi L, Morselli E, Kepp O, Kroemer G. 2009. Targeting post-mitochondrial effectors of apoptosis for neuroprotection. *Biochim Biophys Acta Bioenerg* 1787: 402-413.
 18. Ascah A, Khairallah M, Daussin F, Bourcier-Lucas C, Godin R, Allen BG, Petrof BJ, Des Rosiers C, Burelle Y. 2011. Stress-induced opening of the permeability transition pore in the dystrophin-deficient heart is attenuated by acute treatment with sildenafil. *Am J Physiol Heart Circ Physiol* 300: H144-H153.
 19. Velours J, Paumard P, Soubannier V, Spannagel C, Vaillier J, Arselin G, Graves PV. 2000. Organisation of the yeast ATP synthase F₀: a study based on cysteine mutants, thiol modification and cross-linking reagents. *Biochim Biophys Acta Bioenerg* 1458: 443-456.
 20. Kowaltowski AJ, Castilho RF, Grijalba MT, Bechara EJ, Vercesi AE. 1996. Effect of inorganic phosphate concentration on the nature of inner mitochondrial membrane alterations mediated by Ca²⁺ ions: a proposed model for phosphate-stimulated lipid peroxidation. *J Biol Chem* 27: 2929-2934.
 21. Fanaei H, Karimian SM, Sadeghipour HR, Hassanzade G, Kasaean A, Attari F, Khayat S, Ramezani V, Javadimehr M. 2014. Testosterone enhances functional recovery after stroke through promotion of antioxidant defenses, BDNF levels and neurogenesis in male rats. *Brain Res* 1558: 74-83.
 22. Barreto G, Veiga S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D. 2007. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. *Eur J Neurosci* 25: 3039-3046.
 23. Fedotcheva NI, Sokolov AP, Kondrashova MN. 2006. Non-enzymatic formation of succinate in mitochondria under oxidative stress. *Free Radic Biol Med* 41: 56-64.
 24. Fedotcheva NI, Kazakov RE, Kondrashova MN, Beloborodova NV. 2008. Toxic effects of microbial phenolic acids on the functions of mitochondria. *Toxicol Lett* 180: 182-188.
 25. Vasan SS. 2011. Semen analysis and sperm function tests: how much to test?. *Indian J Urol* 27: 41-48.
 26. Ali ST, Rakkah NI. 2007. Neurophysiological role of sildenafil citrate (Viagra) on seminal parameters in diabetic males with and without neuropathy. *Pak J Pharm Sci* 20: 36-42.
 27. Aremu AO, Cheesman L, Finnie JF, Van Staden J. 2011. *Mondia whitei* (Apocynaceae): a review of its biological activities, conservation strategies and economic potential. *S Afr J Bot* 77: 960-971.
 28. Gbadamosi IT, Erinoso SM. 2015. *In vitro* antioxidant and antimicrobial activities of *Mondia whitei* (Hook. f.) skeels. *J Basic Appl Sci* 11: 428-433.