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Original Article

Safety evaluation of Bon-santé cleanser® polyherbal in male Wistar rats: Further investigations on androgenic and toxicological profile

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ABSTRACT

Background: The global increase in acceptance and use of herbal remedies in recent times is still accompanied with poor knowledge of their potential adverse effects and the toxicological implications of their use are underestimated.
Methods: Bon-santé Cleanser® (BSC), a polyherbal containing *Anogeissus leiocarpus*, *Terminalia ivorensis*, *Massularia acuminate and Macuna pruriens*, is an "energizer and hormone booster". We assessed the effect of BSC on reproductive function after administration for 60 days in male Wistar rats. Rats (150 −300 g) were assigned into four groups of 8/group. Control received distilled water (10 ml/kg) while other groups received BSC 250, 500 and 1000 mg/kg/day *p.o.* respectively. Animals were euthanized by cervical dislocation and samples collected for analysis.
Results: BSC (250 mg/kg) elevated ($p < 0.05$) follicle stimulating hormone and luteinizing hormone levels respectively. BSC decreased sperm motility and the live-dead ratio at 1000 mg/kg and reduced reproductive hormone at 500 mg/kg and 1000 mg/kg respectively. BSC at 500 mg/kg increased ($p < 0.05$, $F = 3.18–13.21$) testicular reduced glutathione level (50.3%) and catalase (43.7%) but not activities of superoxide dismutase, glutathione S-transferase, and malondialdehyde level. Further, BSC influenced Mg, Zn, Cu, P, Mn, Ni and Fe levels ($p < 0.05$). BSC (1000 mg/kg) decreased testis weight ($p < 0.05$) and induced mild inflammation characterized by atrophic tubules.
Conclusion: Overall, our data suggest BSC at low doses may increase reproductive hormones regulated by FSH and LH as observed in this study. However, BSC administration should be done with caution as it may induce reproductive toxicity in large doses.

1. Introduction

The consumer use of herbal products is a serious and growing public health problem despite the relative lack of credible scientific evidence that supports their therapeutic efficacy. The World Health Organization (WHO) has placed interest and commemorates the judicious applications of alternative and complementary medicines that might have passed or become justified for their various indications.[1] Similarly, other food and drug regulatory agencies have embraced this suggestion and ordered such for commercialization. This explains an increasing demand for pharmacologic screening and increases discussion on the safety assessment of herbal remedies. There has been convergent reasoning to provide powerful methodologies for proving efficacy, ensuring quality, standardizing good manufacturing practices, testing for safety, and conducting pharmacovigilance surveillance for adverse effects of polyherbal formulations similar to conventional drugs. This encourages such campaign for a holistic approach of the synergistic or antagonistic effects of several available polyherbal preparations.[2,3] More so,

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since every polyherbal possesses inherent toxicity,[4] the lack of a stringent and harmonized quality control and effective monitoring system predispose herbal medications to contamination or adulteration that could prove harmful to humans. Thus, consumers who have the erroneous belief that herbal products are scientifically proven to be effective or are safe because the products are natural may be at serious risks. Agents such as pesticides, toxic herbs, heavy metals and conventional drugs are commonly encountered toxicological concerns in herbal preparations.[5] Suggestions are that some biological trace elements are capable of modifying the molecular structures of subcellular constituents and membranes[6,7] or play biochemical roles that reflect their involvement in a large number of enzymes.[8] This is supported by the determination of trace elements in medicinal materials[9,10] with evidence of their involvement on the reproductive systems.[11–13] This is particularly true for polyherbal medicines in which constituents have a long traditional use but without ever having been submitted to formal tests of safety compared to conventional medicines.[14] Contrastingly, consumers have the erroneous belief that herbal products are safe because they are natural and or synonymous with their orthodox counterparts.[15,16] In addition, there are speculations that agricultural practices, industrial emissions being accounted as indirect contributors[16] aside some drugs[17] to influence their potentials. In this respect, regulatory authorities including scientific community usually scrutinize their existence.[1]

Bon-santé Cleanser® (BSC), a polyherbal formula, is manufactured by Dabiron Natural Life Care, Nigeria. BSC polyherbal remedy compositions include *Massularia acuminate (G. Don.) Bullock ex* Hoyle, Macuna *pruriens (L.,) DC*, *Anogeissus leiocarpus (DC.)*, and *Terminalia ivorensis (A. Chev.)* formulated into capsule. Interestingly, both folkloric medicine uses and several proposed properties of the potentials of the extracts of the aforementioned medicinal plantsincluding their phytoconstituents have been verified scientifically in rodents.[18–28] For instance, *M. acuminate (G. Don.) Bullock ex Hoyle*, a member of the family Rubiaceae, is used as a chewing stick and aphrodisiac by the Yorubas in southwest Nigeria and has been found to contain a thiophenolic glycoside.[19,20] It is widely used in Ayurvedic system of medicine to treat various ailments including infertility, gingivitis amongst others.[19,29] More so, preliminary studies in rats suggest that this herb can increase testosterone and sexuality.[18,20] *M. pruriens* (L.) DC is a family of *fabaceae* and forms one of the most important medicinal plants used to treat many ailments. Folklore explores and benefited from its behavioural and aphrodisiac properties.[21] Phytochemical constituents present include alkaloids, anthraquinones, saponins, phenolics, flavonoids, glycosides and tannins according to Yakubu et al[20] and Oriola et al[19] In addition, some authors have demonstrated the presence of flavones and 3,4-dihydroxyphenylalanine (L-dopa).[4,30] *A. leiocarpus and T. ivorensis* A. Chev., both *combretaceae* are used for treating worms and protozoan diseases in animals.[1,31] Also, their bark can be used as a chewing stick and their extracts have shown antibacterial, diarrhea, febrifuges, pain and aphrodisiac properties.[23,25] The *A. leiocarpus* stem barks contain tannins, astringents, castalagin[31] and flavogallonic acid dilactone[28,31] while glycosides, saponins, steroids tannins, astringents were found in *T. ivorensis*.[27] Thus, the androgenic, antipyretic, analgesic, antioxidant and anti-inflammatory potentials of these medicinal plants have been documented.[18,22–29] However, no study has yet justified the reproductive effects of the combination of these medicinal plants as found in BSC or its wholistic toxicological effects.

Recently, we assessed the safety of this preparation since it is being sold in public places and the manufacturers claimed they have many customers.[32] Further, the National Agency for Food and Drug Administration and Control enlists BSC among herbal products in Nigeria. Although, we found that BSC was relatively safe with mild alteration in the liver and heart architectures in rats,[32] but its acclaimed indications, however, cannot be juxtaposed on this fact, since no scientific investigations on efficacy have been carried out.

The present study, therefore, assessed the acclaimed hormone boosting effects of Bon-santé Cleanser® polyherbal capsule and reproductive toxicity potentials after a sixty-day sub-chronic administration in male Wistar rats. Also, we determined the influence on the levels of biological trace elements and commented on the quality assurance.

2. Materials and methods

2.1. Drugs and chemicals

The study was carried out in the Department of Pharmacology, University of Lagos, Lagos Nigeria. Bon-santé Cleanser® capsule was obtained from Dabiron Natural Life Care, Nigeria. Thiobarbituric acid (TBA), Ellman's reagent (DTNB) and 1-Chloro-2,4,-dinitrobenzene (CDBN) from Sigma (USA) were purchased from Sigma Chemical Company (USA). Reduced glutathione (GSH), Metaphosphoric acid and Trichloroacetic acid (TCA) were purchased from J.I. Baker (USA). Bovine serum albumin fraction V (BSA) was purchased from SRL, India. Rat Follicle Stimulating Hormone (FSH) (Cat. No.: Rshakrfs-010R) and Luteinizing Hormone (LH) ELISA (Rshakrlh-010SR) kits were purchased from (Biovendor, Shibayagi Co., Ltd. (Japan). RAT Testosterone (RTC001R) ELISA was obtained from Biovendor, Laboratorni, medicina a.s Karasek (Czech Republic). Sodium hydroxide was obtained from MERCK (Germany). All other chemicals and reagents used were of analytical grades. Atomic UV/Visible Spectrophotometer obtained from JENWAY, Bibby Scientific (Model 7300 and 7305) (USA).

2.2. Extraction and preparation of the final polyherbal formulation

BSC was obtained directly from the Dabiron Natural Life Care in Nigeria. It was assigned Batch number 002 and listed with number A7-5321L by the National Agency for Food and Drug Administration and Control (NAFDAC). BSC contains *A. leiocarpus (DC., family Combretaceae) Guill & Perr.*, *T. ivorensis (A. Chev., family Combretaceae)*, *M. acuminate (G.Don, family Rubiaceae) Bullock ex Hoyle and Macuna pruriens (L., family leguminosae) DC.*in the ratio 4:2:1:1 respectively. The extraction and formulation procedures complied with the regulatory manual of NAFDAC. In this context, a single capsule of BSC (total content 442 mg) was prepared as previously reported[32] and administered via oral gavage according to standard toxicological guidelines.

2.3. Animals

Albino rats of the Wistar strain weighing between 150 and 300 g were purchased from the animal house of the Redeemers University, Ogun State, Nigeria. The rats were housed under controlled conditions in the experimental animal handling facility of the College of Medicine, University of Lagos, Nigeria. The experimental animal room had a 12 h light/12 h dark schedule and maintained at a temperature of 22 ± 3 °C throughout the study. Animals were fed with commercially available rat pelleted diet (Ladoke Akintola Growers Mash, Nigeria) and were allowed access to water *ad libitum* throughout the period of the experiment. The experimental protocols were approved by the Institutional Animal Care and Use Committee, Department of Pharmacology, Therapeutic and Toxicology, College of Medicine, University of Lagos. Animals were certified fit for the experiment by the Institution's Animal Health Officers before the commencement of the study. Beddings were

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