ABOUT AJB

The African Journal of Biotechnology (AJB) (ISSN 1684-5315) is published weekly (one volume per year) by Academic Journals.

African Journal of Biotechnology (AJB), a new broad-based journal, is an open access journal that was founded on two key tenets: To publish the most exciting research in all areas of applied biochemistry, industrial microbiology, molecular biology, genomics and proteomics, food and agricultural technologies, and metabolic engineering. Secondly, to provide the most rapid turn-around time possible for reviewing and publishing, and to disseminate the articles freely for teaching and reference purposes. All articles published in AJB are peer-reviewed.

Contact Us

Editorial Office: ajb@academicjournals.org
Help Desk: helpdesk@academicjournals.org
Website: http://www.academicjournals.org/journal/AJB
Submit manuscript online http://ms.academicjournals.me/
Editorial Board

Prof. Sagadevan G. Mundree
Department of Molecular and Cell Biology
University of Cape Town
Private Bag Rondebosch 7701
South Africa

Dr. Martin Fregene
Centro Internacional de Agricultura Tropical (CIAT)
Km 17 Cali-Palmira Recta
AA6713, Cali, Colombia

Prof. O. A. Ogunseitan
Laboratory for Molecular Ecology
Department of Environmental Analysis and Design
University of California,
Irvine, CA 92697-7070. USA

Dr. Ibrahima Ndoye
UCAD, Faculte des Sciences et Techniques
Departement de Biologie Vegetale
BP 5005, Dakar, Senegal.
Laboratoire Commun de Microbiologie
IRD/ISRA/UCAD
BP 1386, Dakar

Dr. Bamidele A. Iwalokun
Biochemistry Department
Lagos State University
P.M.B. 1087. Apapa – Lagos, Nigeria

Dr. Jacob Hodeba Mignouna
Associate Professor, Biotechnology
Virginia State University
Agricultural Research Station Box 9061
Petersburg, VA 23806, USA

Dr. Bright Ogheneovo Agindotan
Plant, Soil and Entomological Sciences Dept
University of Idaho, Moscow
ID 83843, USA

Dr. A.P. Njukeng
Département de Biologie Végétale
Faculté des Sciences
B.P. 67 Dschang
Université de Dschang
Rep. du CAMEROUN

Dr. E. Olatunde Farombi
Drug Metabolism and Toxicology Unit
Department of Biochemistry
University of Ibadan, Ibadan, Nigeria

Dr. Stephen Bakiamoh
Michigan Biotechnology Institute International
3900 Collins Road
Lansing, MI 48909, USA

Dr. N. A. Amusa
Institute of Agricultural Research and Training
Obafemi Awolowo University
Moor Plantation, P.M.B 5029, Ibadan, Nigeria

Dr. Desouky Abd-El-Haleem
Environmental Biotechnology Department &
Bioprocess Development Department,
Genetic Engineering and Biotechnology Research
Institute (GEBRI),
Mubarak City for Scientific Research and Technology
Applications,
New Burg-Elarab City, Alexandria, Egypt.

Dr. Simeon Oلون Kotchoni
Department of Plant Molecular Biology
Institute of Botany, Kirschallee 1,
University of Bonn, D-53115 Germany.

Dr. Eriola Betiku
German Research Centre for Biotechnology,
Biochemical Engineering Division,
Mascheroder Weg 1, D-38124,
Braunschweig, Germany

Dr. Daniel Masiga
International Centre of Insect Physiology and Ecology,
Nairobi,
Kenya

Dr. Essam A. Zaki
Genetic Engineering and Biotechnology Research
Institute, GEBRI,
Research Area,
Borg El Arab, Post Code 21934, Alexandria
Egypt
Dr. Alfred Dixon  
*International Institute of Tropical Agriculture (IITA)*  
PMB 5320, Ibadan  
Oyo State, Nigeria

Dr. Sankale Shompole  
Dept. of Microbiology, Molecular Biology and Biochemistry,  
University of Idaho, Moscow,  
ID 83844, USA

Dr. Mathew M. Abang  
Germlasm Program  
International Center for Agricultural Research in the Dry Areas  
(ICARDA)  
P.O. Box 5466, Aleppo, SYRIA.

Dr. Solomon Olawale Odemuyiwa  
Pulmonary Research Group  
Department of Medicine  
550 Heritage Medical Research Centre  
University of Alberta  
Edmonton  
Canada T6G 2S2

Prof. Anna-Maria Botha-Oberholster  
Plant Molecular Genetics  
Department of Genetics  
Forestry and Agricultural Biotechnology Institute  
Faculty of Agricultural and Natural Sciences  
University of Pretoria  
ZA-0002 Pretoria, South Africa

Dr. O. U. Ezeronye  
Department of Biological Science  
Michael Okpara University of Agriculture  
Umudike, Abia State, Nigeria.

Dr. Joseph Hounhouigan  
Maitre de Conférence  
Sciences et technologies des aliments  
Faculté des Sciences Agronomiques  
Université d’Abomey-Calavi  
01 BP 526 Cotonou  
République du Bénin

Prof. Christine Rey  
Dept. of Molecular and Cell Biology,  
University of the Witwatersand,  
Private Bag 3, WITS 2050, Johannesburg, South Africa

Dr. Kamel Ahmed Abd-Elsalam  
Molecular Markers Lab. (MML)  
Plant Pathology Research Institute (PPathRI)  
Agricultural Research Center, 9-Gamma St., Orman,  
12619,  
Giza, Egypt

Dr. Jones Lemchi  
International Institute of Tropical Agriculture (IITA)  
Onne, Nigeria

Prof. Greg Blatch  
Head of Biochemistry & Senior Wellcome Trust Fellow  
Department of Biochemistry, Microbiology & Biotechnology  
Rhodes University  
Grahamstown 6140  
South Africa

Dr. Beatrice Kilel  
P.O Box 1413  
Manassas, VA 20108  
USA

Dr. Jackie Hughes  
Research-for-Development  
International Institute of Tropical Agriculture (IITA)  
Ibadan, Nigeria

Dr. Robert L. Brown  
Southern Regional Research Center,  
U.S. Department of Agriculture,  
Agricultural Research Service,  
New Orleans, LA 70179.

Dr. Deborah Rayfield  
Physiology and Anatomy  
Bowie State University  
Department of Natural Sciences  
Crawford Building, Room 003C  
Bowie MD 20715, USA
Dr. Geremew Bultosa  
Department of Food Science and Post harvest Technology  
Haramaya University  
Personal Box 22, Haramaya University Campus  
Dire Dawa, Ethiopia

Dr. José Eduardo Garcia  
Londrina State University  
Brazil

Prof. Nirbhay Kumar  
Malaria Research Institute  
Department of Molecular Microbiology and Immunology  
Johns Hopkins Bloomberg School of Public Health  
E5144, 615 N. Wolfe Street  
Baltimore, MD 21205

Prof. M. A. Awal  
Department of Anatomy and Histplogy, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Prof. Christian Zwieb  
Department of Molecular Biology  
University of Texas Health Science Center at Tyler  
11937 US Highway 271  
Tyler, Texas 75708-3154  
USA

Prof. Danilo López-Hernández  
Instituto de Zoología Tropical, Facultad de Ciencias, Universidad Central de Venezuela. Institute of Research for the Development (IRD), Montpellier, France

Prof. Donald Arthur Cowan  
Department of Biotechnology, University of the Western Cape Bellville 7535 Cape Town, South Africa

Dr. Ekhaise Osaro Frederick  
University Of Benin, Faculty of Life Science  
Department of Microbiology  
P. M. B. 1154, Benin City, Edo State, Nigeria.

Dr. Luisa Maria de Sousa Mesquita Pereira  
IPATIMUP R. Dr. Roberto Frias, s/n 4200-465 Porto  
Portugal

Dr. Min Lin  
Animal Diseases Research Institute  
Canadian Food Inspection Agency  
Ottawa, Ontario, Canada K2H 8P9

Prof. Nobuyoshi Shimizu  
Department of Molecular Biology, Center for Genomic Medicine  
Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku  
Tokyo 160-8582, Japan

Dr. Adewunmi Babatunde Idowu  
Department of Biological Sciences  
University of Agriculture Abia  
Abia State, Nigeria

Dr. Yifan Dai  
Associate Director of Research  
Revivicor Inc.  
100 Technology Drive, Suite 414  
Pittsburgh, PA 15219  
USA

Dr. Zhongming Zhao  
Department of Psychiatry, PO Box 980126, Virginia Commonwealth University School of Medicine, Richmond, VA 23298-0126, USA

Prof. Giuseppe Novelli  
Human Genetics, Department of Biopathology, Tor Vergata University, Rome, Italy

Dr. Moji Mohammadi  
402-28 Upper Canada Drive  
Toronto, ON, M2P 1R9 (416) 512-7795  
Canada
Prof. Jean-Marc Sabatier
Directeur de Recherche Laboratoire ERT
Ingénierie des Peptides à Visée Thérapeutique,
Université de la Méditerranée-Ambrilia Biopharma
Inc.,
Faculté de Médecine Nord, Bd Pierre Dramard, 13916,
Marseille cédex 20.
France

Dr. Fabian Hoti
PneumoCarr Project
Department of Vaccines
National Public Health Institute
Finland

Prof. Irina-Draga Caruntu
Department of Histology
Gr. T. Popa University of Medicine and Pharmacy
16, Universitatii Street, Iasi,
Romania

Dr. Dieudonné Nwaga
Soil Microbiology Laboratory,
Biotechnology Center. PO Box 812,
Plant Biology Department,
University of Yaoundé I, Yaoundé,
Cameroon

Dr. Gerardo Armando Aguado-Santacruz
Biotechnology CINVESTAV-Unidad Irapuato
Departamento Biotecnologia
Km 9.6 Libramiento norte Carretera Irapuato-León
Irapuato,
Guanajuato 36500
Mexico

Dr. Abdolkaim H. Chehregani
Department of Biology
Faculty of Science
Bu-Ali Sina University
Hamedan,
Iran

Dr. Abir Adel Saad
Molecular oncology
Department of Biotechnology
Institute of graduate Studies and Research
Alexandria University,
Egypt

Dr. Azizul Baten
Department of Statistics
Shah Jalal University of Science and Technology
Sylhet-3114,
Bangladesh

Dr. Bayden R. Wood
Australian Synchrotron Program
Research Fellow and Monash Synchrotron
Research Fellow Centre for Biospectroscopy
School of Chemistry Monash University Wellington Rd.
Clayton,
3800 Victoria,
Australia

Dr. G. Reza Balali
Molecular Mycology and Plant Pathology
Department of Biology
University of Isfahan
Isfahan
Iran

Dr. Beatrice Kilel
P.O Box 1413
Manassas, VA 20108
USA

Prof. H. Sunny Sun
Institute of Molecular Medicine
National Cheng Kung University Medical College
1 University road Tainan 70101,
Taiwan

Prof. Ima Nirwana Soelaiman
Department of Pharmacology
Faculty of Medicine
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur,
Malaysia

Prof. Tunde Ogunsanwo
Faculty of Science,
Olabisi Onabanjo University,
Agboowo.
Nigeria

Dr. Evans C. Egwim
Federal Polytechnic,
Bida Science Laboratory Technology Department,
PMB 55, Bida, Niger State,
Nigeria
Prof. George N. Goulielmos  
Medical School,  
University of Crete  
Voutes, 715 00 Heraklion, Crete,  
Greece

Dr. Uttam Krishna  
Cadila Pharmaceuticals limited ,  
India 1389, Tarsad Road,  
Dholka, Dist: Ahmedabad, Gujarat,  
India

Prof. Mohamed Attia El Tayeb Ibrahim  
Botany Department, Faculty of Science at Qena,  
South Valley University, Qena 83523,  
Egypt

Dr. Nelson K. Ojijo Olang’o  
Department of Food Science & Technology,  
JKUAT P. O. Box 62000, 00200, Nairobi,  
Kenya

Dr. Pablo Marco Veras Peixoto  
University of New York NYU College of Dentistry  
345 E. 24th Street, New York, NY 10010  
USA

Prof. T E Cloete  
University of Pretoria Department of Microbiology and  
Plant Pathology,  
University of Pretoria,  
Pretoria,  
South Africa

Prof. Djamel Saidi  
Laboratoire de Physiologie de la Nutrition et de Sécurité  
Alimentaire Département de Biologie,  
Faculté des Sciences,  
Université d’Oran, 31000 - Algérie  
Algeria

Dr. Tomohide Uno  
Department of Biofunctional chemistry,  
Faculty of Agriculture Nada-ku,  
Kobe., Hyogo, 657-8501,  
Japan

Dr. Ulises Urzúa  
Faculty of Medicine,  
University of Chile Independencia 1027, Santiago,  
Chile

Dr. Aritua Valentine  
National Agricultural Biotechnology Center, Kawanda  
Agricultural Research Institute (KARI)  
P.O. Box, 7065, Kampala,  
Uganda

Prof. Yee-Joo Tan  
Institute of Molecular and Cell Biology 61 Biopolis Drive,  
Proteos, Singapore 138673  
Singapore

Prof. Viroj Wiwanitkit  
Department of Laboratory Medicine,  
Faculty of Medicine, Chulalongkorn University,  
Bangkok  
Thailand

Dr. Thomas Silou  
Universit of Brazzaville BP 389  
Congo

Prof. Burtram Clinton Fielding  
University of the Western Cape  
Western Cape,  
South Africa

Dr. Brnčić (Brncic) Mladen  
Faculty of Food Technology and Biotechnology,  
Pierottijeva 6,  
10000 Zagreb,  
Croatia.

Dr. Meltem Sesli  
College of Tobacco Expertise,  
Turkish Republic, Celal Bayar University 45210,  
Akhisar, Manisa,  
Turkey.

Dr. Idress Hamad Attitalla  
Omar El Mukhtar University,  
Faculty of Science,  
Botany Department,  
El-Beida, Libya.

Dr. Linga R. Gutha  
Washington State University at Prosser,  
24106 N Bunn Road,  
Prosser WA 99350-8694
Dr Helal Ragab Moussa  
Bahnay, Al-bagour, Menoufia, Egypt.

Dr VIPUL GOHEL  
DuPont Industrial Biosciences  
Danisco (India) Pvt Ltd  
5th Floor, Block 4B, DLF Corporate Park  
DLF Phase III  
Gurgaon 122 002  
Haryana (INDIA)

Dr. Sang-Han Lee  
Department of Food Science & Biotechnology, Kyungpook National University  
Daegu 702-701, Korea.

Dr. Bhaskar Dutta  
DoD Biotechnology High Performance Computing Software Applications Institute (BHSAI)  
U.S. Army Medical Research and Materiel Command  
2405 Whittier Drive  
Frederick, MD 21702

Dr. Muhammad Akram  
Faculty of Eastern Medicine and Surgery, Hamdard Al-Majeed College of Eastern Medicine, Hamdard University, Karachi.

Dr. M. Muruganandam  
Department of Biotechnology  
St. Michael College of Engineering & Technology, Kalayarkoil, India.

Dr. Gökhan Aydin  
Suleyman Demirel University, Atabey Vocational School, Isparta-Türkiye,

Dr. Rajib Roychowdhury  
Centre for Biotechnology (CBT), Visva Bharati, West-Bengal, India.

Dr Takuji Ohyama  
Faculty of Agriculture, Niigata University

Dr Mehdi Vasfi Marandi  
University of Tehran

Dr Fügen DURLU-ÖZKAYA  
Gazi University, Tourism Faculty, Dept. of Gastronomy and Culinary Art

Dr. Reza Yari  
Islamic Azad University, Boroujerd Branch

Dr Zahra Tahmasebi Fard  
Roudehen branche, Islamic Azad University

Dr Albert Magri  
Giro Technological Centre

Dr Ping ZHENG  
Zhejiang University, Hangzhou, China

Dr. Kgomotso P. Sibeko  
University of Pretoria

Dr Greg Spear  
Rush University Medical Center

Prof. Pilar Morata  
University of Malaga

Dr Jian Wu  
Harbin medical university , China

Dr Hsiu-Chi Cheng  
National Cheng Kung University and Hospital.

Prof. Pavel Kalac  
University of South Bohemia, Czech Republic

Dr Kürsat Korkmaz  
Ordu University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition

Dr. Shuyang Yu  
Department of Microbiology, University of Iowa  
Address: 51 newton road, 3-730B BSB bldg. Iowa City, IA, 52246, USA
Dr. Mousavi Khaneghah  
*College of Applied Science and Technology-Applied Food Science, Tehran, Iran.*

Dr. Qing Zhou  
*Department of Biochemistry and Molecular Biology, Oregon Health and Sciences University Portland.*

Dr Legesse Adane Bahiru  
*Department of Chemistry, Jimma University, Ethiopia.*

Dr James John  
*School Of Life Sciences, Pondicherry University, Kalapet, Pondicherry*
ARTICLES

Acute and sub-chronic toxicity study of the extract and powder of Operculina macrocarpa (L.) Urb. in mice
Carpejane Ferreira da Silva, Ana Célia Rodrigues Athayde, Marcílio Fontes Cezar, Onaldo Guedes Rodrigues, Sergio Santos Azevedo, José Galberto Martins da Costa and Fabrício Kleber de Lucena Carvalho

Ex vivo trypanostatic effect of stem-bark extracts of Securidaca longipedunculata (Fres. Holl) against Trypanosoma brucei brucei
Abdullah Mohammad Tauheed, Mohammed Musa Suleiman, Mohammed Mamman and Idris Alao Lawal
Acute and sub-chronic toxicity study of the extract and powder of *Operculina macrocarpa* (L.) Urb. in mice

Carpejane Ferreira da Silva¹, Ana Célia Rodrigues Athayde², Marcílio Fontes Cezar³, Onaldo Guedes Rodrigues³*, Sergio Santos Azevedo², José Galberto Martins da Costa⁴ and Fabrício Kleber de Lucena Carvalho¹

¹Department of Veterinary Medicine, Federal University of Campina Grande, UFCG, Patos, Paraíba, Brazil.
²Academic Unit of Biological Sciences, Federal University of Campina Grande, UFCG, Patos, Paraíba, Brazil.
³Academic Unit of Veterinary Medicine, Federal University of Campina Grande, Patos, Paraíba, Brazil.
⁴Department of Biological Chemistry, Regional University of Cariri, Crato, Ceara, Brazil.

Received 14 September 2016 Accepted 23 November, 2016

The aim of the study was to evaluate the experimental acute and sub-chronic toxicities of *Operculina macrocarpa* with plant extract and powder, respectively, in male and female mice. Phytochemical prospection was performed with extract and administration in single doses by intraperitoneal route to six groups and control at 1230, 970, 700, 350, 120 and 30 mg/kg doses and distilled water (0.1 ml/10 g), respectively. Sub-chronic doses of 1230, 700, 30 mg/kg/day and satellite (1230 mg/kg/day) were administered orally in feed. Major endpoints included alterations in the central and autonomic nervous system, water and food intake, body weight, hematological and biochemical parameters. Phytochemical screening identified compounds: Alkaloids, flavonoids, xanthones, leucoanthocyanidins and tannins condensate. In the acute study, mortality was observed with toxicity signs to the central nervous system (CNS) at LD₅₀ of 270 mg/kg. There were no significant changes in water and food intake, body weight, haematological and biochemical parameters, and histopathological examination in the sub-chronic study (p value). Results indicate that the oral administration of *O. macrocarpa* powder in feed is less toxic and relatively safer.

**Key words:** Mice, *Operculina macrocarpa*, hematological parameters, toxicity.

**INTRODUCTION**

*Operculina* sp. belongs to Convolvulaceae family, including 55 genera and approximately 1930 species (Austin, 1997) and spread worldwide (Heywood, 1993). In Brazil, *Operculina* sp. is found in several states with many different popular names, such as Jalapa do Brasil, Batata de Purga (a reference to one of its popular uses, as a laxative), Ipu, Purga de Amaro Leite, Briônia da América, Jalapa de São Paulo, Escamonéia da América.
and Xalapa (Planchnon et al., 1937). The name of this family is derived from the Latin word \textit{convolvo}, meaning intertwine, and it refers to the way this plant grows because a large number of these plant species are fickle climbing plants that grow and tangle as a support (Pereda-Miranda and Bah, 2003). One of the most remarkable features of Convolvulaceae is the presence of rows of cells that secrete resin glycosides in foliar tissues and in the roots. These resins are one of the chemotaxonomic characteristics of this family, and the use in traditional medicine of some of its genera (\textit{Convolvulus}, \textit{Exogonium}, \textit{Ipomoea}, \textit{Merremia} and \textit{Operculina}) is associated with the purgative properties of their resins (Pereda-Miranda et al., 2006).

Popularly known as batata de purga, \textit{Operculina macrocarpa} (L.) Urb. is commonly found in northeastern Brazil (Matos, 1982). It presents tuberous large starchy milky roots, and it can be easily purchased for medicinal purposes. This species is a climbing ornamental plant mainly because of its fruits. Each fruit contains one to four black hard seeds. \textit{O. macrocarpa} is a biennial species, that is, its aerial part dies every two years, presenting palmatiform leaves, white flowers and rounded fruits (Lorenzi and Matos, 2002). Batata de purga is widely used by the local populations because of its laxative, purgative and depurative effects on skin diseases and in the treatment of leucorrhrea (Belizário, 2012). Despite being listed in pharmacopeias (Brazilian Pharmacopeia 1929 and 1959), its phytochemical study is still incomplete.

It contains starch and 12% resin, which is formed by a complex mixture of substances such as glycoside polymers, of purgative effect, and it is known for its laxative property or, in higher doses, for its drastic purgative and anthelmintic effects (Araujo et al., 2012). All homemade or pharmaceutical preparations of batata de purga should be used with caution because in doses higher than the recommended, they can cause severe intoxication, translate into strong cramps and intense diarrhoea with risk of rapid dehydration (Lorenzi and Matos, 2002).

The use of medicinal plants and their derivatives have been quite significant in recent years. However, popular and even traditional uses are not sufficient to ethically validate medicinal plants as effective and safe medicines (Agra et al., 2008). Toxicological studies aims to assess the erroneous idea that herbal products, because they are natural, are devoid of toxic or adverse effects, and the popular use of medicinal plants serves as validation of the effectiveness of these medicines (Silveira et al., 2008). Thus, considering the ethnopharmacological reasons and toxicological information available, the toxicological study of medicinal plants is essential regardless of the pharmacological results. Therefore, the purpose of this study was to assess the experimental acute toxicity of the extract and sub-chronic toxicity of the powder of \textit{O. macrocarpa} in mice.

**MATERIALS AND METHODS**

**Plant material and phytochemical extraction**

The tubercles of \textit{O. macrocarpa} were collected in the municipality of Patos, Paraíba State, northeastern Brazil, located at the geographic coordinates S37°17'36.4" W07° 04'03". The plant material was identified by D.Sc. Maria Teresa Buril, Federal University of Pernambuco, and deposited at the Center for Rural Health and Technology Herbarium, Federal University of Campina Grande (UFCG) authenticated under specimen number 3106.

Phytochemical prospection was carried out with the ethanol extract of \textit{O. macrocarpa} submitted to a series of tests using specific reagents aiming at elucidating the classes of secondary metabolites. This method is based on the visual observation of colorimetric variation and/or intensification and/or precipitate formation, after addition of specific reagents in the sample solutions. For assay performance, 300 mg of the ethanol extract of \textit{O. macrocarpa} were diluted in 30 ml of ethanol 70%. After that, 3 ml aliquots were distributed in test tubes and the assay was carried out according to the method described by Matos (1997).

**Acute toxicity study in mice**

The experimental protocol for the assessment of acute toxicity was guided mainly by the Specific Resolution (RE 90/2004) of the National Agency for Sanitary Surveillance (Brazil, 2004) and by Michelin (2008). This study was in accordance with the accepted guidelines for animal experimentation under number 64/2012.

For the preparation and quantification of the extract, the tubercules were collected, cut in approximately 1 cm thick slices, shade dried and then ground in knife mills to obtain the powder. Two hundred grams of the obtained powder was weighed and dried in forced ventilation oven at 55°C for 24 h, it was then macerated in 1 L of ethanol 96°GL (C2H5O) for 72 h with sporadic homogenization before filtration with filter paper (Matos, 1997).

After this process, 200 ml of the liquid obtained through filtration was placed in a rotary evaporator for concentration, where 16 ml of ethanol extract (EE) was obtained and concentration was determined according to Matos (1997). A concentration of 3.52 mg/ml was found, which was later used for the calculation of the dose to be administered to the mice by intraperitoneal route.

All animals used in this study were healthy 45-day-old outbred Swiss mice (\textit{Mus musculus}), of either sex, weighing between 22 and 44 g. Animals were segregated according to gender, housed in polypropylene cages, and kept in a room with dark/light cycle (12/12 h) and 28±3°C average temperature with water and food \textit{ad libitum} at the Center for Breeding and Experimentation with Laboratory Animals, Federal University of Campina Grande (UFCG). The ethanol extract of \textit{O. macrocarpa} was administered by intraperitoneal route to groups of 12 animals each (6 male and 6 female mice), which were initially five groups, with standardized volume for dose calculation of 10 ml/kg (0.1 ml/10 g), all in single doses. Subsequently lower concentrations of the extract were used for two groups: G5 and G6 received average doses of 120 and 30 mg/kg, respectively.

Animals were examined at times 0, 15, 30 and 60 min; 4, 8, 12 and 24 h; and daily for 14 days post-treatment. Animals were observed for general behavioral changes regarding signs of toxicity according to the methodology adapted from Mariz (2007) and Atsama et al. (2011) aiming to identify possible alterations in the central nervous system (CNS) and autonomic nervous system (ANS) using the following parameters: general behavior, response to touch and tail squeezing, twisting, straightening, body tone,
ataxia, tremors, hypnosis, lacrimation, ptosis, micturition, diarrhea, piloerection, respiration, cyanosis and drooling. Animal mortality was recorded to determine the lethal dose (LD₅₀) according to Litchfield and Wilcoxon (1949).

Sub-chronic toxicity study in mice

All procedures used for sub-chronic toxicity assessment followed the same protocol mentioned for the acute toxicity evaluation as well as that by Lagarto et al. (2011). Animals were acclimatized for at least one week prior to toxicity testing. Before feed formulation, the average daily food consumption of each group was calculated for determination of each component percentage. The components of feed formulation were distributed as follows: G1 (control) received commercial feed (Presence® animal nutrition, Paulinia, SP, BRA) ad libitum; G2, G3 and G5 received 245.7, 255.3 and 244.5 g of commercial feed; 24.3, 14.7 and 25.5 g of O. macrocarpa powder; and 30, 30 and 30 g of corn starch, totaling 300 g of feed; G4 received 349 g of commercial feed; 1 g of O. macrocarpa powder; and 150 g of corn starch, totaling 500 g of feed. Manual preparation of feed was carried out by previously grinding the commercial feed in a knife mill in the amounts calculated according to the doses established for each group. O. macrocarpa powder was then proportionally added so that the animals could consume daily the previously calculated doses. This mixture was homogenized in a manual mixer, adding 200 ml of water and the corn starch to obtain proper consistency, and was then pelleted by hand using 20 ml disposable syringes. After preparation, the feed was shade dried at room temperature (Benício, 2008). Fifty healthy 2-month-old Swiss (Mus musculus) mice, of either sex, weighing 22 to 44 g were previously acclimatized for five days before the conduction of the biological assays and kept as cited for the acute toxicity study. Animals were divided into five groups of 10 (5 males and 5 females). The groups were named and received treatments as follows: G1 (control)—only commercial feed; G2- addition of 24.3 g of O. macrocarpa powder to obtain the expected average daily consumption of 1230 mg/kg; G3- addition of 14.7 g of plant powder for the expected average daily consumption of 700 mg/kg; G4- addition of 1 g of plant extract for the expected average daily consumption of 50 mg/kg and Group G5 (satellite) had 25.5 g of O. macrocarpa powder added to the feed to obtain the expected average daily consumption 1230 mg/kg. The satellite group remained under study for additional 15 days after the end of the 30-day experiment period for assessment of eventual reversibility of the effects of toxicity and emergence of late effects produced by the powder administered with highest dose. The doses used for the sub-chronic study were obtained from the acute testing: the lowest, the highest, and an approximate intermediate dose (Brasil, 2004). The parameters to be evaluated were distributed among those assessed daily and weekly every five days. Animals were observed for possible changes in the CNS and ANS by the same methodology used for the acute toxicity study.

Water and food was provided as pellets from the first testing day (day 0), providing graduated filled bottles. The average volume consumed by the mice was registered on the following day and the feed was weighed daily and accounted for on the day after. The weight gain curve was drawn weekly using the calculated 5-day weekly total weight of each group. After 30 days of experiment, animals from the treated and control groups fasted for 12 h prior to blood collection. Blood samples were then collected from 40% of the surviving animals (Mariz, 2007), by decapitation and cervical traction (Altsamoa et al., 2011). Blood from each animal was placed into properly identified tubes (Labor Import, São Paulo, SP, BRA) containing ethylenediaminetetraacetic acid (EDTA) acid for assessment of haematological parameters: red blood cell (RBC) count; determination of hematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC); leukocyte, lymphocyte and neutrophil counts (Jain, 1993). For biochemical analysis, serum was obtained for determination of alanine aminotransferase (ALT), total proteins (TP), and albumin (Jain, 1993). Analyses were carried out immediately after collection in commercial veterinary laboratory according to the protocol of specific commercial kits (Labtest® Diagnóstica S.A, Lagoa Santa, MG, BRA) and semi-automatic analyzer (BIO-200® Products Laboratories Ltda, SP, BRA).

For histopathological analysis, blood was collected from the same animals (40% of each group) randomly chosen (Mariz, 2007). They were subjected to necropsy and had their viscera: heart, lung, kidney, liver, stomach, large and small intestines, collected (Brazil, 2004). The viscera were immersed in fixative solution of 10% buffered formaldehyde; after fixation, they were subjected to histological processing and staining with hematoxylin-eosin. The satellite group animals were euthanized 15 days after the sub-chronic treatment for evaluation of hematological, biochemical and histopathological findings.

Statistical analysis

All results are presented as mean ± standard deviation. Before selecting the statistical test for intergroup comparison, the Shapiro-Wilk normality test was performed. The variables presenting normal distribution were used in the analysis of variance (ANOVA), with multiple comparisons assessed by the Tukey test; and the variables presenting abnormal distribution were analyzed by the Kruskal-Wallis test, with multiple comparisons by the Dunn test (Zar, 1999). Comparison of treatment times (weeks) was performed using the Friedman test. Differences were considered statistically significant at P ≤ 0.05 using the SPSS 13.0 for Windows and BioEstat 5.03 software.

RESULTS

Phytochemical prospection

The phytochemical prospection of the ethanol extract of O. macrocarpa revealed some classes of compounds such as alkaloids, flavonoids (flavanones, flavones and flavonoid), xanthones, leucoanthocyanidins and tannins condensate.

Acute toxicity study

Intraperitoneal administration of EE at 1230, 970 and 700 mg/kg doses produced treatment related effects from the first minutes, and after one hour at the dose of 350 mg/kg, all followed by mortality and with no sign of effects over time. The dose of 120 mg/kg caused two deaths while the dose of 30 mg/kg and the control caused neither death nor alterations (Table 1). The median lethal dose (LD₅₀) was 270 mg/kg, varying from 160 to 470 mg/kg.

Sub-chronic toxicity study

Oral administration of O. macrocarpa powder in feed in
Table 1. Acute toxicity of the ethanol extract of *Operculina macrocarpa* (L.) Urb. administered by intraperitoneal route in mice.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>M/T</th>
<th>Latency</th>
<th>Signs (parameters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1 = 1230</td>
<td>12/12</td>
<td>&lt; 1 h</td>
<td>↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone</td>
</tr>
<tr>
<td>G 2 = 970</td>
<td>12/12</td>
<td>&lt; 1 h</td>
<td>↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone</td>
</tr>
<tr>
<td>G 3 = 700</td>
<td>12/12</td>
<td>&lt; 1 h</td>
<td>↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone</td>
</tr>
<tr>
<td>G 4 = 350</td>
<td>8/12</td>
<td>&gt; 1 h &lt; 24 h</td>
<td>↓General behavior, hypnosis, ataxia, ↓response to touch, ↓tail squeezing, ↓respiration, ↓body tone</td>
</tr>
<tr>
<td>G 5 = 120</td>
<td>2/12</td>
<td>&gt; 12 h &lt; 24 h</td>
<td>Sudden mortality</td>
</tr>
<tr>
<td>G 6 = 30</td>
<td>0/12</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>G control l</td>
<td>0/12</td>
<td>-</td>
<td>None</td>
</tr>
</tbody>
</table>
| LD<sub>50</sub> = 270 (mg/kg); Confidence limit of 95% = 470 - 160 (mg/kg)

The ethanol extract of *O. macrocarpa* tubercle was administered by intraperitoneal route to groups of 12 mice (6 males and 6 females). All treated animals were observed for 14 days for signs of toxicity (changes in behavior and mortality). M/T, mortality/treated mice; none, no signs of toxicity were observed during the study period; latency, time period within which signs of toxicity are shown; ↓, reduction.

Table 2. Water intake (ml) of mice treated orally with *O. macrocarpa* powder added to feed for 30 days.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control</th>
<th>1230 (mg/kg/day)</th>
<th>700 (mg/kg/day)</th>
<th>30 (mg/kg/day)</th>
<th>Satellite - 1230 (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>46 ± 16.0</td>
<td>38 ± 16.0</td>
<td>54 ± 28.4</td>
<td>56 ± 30.0</td>
<td>88 ± 33.4</td>
</tr>
<tr>
<td>2</td>
<td>44 ± 17.0</td>
<td>40 ± 19.0</td>
<td>46 ± 15.0</td>
<td>42 ± 17.0</td>
<td>84 ± 23.2*</td>
</tr>
<tr>
<td>3</td>
<td>46 ± 14.0</td>
<td>38 ± 12.0</td>
<td>38 ± 12.0</td>
<td>47 ± 30.0</td>
<td>89 ± 7.30*</td>
</tr>
<tr>
<td>4</td>
<td>37 ± 15.4</td>
<td>31 ± 20.0*</td>
<td>35 ± 15.1*</td>
<td>38 ± 17.3</td>
<td>76 ± 25.2*</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>57 ± 23.2</td>
<td>40 ± 35.1</td>
<td>35 ± 20.0</td>
<td>66 ± 16.2</td>
<td>60 ± 23.0</td>
</tr>
<tr>
<td>2</td>
<td>95 ± 12.5</td>
<td>63 ± 34.0</td>
<td>36 ± 31.0*</td>
<td>61 ± 17.4</td>
<td>113 ± 35.0</td>
</tr>
<tr>
<td>3</td>
<td>62 ± 30.1</td>
<td>37 ± 19.0</td>
<td>28 ± 16.4</td>
<td>69 ± 30.0</td>
<td>68 ± 27.0</td>
</tr>
<tr>
<td>4</td>
<td>62 ± 16.3</td>
<td>32 ± 20.0*</td>
<td>41 ± 17.3*</td>
<td>53 ± 8.0</td>
<td>69 ± 27.0</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation of 10 animals (5/gender). *p <0.05, significantly different from the control.

The ethanol extract did not produce behavioral changes or mortality in the groups studied. There was no statistically significant difference (p > 0.05) in water and food intake in the course of time for all the groups assessed, for both male and female mice (Tables 2 and 3). Comparison between the treated and control groups over time showed significant differences in the water intake of male mice during the second and third weeks between the satellite (1230 mg/kg/day) and the control, and in the fourth week between the two highest doses (1230 and 700 mg/kg/day) and between the satellite and the control. With respect to the female mice, there were statistical differences in the second week between the dose of 700 mg/kg/day and the control and in the fourth week between the two highest doses when compared with the control (Table 2).

Statistically significant differences (p<0.05) were observed in the food intake of male mice in the second week between the 700 mg/kg/day dose and the satellite when compared with the control; in the third week between the 700 and 30 mg/kg/day doses when compared with the control; in the fourth week between the 700, 30 mg/kg/day and the satellite when compared with the control. Regarding the female mice, there were differences only in the second week between the dose of 1230 mg/kg/day and the satellite when compared with the control (Table 3).

The body weight of mice presented variations between the groups observed along the four weeks. The final assessment at the end of the fourth week showed statistically significant difference (p < 0.05) of total weight between the 700 and 30 doses and the satellite (1230 mg/kg/day).
Table 3. Food intake (g) of mice treated orally with *O. macrocarpa* powder added to feed for 30 days.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control</th>
<th>1230 (mg/kg/day)</th>
<th>700 (mg/kg/day)</th>
<th>30(mg/kg/day)</th>
<th>Satellite - 1230 (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23 ± 13.2</td>
<td>22 ± 12.5</td>
<td>21 ± 6.0</td>
<td>26 ± 6.2</td>
<td>26 ± 13.0</td>
</tr>
<tr>
<td>2</td>
<td>15 ± 2.1</td>
<td>13 ± 2.5</td>
<td>27 ± 5.0*</td>
<td>21 ± 12.0</td>
<td>22 ± 5.0*</td>
</tr>
<tr>
<td>3</td>
<td>16 ± 4.0</td>
<td>14 ± 5.4</td>
<td>23 ± 2.0*</td>
<td>28 ± 9.0*</td>
<td>21 ± 5.0</td>
</tr>
<tr>
<td>4</td>
<td>14 ± 2.1</td>
<td>15 ± 2.0</td>
<td>23 ± 1.3*</td>
<td>21 ± 3.0*</td>
<td>22 ± 5.0*</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24 ± 6.3</td>
<td>20 ± 12.0</td>
<td>25 ± 15.0</td>
<td>35 ± 19.1</td>
<td>33 ± 11.3</td>
</tr>
<tr>
<td>2</td>
<td>29 ± 6.0</td>
<td>18 ± 2.3*</td>
<td>20 ± 8.4</td>
<td>34 ± 15.2</td>
<td>36 ± 11.0*</td>
</tr>
<tr>
<td>3</td>
<td>23 ± 3.5</td>
<td>19 ± 3.3</td>
<td>21 ± 6.0</td>
<td>34 ± 19.1</td>
<td>34 ± 12.1</td>
</tr>
<tr>
<td>4</td>
<td>22 ± 1.3</td>
<td>18 ± 3.0</td>
<td>18 ± 4.0</td>
<td>20 ± 12.0</td>
<td>27 ± 11.0</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation of 10 animals (5/gender). *p <0.05, significantly different from the control.

Figure 1. Relative weight of the mice treated orally with *O. macrocarpa* powder added to feed (30-1230 mg/kg/day) for four weeks. Each point represents the total weight of the group at the end of each week, n=10 (5/gender). *p <0.05, significantly different from the control group.

Oral sub-chronic administration of *O. macrocarpa* powder to feed for 30 days did not cause any change in the haematological profile (erythrocytes, hematocrit, haemoglobin, MCV, MCH, MCHC, leukocytes, lymphocytes and neutrophils) of the treated groups; all parameters were within the physiological range of reference according to Jain (1993) throughout the treatment period (Table 4).

There were no statistically significant differences (p > 0.05) between treatments regarding the biochemical parameters evaluated. Parameters were within the physiological range of reference according to Thrall et al. (2006) for alanine aminotransferase (ALT). They showed a slight increase in relation to the 7 g/dl limit according to Thrall et al. (2006), reaching 8 g/dl in the treatment with the smallest dose (30 mg/kg/day) for total proteins (TP). They presented a small reduction in relation to the inferior
Table 4. Selected haematological and biochemical parameters of the sub-chronic study of groups treated with *O. macrocarpa* powder added to feed for 30 days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1230 (mg/kg/day)</th>
<th>700 (mg/kg/day)</th>
<th>30 (mg/kg/day)</th>
<th>Satellite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (x10^6/µl)</td>
<td>8.38 ± 0.85</td>
<td>8.68 ± 0.70</td>
<td>8.73 ± 0.22</td>
<td>9.00 ± 1.10</td>
<td>8.88 ± 0.83</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.0 ± 0.96</td>
<td>13.0 ± 0.62</td>
<td>13.9 ± 0.42</td>
<td>14.4 ± 1.25</td>
<td>14.8 ± 1.34</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39.1 ± 0.64</td>
<td>38.9 ± 1.03</td>
<td>40.3 ± 1.32</td>
<td>41.6 ± 5.10</td>
<td>41.3 ± 3.86</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>45.6 ± 1.11</td>
<td>45.8 ± 1.38</td>
<td>46.6 ± 1.14</td>
<td>46.3 ± 1.00</td>
<td>46.4 ± 0.46</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.0 ± 0.31</td>
<td>15.4 ± 0.52</td>
<td>15.9 ± 0.43</td>
<td>15.7 ± 0.50</td>
<td>15.9 ± 0.47</td>
</tr>
<tr>
<td>CHCM (%)</td>
<td>33.1 ± 2.85</td>
<td>33.6 ± 0.88</td>
<td>33.7 ± 1.24</td>
<td>34.0 ± 1.00</td>
<td>34.1 ± 0.81</td>
</tr>
<tr>
<td>Leucocytes (x10^3/µl)</td>
<td>7.50 ± 0.49</td>
<td>7.00 ± 1.83</td>
<td>7.58 ± 1.64</td>
<td>7.30 ± 2.70</td>
<td>8.50 ± 1.91</td>
</tr>
<tr>
<td>Neutrophils seg. (%)</td>
<td>23.3 ± 5.38</td>
<td>25.0 ± 3.56</td>
<td>30.3 ± 3.70</td>
<td>35.0 ± 12.4</td>
<td>26.0 ± 4.12</td>
</tr>
<tr>
<td>Lymphocytes typ (%)</td>
<td>77.0 ± 6.10</td>
<td>75.5 ± 4.20</td>
<td>71.0 ± 5.00</td>
<td>63.8 ± 14.2</td>
<td>70.0 ± 5.60</td>
</tr>
<tr>
<td>ALT (UI/L)</td>
<td>94 ± 65.0</td>
<td>181 ± 108</td>
<td>104 ± 62</td>
<td>90 ± 39</td>
<td>116 ± 23</td>
</tr>
<tr>
<td>TP (g/dL)</td>
<td>7.23 ± 0.50</td>
<td>7.95 ± 0.82</td>
<td>7.43 ± 0.68</td>
<td>8.00 ± 0.78</td>
<td>6.83 ± 0.94</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.41 ± 0.56</td>
<td>2.37 ± 0.61</td>
<td>2.78 ± 0.17</td>
<td>2.59 ± 0.25</td>
<td>2.47 ± 0.30</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard deviation of 40% of the group (10 animals/group). MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; ALT, alanine aminotransferase; TP, total protein; seg, segmented; typ, typical. There was no statistically significant difference between treatments.

The limit of 3 g/dl according to Thrall et al. (2006), reaching 2.37 g/dl in the group treated with the highest dose (1230 mg/kg/day) for albumin (Table 4). Histopathological assessment of tissue samples collected showed no abnormality for all the treated groups.

**DISCUSSION**

*O. macrocarpa* popularly known as batata de purga, is widely used by the local populations because of its laxative, purgative and anthelmintic effects (Lorenzi and Matos, 2002), and the latter has been proved by studies to be effective in the control of gastrointestinal parasites of goats, *in vitro* using the extract (Gomes et al., 2010) and *in vivo* using the powder (Silva et al., 2010). However, in high doses, its use can cause severe toxicity, translated into strong cramps and intense diarrhea with risk of rapid dehydration (Lorenzi and Matos, 2002). Various therapeutic applications are attributed to the metabolites found in the phytochemical prospect of *O. macrocarpa* such as tannins, which provide antimicrobial and antifungal effects by protein precipitation (Vieira et al., 2015), and several antioxidant activities are conferred to flavonoids (Karimi and Moradi, 2015).

The Convolvulaceae family presents a great diversity of metabolites, but the glycoresins and phenolic substances are characteristic of this family. This family also presents secondary metabolites of low molecular weight containing nitrogen groups such as: ergolines, pyrrolidines, lipophilic and hydrophilic tropanes, indolizidine and pyrrolizidine alkaloids, cyanogenic glycosides, and different types of flavonoids and amides (Michelin, 2008).

In the acute toxicity study, the extract of this plant administered by intraperitoneal route caused mortality at higher doses with signs of toxicity that affect the central nervous system (CNS), presenting a LD₅₀ of 270 mg/kg, of very high toxicity by this route according to Stacey (1993). The intraperitoneal route (which cannot be used in humans) is usually selected to determine the inherent toxicity of chemicals because the effects of an oral dose are subject to systemic bioavailability and hepatic detoxification, and acute dose study provides guidance for the selection of doses for the study of sub-chronic doses, which may be more clinically relevant (Li et al., 2010).

*O. macrocarpa* seems to be less toxic when administered orally, as demonstrated by Michelin (2004) using the gross extract at the dose of 3800 mg/kg, presenting moderate toxicity; and by Stacey (1993) using the powder >5000 mg/kg, presenting slight toxicity; and yet by the present sub-chronic study using the powder added to feed at the dose of 1230 mg/kg/day obtained by the acute study for thirty days, not presenting mortality or clinical signs of toxicity. According to toxicology, any substance can be considered a toxic agent depending on the exposure conditions, such as the dose administered or absorbed, time and frequency of exposure, and the process by which it is administered. The toxicity of a substance can be considered as the ability to cause serious injury or death (Ruppenthal, 2013).

In the evaluation of water and food intake, no statistically significant differences (p=0.05) were observed in the groups treated over time, even with the highest dose, confirming that, orally and in the form of powder added to the feed, *O. macrocarpa* did not produce toxic effects that could reduce consumption during the 30-day study. Nevertheless, there were some differences between the treated groups when compared...
with the control for both males and females, and between the high, intermediate and low doses. This probably occurred because experimental animals exhibit variations depending on environmental factors, diet, and biotherium conditions, according to Harkness and Wagner (1993).

Variations occurred over time in the assessment of the total body weight \((n = 10)\) of treated groups; however, after four weeks, the weights of the groups that received the powder added to the feed, mainly the dose of 1230 mg/kg/day and the satellite (1230 mg/kg/day), were higher than the control. This corroborates the results of Michelin (2004), who found no significant difference in the weight of animals treated with the powder. The body weight changes have been used as an indicator of adverse effects of drugs and chemicals and Li et al. (2010) demonstrated the non-toxicity of \(O.\) \textit{macrocarpa} considering that it did not affect the body weight of treated animals after 30 days of daily consumption.

According to Li et al. (2010), the hematopoietic system is one of the most sensitive targets of toxic chemicals and an important indicator of physiological and pathological state in humans and animals. In the present study, the data of hematological parameters showed no differences \((p > 0.05)\) between the treated and control groups. These results indicate that the powder of \(O.\) \textit{macrocarpa} added to the feed for 30 days had no effect on the circulating blood cells, or on their production.

In most rodents, the serum activity of ALT increases with hepatocellular damage, where it seems to be specific for rat and mice liver (Thrall et al., 2006) and as a good indicator of hepatic function as biomarkers predicting possible toxicity (Li et al., 2010). In general, any damage to the parenchymal liver cells results in the increase of this transaminase in blood. In this study, ALT remained within the physiological range for mice, suggesting that the sub-chronic administration of \(O.\) \textit{macrocarpa} powder added to the feed for 30 days did not influence the function and metabolism of hepatocytes.

In other chemical parameters such as TP and albumin, a small increase was observed in relation to the superior TP reference limit for mice with the lowest dose (30 mg/kg/day) and a small reduction with respect to the inferior albumin limit in the group treated with the highest dose (1230 mg/kg/day). According to Thrall et al. (2006), in mice, the normal plasma protein concentration varies between different strains.

However, no significant differences were observed in either parameter when compared with the control, giving an indication that there is no presence of hepatic disease, which would lead to decreased levels of total protein by reducing the production; and in principle, the presence of kidney disease that could lead to excessive protein loss can also be discarded. These findings confirm that the sub-chronic administration of \(O.\) \textit{macrocarpa} powder added to the feed for 30 days did not influence the biochemical profile of the treated animals. This hypothesis is consolidated by histopathological analyses, especially those of the heart, lung, kidney, liver, stomach and intestines, which showed normal morphology.

**Conclusion**

This study provides valuable data on the acute toxicity profile of the intraperitoneal administration of ethanol extract and the sub-chronic toxicity of the powder of \(O.\) \textit{macrocarpa} (batata de purga) added to the feed. These data can be very useful for any further study in vivo, especially the powder administered orally. It is because it did not cause mortality and did not significantly alter body weight gain, hematological and biochemical parameters, and caused no histopathological damage to the organs analyzed for 30 days. Also, it seemed less toxic and relatively safer by this route, with new studies with different formulations to be developed.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The authors are grateful to D.Sc. Maria Teresa Buril for the identification of plants and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES.

**REFERENCES**


Full Length Research Paper

Ex vivo trypanostatic effect of stem-bark extracts of Securidaca longipedunculata (Fres. Holl) against Trypanosoma brucei brucei

Abdullah Mohammad Tauheed1*, Mohammed Musa Suleiman1, Mohammed Mamman1,2 and Idris Alao Lawal3

1Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria.
3Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria.

Received 21 August, 2015; Accepted 26 May, 2016

Current treatment of trypanosomosis in sub-Saharan Africa is associated with widespread inefficiency. There is therefore the need to find more effective drugs against the disease from promising traditional medicinal herbs. This work is aimed at evaluating ex vivo anti-trypanosomal effect of stem-bark extracts of S. longipedunculata against T. brucei brucei. One hundred microlitre of crude methanol, ethyl acetate and aqueous methanol extracts of S. longipedunculata at concentrations of 3 and 6 mg/ml each were mixed with 50 µl of blood containing 8.1 × 10^6 trypanosomes and incubated at 37°C for 90 min. Similarly, diminazene aceturate (10 µg/ml), physiological saline solution (50 µl) and blood (100 µl) containing trypanosomes only served as treated, negative and untreated controls, respectively. Motility of the parasite was monitored under light microscope (×400) at 5 min interval throughout the 90 min observation period. All experiments were done in duplicate. The mixtures were subsequently inoculated into rats that were not previously infected with trypanosomes. Phytochemical screening of the extracts revealed the presence of carbohydrates, cardiac glycosides, saponins, steroid, triterpenes, flavonoids and tannins. However, aqueous and ethyl acetate fractions were devoid of flavonoids. The crude methanol immobilized the parasites within 75 min, while ethyl acetate and aqueous extracts induced slight reduction in motility of the parasite at 90 min of incubation. However, inoculated rats developed infection and succumbed to the infection. It is concluded that the stem-bark of the plant possesses trypanostatic, but not trypanocidal, activity against the parasite.

Key words: Antitrypanosomal, drug incubation infectivity test, in vitro, phytochemical screening, stem-bark.

INTRODUCTION

African trypanosomosis is a disease of humans and livestock caused by several species of flagellated single-celled protozoan parasites belonging to the genus Trypanosoma (Kuriasko et al., 2012). Trypanosomosis is

*Corresponding author. E-mail: mtauheed40@yahoo.com. Tel: +2348033190282.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
an important constraint to livestock development in sub-Saharan Africa with estimated direct annual economic loss exceeding US$1 billion (Simukoko et al., 2007). In animals, trypanosomosis is characterized by pyrexia, anaemia, loss of condition, reduced productivity, cachexia and mortality (Losos and Ikede, 1972; Anosa, 1983; Murray et al., 1983; Nwosu and Ikeme, 1992; Igbokwe, 1994). The disease is ranked among the top 10 global cattle diseases affecting livestock production in sub-Saharan Africa (Perry et al., 2002). The scarcity of modern effective drugs for the treatment and management of trypanosomosis, combined with their high cost have created a growing public interest in alternative natural drugs from botanicals (Ete et al., 2012).

In countries where trypanosomosis is endemic, plants have been used traditionally for centuries and are still widely used to treat this illness and other parasitic diseases (Tagboto and Townsend, 2001). *Securidaca longipedunculata* (Polygalaceae) is a small tree of up to 6 to 9 m high with a pale grey, smooth bark and oblong, more less hairless alternate leaves of varying size and shape and crowded towards the stem tips (Van Wyk et al., 2009). Root, stem-bark, leaves of the plant and whole plant are used for medicinal purposes by local folk (Nadembega et al., 2011; Wanzala et al., 2012; Borokini et al., 2013; Mustapha, 2013). The plant is widely used in African traditional medicine as a general remedy for cough, malaria, backache, venereal disease, snakebite, erectile dysfunction and tuberculosis (Watt and Breyer-Brandwijk, 1962; Atawodi et al., 2002; Ajali and Chukwurah, 2004; Adebiyi et al., 2006; Marion Meyer et al., 2008). *S. longipedunculata* is known as violet tree, fibre tree or Rhodesian violet in English, and popularly known as *Uvar magunguna* (mother of all medicine) in Hausa speaking communities of Northern Nigeria (Ajali and Chukwurah, 2004; Adebiyi et al., 2006; Orwa et al., 2009). The aim of this study is to determine the anti-trypanosomal effect of the stem-bark extracts of *S. longipedunculata* against *T. brucei brucei*.

### MATERIALS AND METHODS

#### Plant collection and identification

Fresh stem-bark of *S. longipedunculata* was collected in Zaria, Nigeria. The plant was identified in the Herbarium, Department of Biological Science, Ahmadu Bello University (A.B.U.), Zaria, Nigeria where a voucher number specimen of 900213 was allotted. The identified stem-bark was dried in an open air in the laboratory and the dried sample was kept in polythene bags until required for preparation of the extract.

#### Plant extraction, concentration and fractionation

Seven hundred and ninety grams of the dried powder of the plant was extracted with absolute methanol in a soxhlet extractor. The liquid extract was concentrated to dryness over a water-bath.

Seventy-three grams of the crude methanol extract was dissolved in 300 ml of distilled water and the solution was transferred to 1 L separating funnel. The mixture was partitioned twice its volume with ethyl acetate. The lower denser aqueous fraction was collected into a separate conical flask and upper portion (ethyl acetate fraction) was dispensed into a clean conical flask. The aqueous portion of the extract was further partitioned with ethyl acetate and similar fractions were combined. The fractions were concentrated to dryness over a water-bath.

### Phytochemical screening

The extract and fractions of *S. longipedunculata* were evaluated for the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloid, saponins, cardiac glycosides, steroids and triterpenes using standard procedures (Trease and Evans, 1983).

### Experimental animals

Twenty Wistar rats of both sexes weighing between 170 and 190 g were obtained from the Animal House, Department of Physiology, Faculty of Medicine, A.B.U., Zaria, and were allowed to aclimatize for two weeks in the laboratory at the Department of Veterinary Pharmacology and Toxicology, A.B.U., Zaria. They were housed in clean plastic cages with wood shavings as bedding. The bedding was changed twice a week. The rats were fed on standard rat feed and given access to clean water ad libitum. The animal experiment was approved by A.B.U., Zaria Animal Welfare Committee (Vet-Med/15632/10-11).

#### Test organism

*Tryptansoma brucei brucei* was obtained from the Department of Veterinary Parasitology and Entomology, A.B.U., Zaria. The parasite was maintained in rats by continuous passage. Each cycle of passage was done when parasitaemia was in the range of 35 to 40 parasites per field, which corresponded to an interval of 6 days post-infection. For several passages, about 3 ml of blood was obtained from an infected rat by cardiac puncture after light chloroform anaesthesia into 5 ml syringe and emptied into a vial containing 9 ml of physiological saline solution (PSS). About 1 x 10⁶ trypanosomes contained in 0.2 ml was used to infect a trypanosome-free rat by i.p route (Figure 1).

#### In vitro screening

In vitro antityranosomal screening test was done in duplicate using the rapid matching method (Herbert and Lumsden, 1976) and the drug incubation infectivity test (Vincent et al., 2008). Briefly in the rapid matching method, 100 µl of blood containing 8.1 x 10⁵ parasites was individually mixed with 50 µl of 3 and 6 mg/kg solutions of the crude methanol extract, ethyl acetate and aqueous methanol fractions in a 48-microtitre plate. The mixture was rocked gently and then incubated at 37°C for 5 min. Similarly, 100 µl of blood containing 8.1 x 10⁵ trypanosome was mixed with diminazene aceturate 10 µg/ml and PSS (50 µl); and blood (100 µl) containing trypanosome only served as treated, negative and untreated controls, respectively. At the end of the incubation period, 2 µl of individual test mixtures were separately observed under a light microscope at x400 magnification. The parasites were observed every 5 min for a total of 90 min. A reduction or complete cessation of motility of parasites in the treated blood samples when compared to that of untreated control was taken as a measure of trypanocidal activity.
Drug incubation infectivity test (DIIT)

About 0.2 ml of the concentration of extract that was observed to have immobilized the parasites was immediately taken into a clean 1 ml syringe and inoculated into 2 rats each not previously infected with trypanosome. Those extracts and concentrations that neither reduced the motility of nor immobilized the parasites at the end of 90 min observation were also inoculated i.p into 2 rats each not previously exposed to trypanosomal infection. The rats were observed daily for development of parasitaemia for 3 weeks.

Determination of parasitaemia in experimental rats

Parasitaemia was monitored in blood obtained from the tail of infected rats. The number of parasites per ml of blood was determined microscopically at ×400 magnification using the "Rapid Matching" method of Herbert and Lumsden (1976).

Data analysis

Data for pre-patent periods were expressed as mean (±SEM) and were subjected to analysis of variance followed by Tukey post hoc using GraphPad Prism version 4. Values of p < 0.05 were considered statistically significant.

RESULTS

Plant yield

Nine hundred and eighty-six gram (986 g) of the pulverized stem-bark of *S. longipedunculata* (SL) gave 109.49 g (13.9%) of crude methanol extract. Upon partitioning with different solvents, the crude methanol yielded 4.83 g (6.7%) and 18.44 g (25.4%) of ethyl acetate and aqueous methanol fractions, respectively.

Phytochemical screening

Preliminary phytochemical screening of the extract and fractions revealed the presence of carbohydrates, cardiac glycosides, saponons, steroids, triterpenes, flavonoids and tannins. However, the ethyl acetate and aqueous methanol fractions did not contain flavonoids.

In vitro study

Complete cessation of motility was observed in trypanosomes that were treated with crude methanol extract of SL at concentrations of 3 and 6 mg/ml at 75 min of observation (Table 1). The ethyl acetate and aqueous methanol fractions at concentrations of 3 and 6 mg/ml slightly reduced parasites motility at 90 min. However, trypanosomes that were treated with diminazene aceturate (10 µg/ml) were immobilised within 15 min of treatment.

Drug incubation infectivity test

The prepatent period of rats in the crude methanol treated groups was 8 days compared to 6 days recorded in the physiological saline solution and untreated groups. The difference was not statistically significant. Furthermore, rats treated with the ethyl acetate and aqueous methanol fractions were also patent on day 6 post-infection. However, the diminazene aceturate treated rats remained aparasitaemic for 21 days. The differences in the time course of the disease between the crude methanol extract, fractions, physiological saline solution and untreated groups were not statistically significant.

DISCUSSION

Animal trypanosomosis remains one of the most important haemoparasitic diseases bedeviling sub-Saharan African. It is a constraint to improved livestock production in the region. *In vitro* evaluation of plant extract for antitrypanosomal effect is a rapid and cheap test for evaluating the efficacy of plant against trypanosomes. Some plants had been reported to exhibit *in vitro* trypanocidal effect measured by cessation of motility of the parasites (Habila et al., 2011) and minimum inhibitory concentration (Freiburghaus et al., 1996; Aderbauer et al., 2008), while some were reported to exhibit trypanostatic effect measured by reduction in motility of the parasites (Atawodi, 2005). The result of this study showed that crude methanol extract of *S. longipedunculata* was able to immobilize the parasite completely within 75 min observation period. Furthermore, the ethyl acetate and aqueous methanol showed slightly reduced parasite motility. Since
immobilization of parasite motility by extract is used as trypanocidal effect, the stem-bark of *S. longipedunculata* could be said to be trypanocidal in effect.

In the drug incubation infectivity test (DIIT) study, parasites that were immobilized by extract of *S. longipedunculata* were shown to be infective when inoculated into rats. It follows that DIIT can be used to evaluate or confirm the effect of a plant extract for anti-trypanosomal effect. In addition, the course of the disease in the animals was only slightly longer than that of the animals from *in vivo* study, indicating that the virulence of the pathogen was not affected. This is very important because hitherto cessation of parasite motility has been taken as trypanocidal effect (Freiburghaus et al., 1996; Aderbauer et al., 2008; Habila et al., 2011) while reduction in trypanosome motility is taken as trypanostatic (Atawodi, 2005). These conclusions should be made with caution when only *in vitro* conditions, there is initial stage of reversible parasite paralysis before clinical death of the parasite. It is possible that cessation of parasite motility in this study was at the point of paralysis of the parasite.

The trypanocidal effect observed in this study could be attributable to one or combination of secondary metabolites detected in the present study. Furthermore, flavonoids could be said to be the primary secondary metabolite in the stem-bark of *S. longipedunculata* with trypanocidal effect since only crude methanol extract (which contained flavonoids) was able to immobilize the parasite completely. Flavonoids found in higher plants have been reported to possess antimicrobial and antitypanosomal activities (Cordell et al., 2001). Kobo et al. (2014) reported protective effect of flavonoids on erythrocytes of rats infected with *T. brucei brucei*. Since ethyl acetate and aqueous methanol extracts devoid of flavonoids exhibited slightly reduced motility, flavonoid could be said to be responsible for trypanocidal activity in this study.

Moreover, mild antitypanosomal effect shown by ethyl acetate and aqueous methanol fractions of *S. longipedunculata* revealed that other bioactive metabolites detected in the extracts also possessed some degree of activity against *T. brucei brucei*. Trypanocidal activity of leaf extract of *Annona senegalensis* has been attributed to the presence of saponins and tannins (Ogbadoyi et al., 2007). Also, Atawodi et al. (2011) speculated that the antitypanosomal activity of *Boswellia dalzielii* could be due to one or combination of flavonoids, saponins, alkaloids, tannins, resins, steroids and triterpenes detected.

---

### Table 1. Ex vivo effect of extract and fractions of *S. longipedunculata* on the motility of *T. rypanosoma brucei brucei*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations (mg/ml)</th>
<th>Immobilization of parasite motility (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude methanol extract</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>3</td>
<td>90*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>90*</td>
</tr>
<tr>
<td>Aqueous Methanol fraction</td>
<td>3</td>
<td>90*</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>90*</td>
</tr>
<tr>
<td>Diminazene aceturate</td>
<td>0.01</td>
<td>15</td>
</tr>
<tr>
<td>Physiological saline solution</td>
<td>50 µl</td>
<td>No effect</td>
</tr>
</tbody>
</table>

* = Slight reduction in motility.

### Table 2. Effect of extracts of *S. longipedunculata* on pre-patent period of parasite under drug incubation infectivity test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentrations (mg/ml)</th>
<th>Pre-patent period (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude methanol extract</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Aqueous Methanol fraction</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Physiological saline solution</td>
<td>50 µl</td>
<td>6</td>
</tr>
</tbody>
</table>
Therefore, antitrypanosomal potential observed in the present study could be due to one or combined effect of flavonoids, tanins, saponins, triterpenes and or steroids detected. This finding further underpins the fact that secondary metabolites work synergistically to produce an effect. Crude extracts exhibited better bioactivity than individual component (Cock, 2011). Mahomoodally et al. (2010) observed that bioactive metabolites in plant extract worked catalytically and synergistically to produce an effect that surpassed the total activity of the individual constituents.

Since completely immobilized parasite in crude methanol extract of S. longipedunculata in the in vitro test became infective following inoculation into rats, it can be concluded that stem-bark of S. longipedunculata is trypanostatic, but not trypanocidal in action. Elucidating the mechanism of trypanostatic effect of plant extract can facilitate screening of medicinal plants for their trypanocidal potential.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Profuse gratitude to technical staff of Department of Veterinary Pharmacology and Toxicology, and Kate Adeyanju, Department of Parasitology and Entomology, A.B.U., Zaria, for technical assistance.

REFERENCES


and control in Bungoma district, Western Kenya. J. Ethnopharmacol. 140:298-324.

African Journal of Biotechnology

Related Journals Published by Academic Journals

- Biotechnology and Molecular Biology Reviews
- African Journal of Microbiology Research
- African Journal of Biochemistry Research
- African Journal of Environmental Science and Technology
- African Journal of Food Science
- African Journal of Plant Science
- Journal of Bioinformatics and Sequence Analysis
- International Journal of Biodiversity and Conservation