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Neuro-endocrine effects of aqueous extract of *Amaranthus viridis* (Linn.) leaf in male Wistar rat model of cyclophosphamide-induced reproductive toxicity

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ABSTRACT

Cyclophosphamide (CP) is a widely used cytotoxic alkylating agent with antitumor and immunosuppressant properties that is associated with various forms of reproductive toxicity. The significance of natural antioxidants of plant origin should be explored, especially in a world with increasing incidence of patients in need of chemotherapy. The neuro-endocrine effects of aqueous extract of *Amaranthus viridis* (Linn.) leaf (AEAVL) in Wistar rats with CP-induced reproductive toxicity was determined. Forty rats were used for this study such that graded doses of the extract were administered following CP-induced reproductive toxicity and comparisons were made against control, toxic and standard (vitamin E) groups at $p < 0.05$. The synthetic drugs (CP, 65 mg/kg i.p. for 5 days; Vitamin E, 100 mg/kg p.o. for 30 days) as well as the extract (100, 200 and 400 mg/kg p.o. for 30 days) were administered to the rats at 0.2 mL/100 g. CP induced reproductive toxicity as evidenced by significantly lowered levels of FSH, LH and testosterone, perturbation of sperm characterization, deleterious disruptions of the antioxidant system as evidenced by decreased levels of GSH as well as elevation of TBARS activity. Histopathological examination showed hemorrhagic lesions with scanty and hypertrophied parenchymal cells in the pituitary while the testis showed ballooned seminiferous tubules with loosed connective tissues and vacuolation of testicular interstitium. These conditions were significantly reversed ($p < 0.05$) following administration of the graded doses of the extract. It was, therefore, concluded that AEAVL could potentially be a therapeutic choice in patients with CP-induced neuro-endocrine dysfunction and reproductive toxicity.

1. Introduction

Reproductive toxicity is the occurrence of biologically adverse effects on the reproductive system of living organisms that may result from exposure to environmental agents or xenobiotics. The toxicity may be expressed as alterations to the reproductive organs, related endocrine system and or pregnancy outcomes [1]. According to existing literatures, the use of anticancer drugs affects aspects of reproductive function, including fertility, in both human and experimental animals [2–4]. CP is a widely used cytotoxic alkylating agent with antitumor and immunosuppressant properties [5]. It is used for the treatment of various forms of cancer such as chronic and acute leukemia, multiple myeloma, lymphomas, rheumatic arthritis and systemic lupus erythematosus [5]. It is also used as an immunosuppressive agent for organ transplantation [5]. However, CP treatment is associated with various forms of reproductive toxicity despite its wide spectrum of clinical use [6]. Due to severe toxic and undesirable side effects in multiple organ systems, there is a gradual but increasing limitation to the use of this drug for cancer chemotherapy [6,7]. It is therefore imperative to develop strategies that are geared towards minimizing the side effects of this apparently health-beneficial drug in a world of increasing incidence of patients in need of chemotherapy.

Vitamin E, a group of compounds comprising both tocotrienols and tocopherols, is a lipid-soluble antioxidant that protects cell membranes from oxidation by reacting with lipid radicals that are produced in the lipid peroxidation chain reaction as well as expresses its antioxidant functions in the glutathione peroxidase pathway [8–10]. Its most biologically active form is alpha toco-

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pherol [8,11] and is apparently a conventional drug used in the amelioration of xenobiotics-induced reproductive toxicity in animal models [12–14].

The pathophysiology of CP has implicated both oxidative stress and reactive oxygen species (ROS) generation [15–17]. ROS plays a critical role in the pathological mechanism of male reproductive dysfunction [7,18]. These proposed pathophysiological mechanisms of CP point towards the fact (hypothesis) that treatment with a potent antioxidant can possibly help reverse or ameliorate its toxic effects. This study aimed at engaging ethno-botanical approach to test this hypothesis since plant derived medicines, besides their easy availability and being relatively cheaper for the affordability of a common man, is relatively safer than the synthetic alternatives [19] and has been used as a source of inspiration for the development of novel drugs [20].

Amaranthus viridis (Linn.) is an annual herb of the family Amaranthaceae [21]. In Nigeria, it is commonly called "green leaf" or "tete" amongst the Yoruba tribe. The plant is erect, having stems that are up to about 10–100 cm in length with branched glabrous leaves. In Greek, *Amaranthus* is translated as "never-fading flower". In the light of modern research, the various parts of the plant have been explored for health-boosting potentials. In traditional medicine, powdered seeds of the flower have been shown to possess health beneficial effects such as treatment of stomach problems [22] and reduction of labour pain in pregnant women [23]. The stems have potent anti-diuretic potentials while the traditional use of the leaves range from anti-diabetic, anti-rheumatic, laxative, diuretic, analgesic agent as well as an anti-inflammatory agent used in the treatment of asthma, eye and respiratory dysfunctions [24–26]. Also, the plant experimentally evaluated for antioxidant, anti-hyperlipidemia and anti-diabetic properties [27,28], anti-nociceptive [29], hepatoprotective [30] as well as antipyretic and analgesic activities [31].

Despite the experimental evidences portraying a wide range of its health-beneficial effects, our literature survey revealed that (till date) there is no experimental evidence of this plant, *Amaranthus viridis* (Linn.), effects on the neuro-endocrine function in an animal model of drug-induced reproductive toxicity. In order to bridge this gap in knowledge, this study was embarked upon to assess the neuro-endocrine effects of the aqueous extract of *Amaranthus viridis* (Linn.) leaf (AEAVL) in male Wistar rats with cyclophosphamide-induced reproductive toxicity.

2. Materials and methods

2.1. Plant material, drug and biochemical kits

Fresh leaves of *Amaranthus viridis* were obtained from the Farm House of the Department of Crop Science and Production, Faculty of Agricultural Science, Obafemi Awolowo University (OAU), Ile Ife, Osun State, and certified by a Taxonomist in the Department of Botany, OAU, Ile-Ife; where a voucher specimen (IFE – 17424) was deposited.

Salt of cyclophosphamide (CP) was purchased from Zuvius Lifesciences Pvt. Ltd, Mumbai, India while th kits for hormone assay were purchased from Monobind Inc., Lake Forest CA 92630, USA (Accu-Bind Elisa Microwells).

2.2. Plant extraction

Fresh leaves of *Amaranthus viridis* were air-dried, pulverized with an Electric Pulverizer (DIK-2910, Daiki Rika Kogyo Co. Ltd, Tokyo-Japan) and thereafter macerated in water. This mixture was subjected to constant shaking for a period of 24 h with the aid of an Electric Shaker and afterwards filtered under vacuum using Buchner funnel and Whatman number 2 Filter Paper (Whatman PLC, Middlesex, UK). The filtrate was concentrated under vacuum using a Rotary Evaporator (HahnShin Scientific, HS-2005-N) and thereafter freeze-dried in a Lyophilizer (Ilshin Lab. Co. Ltd, Seoul, Republic of Korea). The resulting yield was kept in a desiccator until when needed.

2.3. Detection and quantification of phytochemicals

Alkaloids were qualitatively determined by the method of Halilu and coworkers [32] and quantified as described by Harbone [33]. Also, the presence of flavonoids was determined by the method of Halilu and coworkers [32] and quantified by the method of Obadoni and Ochuko [34]. Tannins was qualitatively determined by the method of Halilu and coworkers [32] and thereafter quantified by the method of Allen and coworkers [35]. Saponins were qualitatively determined using Froth test as described by Benmehdi and coworkers [36] and quantitatively screened by the method of Obadoni and Ochuko [34]. Quantitative determination of cardiac glycosides was by Keller-Kiliani test as described by [37] Anjali and Sheetal. This was thereafter quantified by the method of Harbone [35].

2.4. Stock solutions of cyclophosphamide and aqueous extract of amaranthus viridis (Linn.) leaf

Sixty five (65) mg/kg stock solution of cyclophosphamide (CP) was prepared as follows; 500 mg of CP salt was dissolved in 15 mL of distilled water and administered at 0.2 mL/100 g rat per day via intraperitoneal route. Thus

each 100 g rat received 6.5 mg of CP per day; an equivalent of 65 mg/kg/day of CP.

The extract's stock solution for 100 mg/kg was prepared by dissolving 1 g of AEAVL in 20 mL of distilled water. Consequently, every 100 g rat received 0.2 mL of the extract to prevent the deleterious effects of fluid overload. Accordingly, stock solutions for 200 mg/kg and 400 mg/kg of AEAVL were prepared by dissolving 2 g and 4 g of the extract, respectively, each in 20 mL of distilled water.

2.5. Animal management and experimental design

All experimental protocols were in strict compliance with the guidelines for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals [38] and approved by local Institutional Research Committee. Forty (40) male Wistar rats of about 3 months of age, weighing 150–180 g, were used for this study. They were purchased from the Animal Holdings of the College of Health Sciences, OAU, Ile – Ife, Osun State, Nigeria where the study was carried out. They were housed in plastic cages under natural light and dark cycle and allowed access to standard laboratory rat chow (Caps Feed PLC Osogbo, Nigeria) and water *ad libitum*.

The rats were divided into eight groups of five rats (n = 5) each as follows; Group 1 (Control) received distilled water (0.2 mL/100 g) intraperitoneally for 5 days and thereafter left for a period of 30 days after which they were sacrificed. Group 2 (Toxic) received 65 mg/kg of cyclophosphamide (CP) intraperitoneally for 5 consecutive days after which they were sacrificed. Group 3 (CP + Recovery) were pretreated as group 2 and thereafter left for a recovery period of 30 days after which they were sacrificed. Group 4 (CP + Vitamin E) were pretreated as group 2 and thereafter received 100 mg/kg/day Vitamin E via oral route for 30 days after which they were sacrificed. Groups 5, 6 and 7 were each pretreated as group 2 and thereafter received graded doses of aqueous extract of *Amaranthus viridis* (Linn.) leaf (AEAVL) at 100, 200 and 400 mg/kg/day respectively via oral route for 30 days after which they were sacrificed. Group 8 received AEAVL (only) at 200 mg/kg/day for 30 days and thereafter