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ANTIOXIDANT AND GENOPROTECTIVE ACTIVITIES OF AQUEOUS EXTRACT OF *ANCHOMANES DIFFORMIS* AGAINST LEAD-INDUCED CHROMOSOMAL ABERRATION IN ALBINO RAT

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Abstract

Involvement of antioxidants has recently been linked in anticlastogenicity. The current study therefore, sought to carry out the genoprotective and antioxidant activities of aqueous extract of *Anchomanes difformis* (AEAd) against lead-induced chromosomal aberration in the bone marrow of albino rats. Twenty (20) Albino rats were used in the experiment and they were randomly selected into group of four (4 groups). The groups were; group 1 (positive control), 2 (2.5 mg/kg lead acetate only), 3 (2.5 mg/kg lead acetate and 50 mg/kg AEAd), 4 (2.5 mg/kg lead acetate and 100 mg/kg AEAd) and 5 (2.5 mg/kg lead acetate and 200 mg/kg AEAd). *In vitro* total antioxidant potentials (total phenolic and flavonoid contents) of AEAd were quantified along with various antioxidant activities such as; Fe^{2+}-chelating ability, ferric reducing power (FRAP), nitric oxide (NO) radical scavenging power, hydroxyl (OH^-) radical scavenging ability, 1,1-diphenyl, 2- picrylhydrazyl (DPPH) scavenging ability and 2, 2'-azino-bis (3-ethylbenthiazoline-6-sulphonic acid) (ABTS) scavenging ability. The result indicated that AEAd considerably showed a good free radical scavenging property in a concentrations dependent manner. In addition, the levels of micronucleated polychromatophilic erythroblasts (MNPCEs) in the AEAd-treated groups were significantly ($p>0.05$) lower when compared to group 2. Though, several study have emphasized the toxicity of the plant at higher concentrations but in this study, various moderate concentrations were able to significantly exhibit anti-chromosomal damage which could be accredited to the endowed antioxidant potentials and bioactive components in the plant.

Keywords: *Anchomanes difformis; antioxidant; free radical; chromosomal aberration.*

Introduction

The application of alternative medicine is gaining ground and on the increase from a cultural perspective with transcendence, either as a result of the high cost of formulated pharmaceutical health care or impoverishment. A good number of traditional plant-derived biologically active products have been successfully engaged in the management of different diseases such as cancer, across the globe [1]. World Health Organization, has recently estimated that 80% worldwide rely on herbal medicines for some aspect of infection and diseases [2]. In the last decades, there has been an upsurge of interest in the effect of plant but with little information on chromosomal aberration. Chromosomal aberrations (CA) is one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents [3]. In epidemiological studies, damage to DNA can cause genetic alterations and the resulting alterations of DNA structure are generally incompatible with its vital role in preservation and transmission of genetic information, consequently leads to the development of derangement in the system if genes that control cell growth are involved [4]. An increasing number of cell death and human hereditary diseases that are characterized by severe developmental problems and/or a predisposition to disorder have been linked to Chromosomal damage. Bonassi et al. [5], claim that people with elevated frequencies of CA in their peripheral blood lymphocytes have a significantly elevated risk of developing cancer which has received attention in recent years.

Lead (Pb) compound is the most commonly available toxic and poisonous metal and practically detectable in all spheres of ecological systems [6]. It has also been widely engaged in anthropogenic activities such as leaded gasoline, lead paints, etc [7]. (Koller et al., 2004). The incessant use of Pb has made it possible to be realistically available in the vicinity of factories [8]. In addition, exposure to Pb has been suggested to occur primarily via oral route, with some contribution from inhalation which invariably induce chromosomal alterations in form of micronuclei and sister chromatid exchange as well as mutation leading to cancer or degenerative diseases [6]. However, knowledge of probable mechanisms by which lead causes cancer is still rather complex and not fully understood, but several likely mechanisms have been suggested to expound the oncogenic activities of lead at the cellular level ([9]. A growing evidence based on epidemiological studies as well as clinical investigation trials suggest that natural bioactive antioxidants may as well play a pivotal role in preventing or slowing the progression of any form of cancer as it may occur in the toxicity of Pb [10; 11].

Anchomanes difformis (*Ad*)is an herbaceous plant in a family *Aracea* with prickly green stem, perennial stout, a huge divided leaf and spathe that stem from a horizontal tuber. They are commonly found in West Africa [12]. The rhizomes are eaten but only after special preparation that entails prolong washing and cooking of early shooting stage. The root leaves and stems are purgatives with the aqueous extract of the tubers being used to cure dysentery by traditional healers [13]. It has also been reported in the treatment of kidney-pains, Oedemas and as diuretic for treating urethral discharge, jaundice and as poison antidote [14]. It is sometimes called Forest *Anchomanes* in English while in South West Nigeria it is known as *Ogirisako*, (Igbo language) and *Langbodo* in Yoruba language [15].

Materials and Methods

Chemicals and Reagents used

Chemicals and reagents such as; 1,10-phenanthroline, gallic acid, Folin-Ciocalteau's trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), methanol, $FeCl_3$, sodium carbonate ($NaCO_3$), aluminum chloride ($AlCl_3$), Tris-HCl buffer, Iron II sulphate ($FeSO_4$), sodium hydroxide (NaOH), sodium nitrate ($NaNO_3$), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and other chemicals were of analytical grades and procured from well-established chemical companies. All reagents were prepared in all-glass apparatus using sterilized distilled water.

Collection of Plant Sample

The aerial leaves of *Ad* were collected from the Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. Voucher

specimen was deposited at the Departmental herbarium of Plant Science, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria, for identification and authentication. A voucher number U.H.A.E.51 was assigned by a botanist from the data base.

Preparation of the aqueous extract

The aqueous extract of the powered *Ad* leaves was prepared using the method of Aguawa and Mittal [16]. The leaves were air-dried in the laboratory at ambient temperature (30 ± 2°C) for 10 days, pulverized using a laboratory mechanical grinder and the fine powder obtained stored until further use. 1 g of the powdered sample was dissolved in 100 ml of distilled water (w/v) and boiled for 15 min (Hot water extraction). The mixture was decanted and filtered using sterile Whatman filter paper (grade1). The filtrate was then stored until further use.

Animal treatments

Twenty (20) Wistar Albino rats weighing between 120-180 g were obtained from the Animal house of the Department of Biochemistry, School of Science and Technology, Federal Polytechnic, Ado-Ekiti, Ekiti State. The rats were acclimatized for two weeks and were given standard rodent diet and water *ad libitum* in a clean and disinfected environment.

Experimental protocol

The rats were randomly distributed into five treatment groups of four rats each. The various groups were:
Group 1; group fed with the pelleted animal feed and water *ad libtum* (Positive control) for two weeks;

Group 2; group administered 2.5 mg/kg lead acetate alone (Negative control) for two weeks;

Group 3; group administered 2.5 mg/kg lead acetate with 50 mg/kg of AEAd for two weeks;

Group 4; group administered 2.5 mg/kg lead acetate and 100 mg/kg of AEAd for two weeks;

Group 5; group administered 2.5 mg/kg lead acetate and 200 mg/kg of AEAd for two weeks.

Preparation of blood film samples

Following the last day (fourteenth day) of administration of lead-acetate alongside extract, the rats were sacrificed through cervical dislocation and the femur removed and stripped clean of muscle, according to the method used by Afolabi et al. [6]. Thereafter, slides with film of blood smear were prepared and lightly mixed with a drop of fetal bovine serum (FBS) to form a homogenous mixture. The slides were air-dried and fixed in absolute methanol and stained in 5% Giemsa at pH 6.8.

Chromosomal aberration assay

The method used for obtaining chromosome preparations from the bone marrow cells was based on the method described by [17]. The assay is based on an increase in the frequency of micronucleated/polychromatophilic erythroblast in bone marrow of animals as an indication of induced chromosomal damage. The frequency of polychromatophilic erythroblasts (PCEs) per total erythroblast was determined using a sample size of 1000 erythrocytes per slide and also the number of MNPCEs was determined using 1000 PCE per slide.

Data Analysis

Data were pooled and expressed as mean ± standards deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests was applied for the post hoc test. The p value < 0.05 was considered statistically significant.

Results

The results of phenol and flavonoid contents is shown in figure 1 shows that the AEAd has a considerable amount of phenol and flavonoid which is concentration –dependent. The phenol and flavonoid content increased as concentration of the extract was increased.

The Fe^{2+} chelating power and ferric reducing antioxidant property (FRAP) of the AEAd increased as the concentration of the extract increased from 2-10 mg/ml.

Nitric oxide scavenging activity of the extracts as shown in fig. 3, revealed inhibitory activity of the AEAd with increasing concentration which varied from 2- 10 mg/ml. Also, 2, 2-diphenyl-1-picrylhydrazyl

(DPPH) inhibitory activity as shown in fig. 3 revealed that, the AEAd demonstrated similar inhibitory activity in concentration-dependent manner.
The ABTS Scavenging ability of AEAd as represented in fig. 4 showed that the scavenging effect of the extracts in concentrations ranging from 2 -10 mg/ml on the ABTS radical was concentration-dependent.

Discussion

Over the years, studies have shown that cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases such as cancer, cardiovascular disease, among others [18]. Cancer is the leading cause of death, claiming more than 6 million lives in a year worldwide [19]. Largely, the activities of free radicals have been implicated in the pathogenesis of this menace [20]. Moreover, antioxidants roles in inhibiting and scavenging free radicals, thus providing protection to human system against degenerative diseases have been extensively studied [21].
Moreover, number of studies have suggested the medicinal importance of natural plants in folkloric medicine due to a range of substances that act as antioxidative agents [22]. Polyphenolics such as phenolic compounds and flavonoid include a large class of phytochemicals that are endowed with interesting biological properties [23]. Interestingly, AEAd (Fig. 1) at minimal concentrations showed considerable phytochemical contents in concentration-dependent manners.
Apart from several environmental or hereditary factors, free radicals can also adversely affect various important classes of biological molecules such as nucleic acids, lipids, and proteins, thereby altering their normal redox states leading to increased oxidative stress which underlies pathogenesis of degenerative disorder [24]. Hydroxyl radical released via the catalytic role of Fe^{2+} through Fenton's reaction has been a well-recognized highly reactive free radical [25]. It can strongly react with both organic and inorganic molecules including DNA, proteins, lipids, among others and cause severe damage to the cells than any other ROS [20]. In this study (Fig. 2), AEAd was able to inhibit catalytic process of Fe^{2+}, thus able to play a significant role in protecting humans from deleterious activities of free radicals at a very low concentration as earlier reported by Jacob and [26].
In addition, this study considered the nitric oxide inhibitory potential of AEAd (Fig.3). Elevation of nitric oxide has been implicated in many pathophysiological processes, howbeit, inhibition of nitric oxide or its production has also been described beneficial in the management of degenerative conditions [27]. Subsequently, the reductive potential of AEAd on ABTS radical (Fig. 4) suggest a strong antioxidant capacity, however, the mechanism of actions of the various inhibitory activities of AEAd is not fully understood in this study, however, it could be credited to the redox potentials of the phytochemical constituents as established in Fig. 1
DNA damage when caused, it may remain unrepaired and this may lead to cell death or genomic instability [28]. Direct interactions between DNA/protein and heavy metals at the molecular level have been extensively studied but knowledge of the mechanisms by which lead causes cancer is still rather complex and not fully understood [29]. However, MNPCEs/PCE index has been often used in the estimation of level of DNA damage via the bone marrow. Bone marrow is the most delicate structure in response to cytotoxic effects of carcinogenic agents [30]. More studies into anticlastogenic activities of AEAd (Table 1) in this study revealed substantial reduction in the levels of MNPCEs in the bone marrow erythrocytes of the groups treated with the extract. This is in line with previous study that related the antigenotoxicity potentials of a plant with the level of antioxidant [31].

Conclusion

The study has shown various antioxidant activities of AEAd, which indeed has given an insight on the usage of the plant in the treatment of various ailments among some folks. Though, several study have emphasized the toxicity of the plant at higher concentrations but in this study, various moderate concentrations were able to significantly exhibit anti-chromosomal damage which could be accredited to the endowed antioxidant potentials and bioactive components of the plant. However, further study is suggested so as to understand the mechanism of action through which the plant extract exhibits geno-proctetive property.

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Table 1: Effect of aqueous extract of the leaves on lead-induced chromosomal aberration in bone marrow of rats.

Treatment Groups	Frequency of MNPCEs/1000PCEs (%)	Frequency of NPCE/TEs (%)
Group 1	0.05±0.03[a]	1.00±0.00[a]
Group 2	2.45±0.29[c]	1.03±0.00[c]
Group 3	0.60±0.08[b]	1.01±0.00[b]
Group 4	0.08±0.06[ab]	1.00±0.00[a]
Group 5	0.18±0.03[ab]	1.00±0.00[a]

Data are expressed as mean±SD of quadruplicates (n=4). Superscripts [a, b& c] indicate levels of significance at $p<0.05$ when compared to group 2. **Keys**: PCEs, polychromatophilic erythroblasts; MNPCEs, micronucleated polychromatophilic erythroblasts; TEs, total erythroblasts

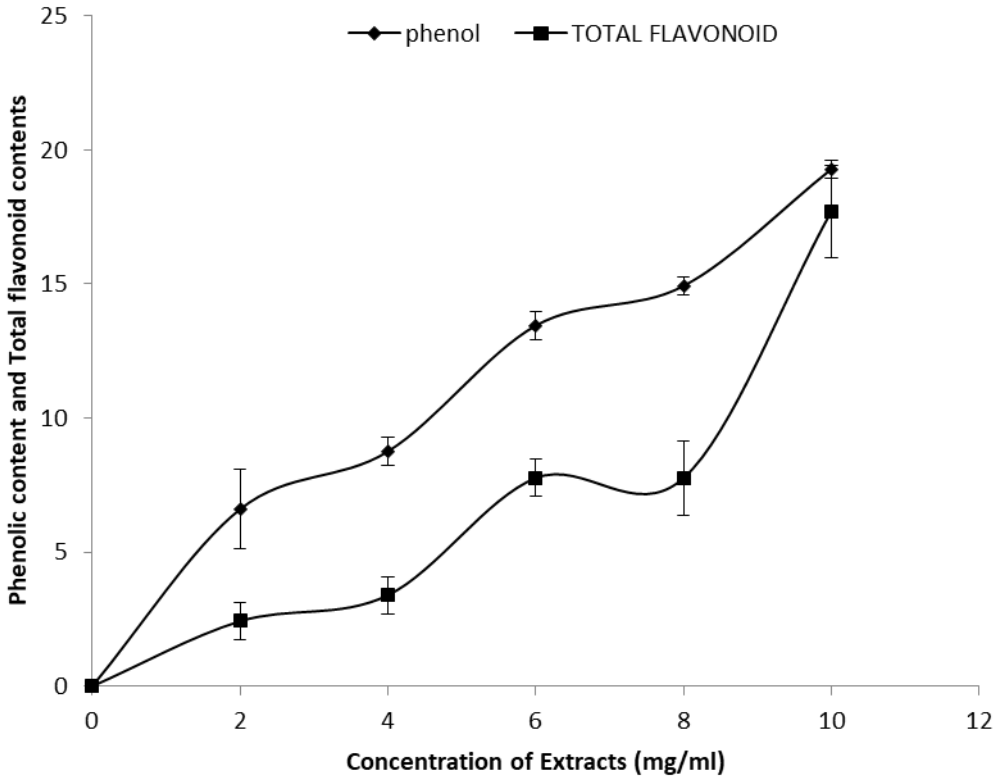

Figure 1: Total phenolic and flavonoid contents (mg/g) of AEAd.

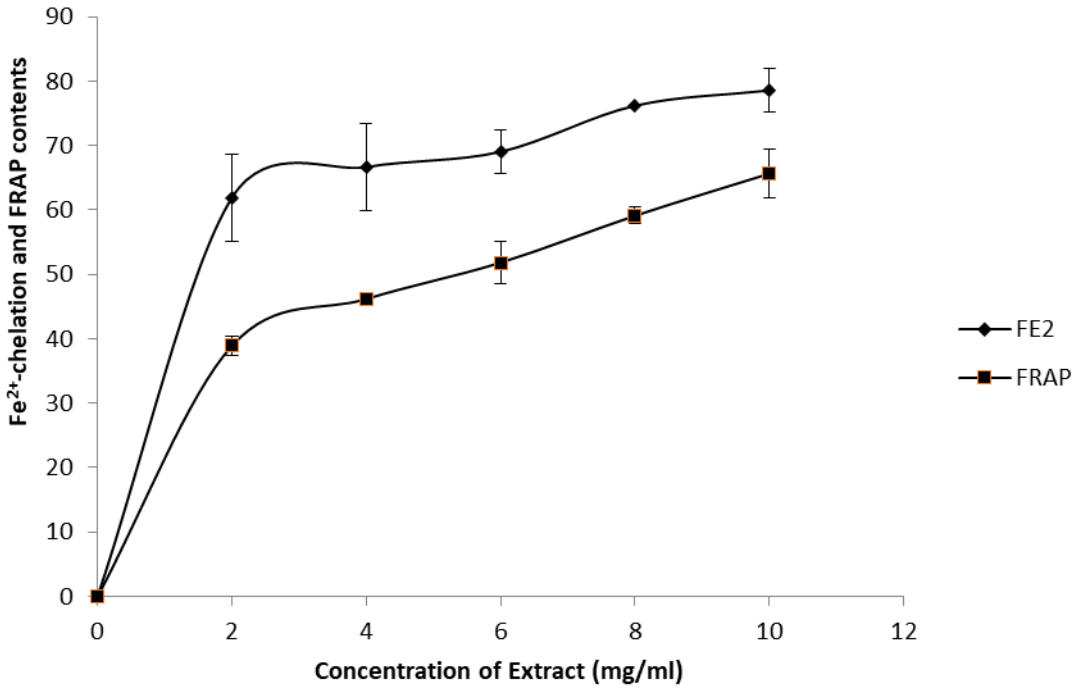

Fig. 2: Fe^{2+} chelating and ferric reducing antioxidant property of AEAd.

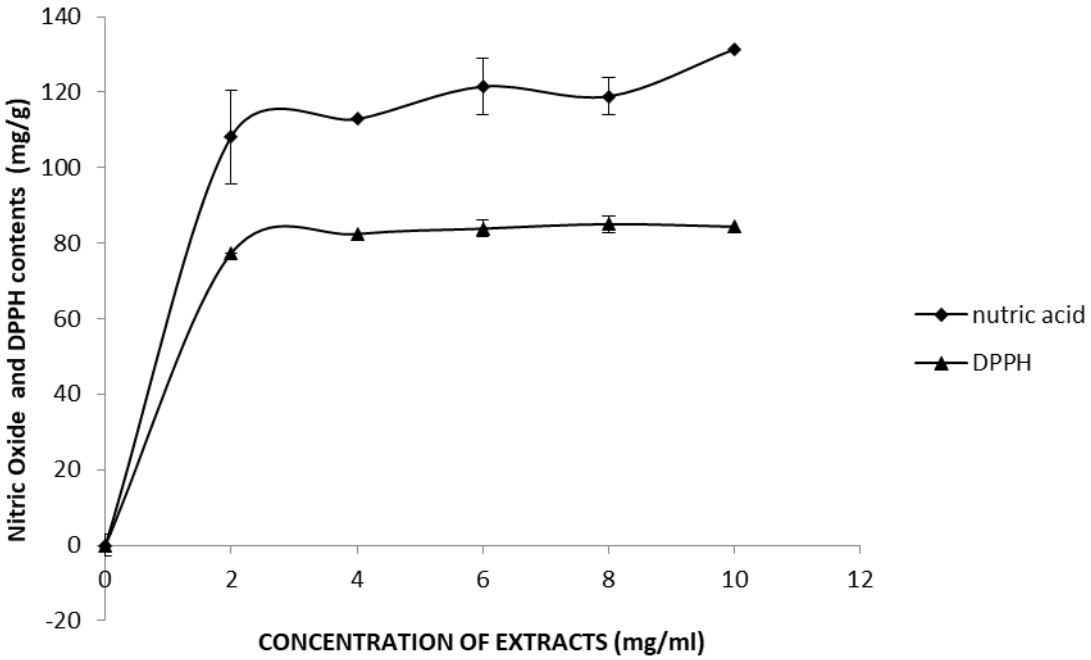

Fig. 3: Nitric oxide and DPPH Scavenging Activities of AEAd.

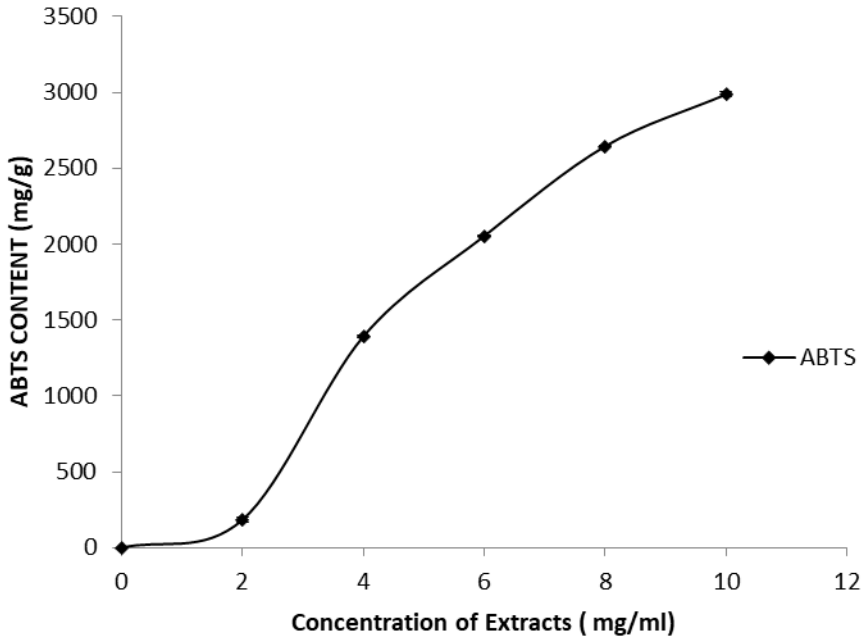

Fig. 4: ABTS scavenging activity of AEAd.