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ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHYLACETATE LEAF EXTRACTS OF *ALCHORNEA CORDIFOLIA* FOUND IN EBONYI STATE, NIGERIA

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ABSTRACT

This research investigated the antioxidant activity of leaf extracts of *Alchornea cordifolia* and their roles in infertility. This study was carried out using albino rats. The dried leaf extracts were prepared with deionised water and ethylacetate. Chemical analyses of the extracts were performed using standard methods. The antioxidant effect of the extracts was monitored in 50 rats placed in 5 groups of 5 rats each. Group 1 acted as normal control while group 2 was the negative control. Groups 3, 4 and 5 were treated with CCl_4 and given the aqueous and ethylacetate extracts at 200, 400 and 800 mg/kg. The results on antioxidant potentials of both extracts showed a significant increase ($P<0.05$) in the level of hepatic MDA on treatment with CCl_4 as seen in the negative control which reduced significantly ($P<0.05$) in a dose dependent manner on administration of both extracts. The results also showed a significant decrease ($P<0.05$) in the levels of the antioxidant indices GSH, SOD and catalase which increased significantly in a dose dependent manner on administration of both extracts with ethylacetate extract showing greater potentials. This analysis has confirmed the use of this plant as an antioxidant herb for treatment of diseases caused by oxidative stress.

From the results of this study, it can be concluded that aqueous and ethylacetate extracts of *A. cordifolia* have potential to improve infertility associated with low spermatogenesis and oxidative stress through its chemical contents and antioxidant activities.

INTRODUCTION

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designated as medicinal plants. Many diseases are caused by oxidative stress. The search for medicinal plants with antioxidant properties have increased in the past decades due to the link between antioxidants and several degenerative diseases with hope of finding an effective remedy for several present-day diseases and means to delay symptoms of aging. Several abnormalities are associated with excessive cellular oxidative stress and aging include type II diabetes, spondylosis, neurodegenerative diseases and some types of cancer (Khan *et al*., 2010). Furthermore, there is also a huge demand for natural antioxidants in food and related industries, for replacing the synthetic preservatives. The enzymatic antioxidant systems such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase play a coordinated role to prevent reactive oxygen species from causing oxidative damage. Superoxide dismutase is an important cellular defence enzyme that catalyses the dismutation of superoxide radicals into water and oxygen while catalases on the other hand are heme-containing proteins that guide against the effect of reactive oxygen species from exerting toxic effects on the cells by converting hydrogen peroxide to water and oxygen molecular (Nair *et al.,* 2008).

Alchornea cordifolia belongs to the family of Euphorbiaceae. The common names are Christmas bush and Dovewood. In Nigeria, it is called"ububo", in Igbo,"ipa esinyin", in Yoruba and "banbani" in Hausa. The plant is a strangling shrub or small evergreen plant that can grow up to 32 feet tall in swampy locations. It is propagated through seed or stem cuttings and grows well in very moist soil. The leaves and stems are used in the traditional medicine of many African countries for the treatment of acute and chronic inflammatory disorders, cancer, veneral diseases, ulcers, canker sores, to prevent miscarriage and cure various reproductive diseases (Amos-tautua *et al.,* 2011). The bark is tinctured with local rum for its aphrodisiac effect, as a local remedy for cold, rheumatism, arthritis and muscle pains (Agbor *et al*., 2004). It is used as an antidote for poison, as a sedative and antispasmodic. The parts mostly used for

medicine are the leaves and stem bark but the leaf is more potent.

The aim of this study was to analysis the leaves of *Alchornea cordifolia* plant for their antioxidant properties in relation to the concentrations of these phytochemicals.

MATERIALS AND METHODS

Collection and Preparation of Test Materials

Fresh leaves of *Alchornea cordifolia* were collected from Ohaukwu local government Area of Ebonyi state and were identified by a Taxonomist. The plant samples were dried under ambient temperature before they were grounded into fine powder using manual grinder.

Test for Phytochemicals

Chemical tests were carried out on the extracts of *A. cordifolia* for the qualitative and quantitative determination of phytochemical constituents as described by Harborne (1973), Trease and Evans (1989) and Pearson (1976).

Experimental Design

The albino rats used for the study were acclimatized in separate cages inside the animal house of Biochemistry/Biotechnology Department of Ebonyi State University for two weeks before the commencement of the administration of the extracts. All the rats in both test and control groups were allowed free access to feed and water, throughout the experimental period. They were divided into five groups with five rats in each group (n=5) for the following studies:

Antioxidant Potentials, effect on reproduction and biochemical studies.

Antioxidant Potentials

The 50 rats used for this study were divided into 5 groups. Group 1 received deionized water to act as control for all the groups while group 2 received a single dose of 1 ml/kg of CCl_4 in olive oil (1:1 volume) through an intraperitoneal (IP) injection, inoculated dose was 0.1 ml. Groups 3, 4 and 5 were injected with CCl_4 and treated with 200, 400 and 800 mg/kg body weight of aqueous and ethylacetate leaf extracts respectively. At the end of the 14 days treatment period, the animals were sacrificed under light anesthesia and the livers were removed, rinsed with saline and used for assessment of lipid peroxidation. The hepatic tissues were homogenized in 10 mM KCl in 1.15 % phosphate buffer and ethylenediamine tetraacetic acid (EDTA; pH7.4) and centrifuged at 5000 x g for 10 min. The supernatant was used to assay the level of thiobarbituric acid reactive

substances (TBARS) and to estimate the amount of GSH, SOD and catalase activities.

Determination of Lipid Peroxidation Activity of *Alchornea cordifolia*

Hepatic MDA was estimated by the method of Fraga *et al.*, (1981).

Principle: Malondialdehyde, a secondary product of lipid peroxidation reacts with thiobarbituric acid (TBA) to give a pink coloured pigment.

To 0.5 ml of liver and testis homogenate respectively, 0.5 ml normal saline and 1 ml of 10% TCA were added, mixed well and centrifuged at 3000 rpm for 20 minutes. To 1.0 ml of serum and tissue supernatant respectively, 0.25 ml of Thiobarbituric acid (TBA) solution were added and the mixture kept in a water bath at 95 ºC for 1 hour. The test tubes were then cooled to room temperature under running water and absorbance measured at 532 nm.

Determination of SOD

The activity of SOD in the liver was assayed by the method of Kakkar *et al*., 1984.

Principle: This is based on the ability of superoxide dismutase to catalyse the dismutation of superoxide into oxygen and hydrogen peroxide.

The assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3), 0.1 ml phenazine methosulphate, 0.3 ml NTB (Nitro blue tetrazoline), 0.2 ml NADH and approximately diluted enzyme preparation and water in a total volume of 3 ml. After incubation at 30 ºC for 90 sec, the reaction was terminated by the addition of 1.0 ml of glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4.0 ml n-butanol. The color intensity of the chromogen in the butanol layer was measured at 560 nm against n-butanol.

Determination of GSH

GSH was determined by the method of Ellman *et al.,* 1958.

Principle: This is based on the formation of relatively stable yellow colour when Ellman‘s reagent is added to sulfhydryl compound (GSH) to form a chromophoric product known as 2-nitro-5-thiobenzoic acid.

About 0.2 ml of liver homogenate was mixed with 1.8 ml of EDTA solution. To this 3.0 ml precipitating reagent was added, mixed thoroughly and kept for 5 minutes before centrifugation. To 2.0 ml of the filtrate, 4.0 ml of disodium hydrogen phosphate solution and 1.0 ml of DTNB (5, 5- dithio-bis-2-nitrobenzoic acid) reagent were added and read at 412 nm.

Determination of Catalase activity of extracts.

Catalase was assayed according to the method of Maehly and Chance (1954).

Principle: This is based on the ability of Catalase enzyme to catalyse the decomposition of hydrogen peroxide to water and oxygen.

The estimation was done spectrophotometrically at 230 nm. The tissue was homogenized in 2 ml phosphate buffer (pH 7.0) at 1-4 ºC and centrifuged at 5000 rpm. The reaction mixture contained 0.01 M phosphate buffer, 2 ml hydrogen peroxide and the enzyme extract. The specific activity of catalase is expressed in terms of mole of H_2O_2 consumed/min/mg of protein.

STATISTICAL ANALYSIS

Results were presented as mean and standard deviation. The statistical analysis was done using one way analysis of variance (ANOVA). The difference between the mean were tested using LSD. A *p*-value of $P<0.05$ was considered to be statistically significant. All antioxidant assays were done in triplicates.

RESULTS

Effect of Extracts on Antioxidant Indices in CCl_4 Treated Albino Rats.

A significant increase ($p<0.05$) in the level of malondialdehyde (MDA) (Figure 1) was observed in the CCl_4 treated rats (negative control) which was reduced on administration of both extracts at a dose dependent manner of 200, 400 and 800 mg/kg respectively.

Reduced glutathione activity (Figure 2) was significantly decreased ($P<0.05$) in CCl_4 treated group as compared to the control. From the results, glutathione activity gradually increased on administration of the both extracts in a dose dependent manner with ethylacetate extract showing the highest effect close to the control.

The result of the effect of the extracts on catalase (Figure 3) showed a significant decrease ($P<0.05$) in the level of catalase activity in CCl_4 treated rats as compared with the control (14 ± 0.03). The activity of catalase gradually increased on administration of both extracts in a dose-dependent manner with ethylacetate showing a greater effect.

Figure 4 shows that there was a significant difference ($P<0.05$) between the normal control group and negative control group in the level of SOD. However the administration of the extracts increased the

SOD activity significantly in a dose - dependent manner.

The result the effect of aqueous and ethylacetate extracts on serum protein showed a significant decrease ($P<0.05$) in protein level in all rats treated with CCl_4. However on administration of the extracts, it increased significantly in a dose- dependent manner.

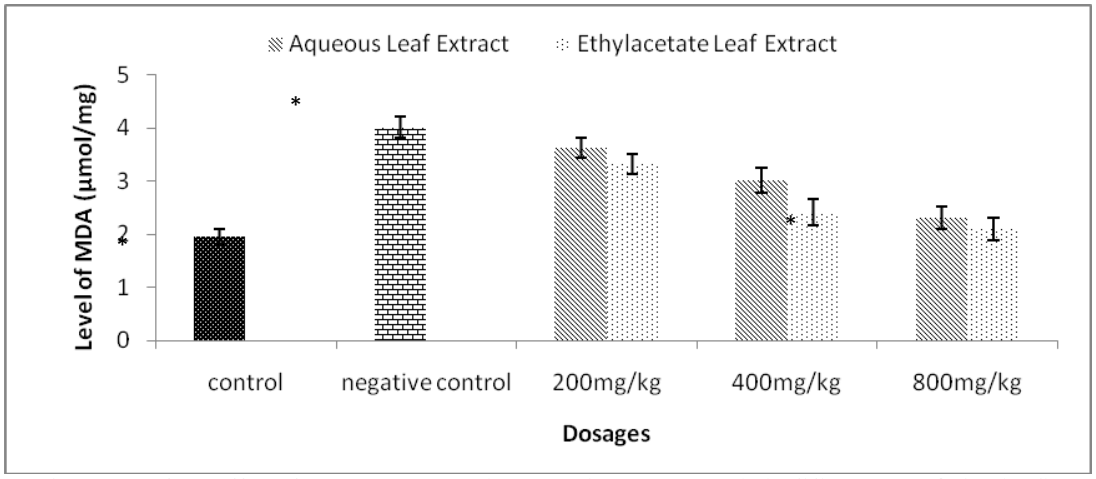

Figure 1: Results of the Effect of Extracts on Hepatic Malondialdehyde (MDA) in CCl_4 Induced Oxidative Stress in Albino Rats.
Plotted values are mean of five determinations ± SD.
*** implies significant difference ($P<0.05$) when compared to the control.**

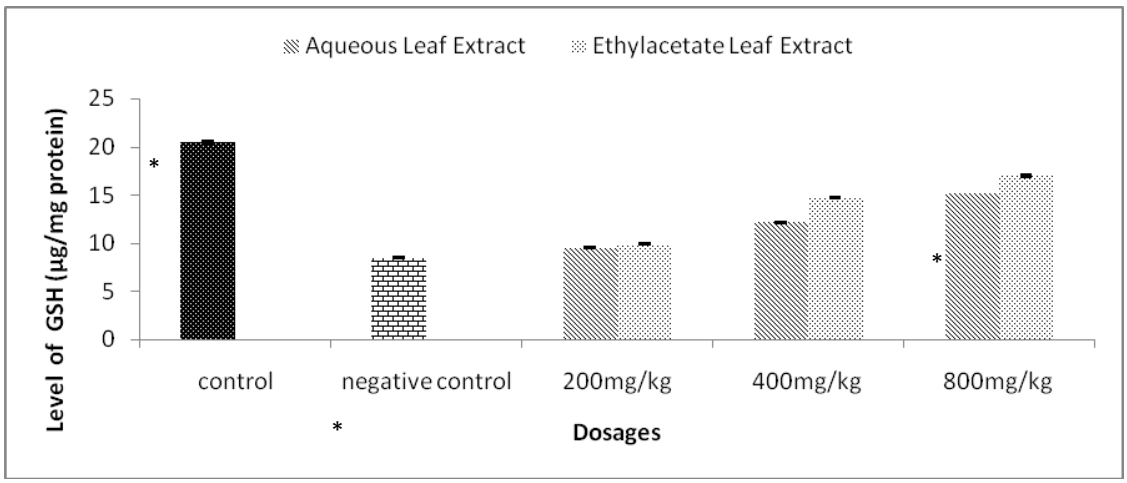

Figure 2: Results of the Effect of Extracts on the Level of Reduced Hepatic Glutathione (GSH) in CCl_4 Induced Oxidative Stress in Albino Rats.

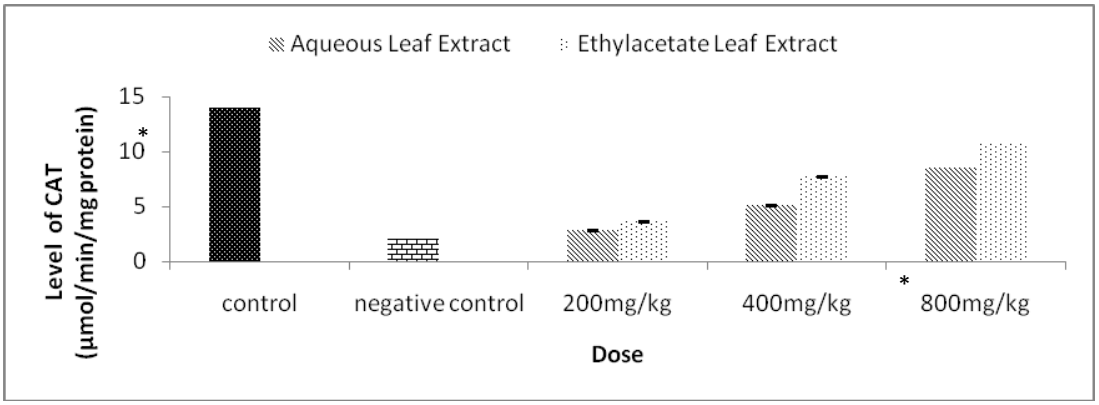

Figure 3: Results of the Effect of Extracts on the Level of Hepatic Catalase (CAT) in CCl_4 Induced Oxidative Stress in Albino Rats.

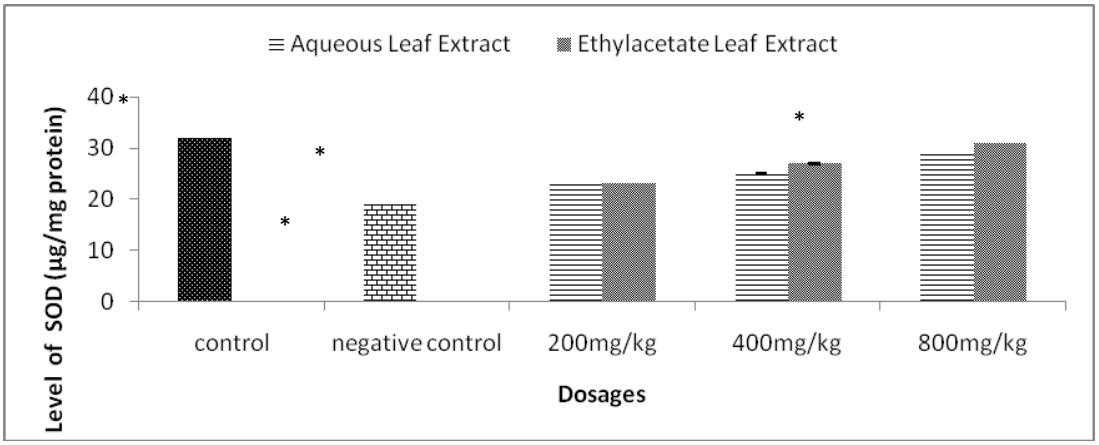

Figure 4: Results of the effect of extracts on the level of hepatic SOD in CCl_4 induced oxidative stress in albino rats.

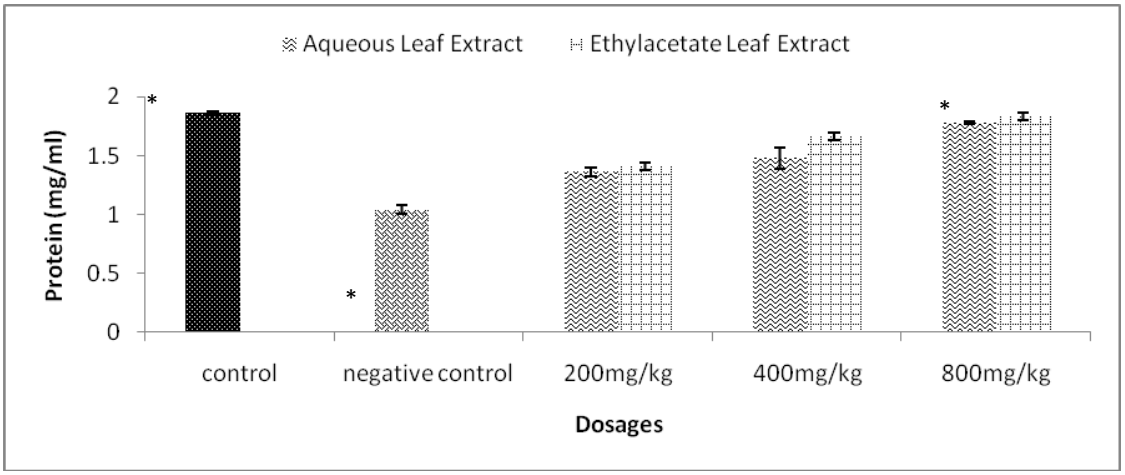

Figure 5: Results of the Effect of Extracts on the Level of Hepatic Protein in CCl_4 Induced Oxidative Stress in Albino Rats.

DISSCUSSION

The use of this plant in enhancing fertility may be explained by its high level of antioxidant activity. The primary defence mechanism of the body is monitored by the indicative expression of MDA, GSH, Catalase and SOD levels which constitutes a mutually supportive team of defence against Reactive Oxygen Species (ROS). Malondialdehyde is the main oxidative product of lipid peroxidation of polyunsaturated fatty acids and high malondialdehyde level is a vital indicator of lipid peroxidation and ultimately tissue damage by series of chain reactions (Miller *et al*., 1996).

The spermatozoa, in common with all cell types have developed an elaborate antioxidant defense system consisting of enzymes such as catalase, Superoxide dismutase and reduced glutathione that scavenges and suppress the formation of reactive oxygen species (Wilcox *et al*., 2004). A significant increase recorded in the level of MDA in the CCl_4 induced rats (negative control) as compared with the control is evidence to decreased production of antioxidants in the treated rat tissues there by shifting the delicate balance in favour of ROS ultimately leading to a pathological damage to sperm cells. This was reduced on administration of aqueous and ethylacetate extracts at a dose dependent manner of 200, 400 and 800 mg per kg respectively.

Glutathione reductase is an intracellular reductant, commonly found in different cells and plays a vital role as a catalyst, in metabolism, in transportation and protection of cells against reactive oxygen species (ROS) and other compounds that are toxic to the cells (Ghosh *et al.,* 2007). Catalase serves the function of rapidly catalyzing the decomposition of hydrogen peroxide into water molecules and less reactive oxygen specie. In the present study, the effectiveness of these extracts was demonstrated using CCl_4 induced peroxidation. Reduced glutathione, Catalase and Superoxide dismutase levels were significantly decreased ($P<0.05$) in CCl_4 induced group as compared to the control. These results are in line with research works by Ebenyi *et al*., 2012 and Ghosh *et al.,* 2007 because it was observed from the results that, enzyme activities gradually increased on administration of the both extracts in a dose dependent manner with ethylacetate extract showing the highest

effect close to the control. Previous study had shown that exposure to CCl_4 during early development can disrupt adult reproductive function by mediated depletion of antioxidant (Hotchkess *et al*., 2008) and elevation of lipid peroxidation. It has been documented by Ghosh *et al.,* 2007 that high levels of the antioxidant enzymes serve as biomarkers of oxidative stress due to alcohol and drug toxicity. It is plausible to suggest that there might be a strong correlation between *A. cordifolia* intake and degenerative disease prevention. The plausible explanation for this phenomenon is that the phenolic compounds present in the extracts are directly involved in the reduction of reactive oxygen species intermediate (Ebenyi *et al.,* 2012). Phenols are very important plant constituent because of their free radical scavenging ability due to their hydroxyl groups. The antioxidant activities results of *A. cordifolia* extracts might justify the rationale for its use in clinical trials in such conditions as Alzheimer's disease, Parkinson, cancer, viral and cardiovascular diseases where free radicals are implicated (Olaleye *et al.*, 2007).

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