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Antioxidant and Antimutagenic properties of Aqueous plant extracts of *Viscum album* against Oxidative Damage in Normal Albino Rats

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ABSTRACT

The aim of this research was to determine the antioxidant and antimutagenic properties of aqueous plant extracts of *Viscum album*. Most of the diseases in the world today have been associated with the influence of reactive oxygen species (ROS) and mutagenic materials. Oxygen free radical induces damage due to peroxidation to biomembranes and also to DNA, which lead to tissue damage, thus resulting in the development of degenerative diseases. Antioxidant is, any substance that, when present at low concentration significantly delays or prevent oxidation of cell content like proteins, lipids, carbohydrates and DNA. Thus antioxidants neutralize the effect of free radicals through different ways and may prevent the body from various diseases. Evidence suggests that Compounds especially from natural sources such as medicinal plants are capable of providing protection against free radicals and mutagens. From the research findings, it could be deduced that *Viscum album* increased the antioxidant enzyme actions and also showed antimutagenic properties. This suggest that the plant can be used as therapeutic agent against a number of diseases.

Keywords : *Viscum album*; peroxidation; Reactive Oxygen Species; mutagens; fixative solution.

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1. INTRODUCTION

In recent years, considerable attention has been devoted to medicinal plants particularly rich in polyphenols, mainly flavonoids and phenolic acids, which exhibit antioxidant properties due to their hydrogen-donating and metal-chelating capacities as potential chemopreventive agents (Grzegorczyk, I, 2007, Rice-Evans, 2004, Chanda, S and Dave, R., 2009). The phenolic compounds have demonstrated protective effects against deleterious effects of genotoxic carcinogens by scavenging reactive oxygen species (ROS) and enhancing host antioxidant defense systems (Subapriya, R. et al., 2004, Weisburger, J.H, 2001,). It is known that many plant infusions have a large number of these molecules (Gurib-Fakim, A., 2006) and, hence, it is reasonable to investigate whether plants have the capacity to prevent the genotoxic potency of specific mutagens or carcinogens that are known to generate free radicals in cells.

One medicinal plant of interest for such investigation is *Viscum album*. *Viscum album* is a highly specialized angiosperm of the family of Loranthaceac. It is a semi-parasitic plant (Deeni and Sadiq, 2002). . *Visum album* has been used in the treatment and management of many diseases such as nervous disorders, spasmodic, diuretic, hypotension, diabetes mellitus, gastrointestinal disorders, hot flushing in menopause for many years (Ohiri, 2003, Oluwaseun and Ganiyu, 2008). Mistletoe extracts could also reduce harmful and mutagenic effects of reactive oxygen species (ROS) generated during radiotherapy and chemotherapy (Onay-Ucar et al., 2006).

Several experimental studies have demonstrated the antioxidant properties of *Viscum album* extract and some of its constituents, but no detailed study has been carried out on the protective effect of *Viscum album* leaf and stem extracts against genotoxins. Therefore, the present study was undertaken to evaluate the antimutagenic and antioxidant potential of the aqueous plant extracts of *Viscum album* in normal rats. Herbal protection was assessed by monitoring endogenous antioxidants superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) and lipid peroxidation in the brain and serum and antimutagenic properties using Allium Cepa.

2. MATERIALS AND METHODS

Preparation of plant extracts:

Fresh leaves of *Viscum album* was obtained in Mushin market and was botanically identified by Mr. Adeleke in the department of pharmagnosy, college of medicine, University of Lagos. The leaves were removed from the stem and were weighed. Both the leaves and stem weighed 178g and 284g respectively.. The different portions were sun-dried and extracted by aqueous extraction. The different filtrates were then freeze- dried to obtain a powdered form. The powder yield of the leaf and stem were 8g and 5g respectively.

Animals:

15 albino rats were bought from the Animal Laboratory center, college of medicine, University of Lagos. The rats weighed between 150-200g and were acclaimatized for two weeks (2weeks). The rats were fed with well formulated rat feed and water.

Determination of Antioxidant Enzymes

Preparation of Homogeneous samples

The brain of the rats was used. The rats' brains were washed in ice cold 1.15% KCL solution, blotted and weighed. They were then homogenized with 0.1M phosphate buffer (P^H = 7.2) and laboratory sand in a mortar and pestle. The homogenate was then centrifuged at 2500rpm for 15mins. The supernatant was then removed and stored at 4^{o}C and then used for analysis.

Superoxide dismutase Activity

The method of Sun and Zigma, 1978 was adopted. The reaction mixture contained 2.95ml 0.05M Na_2CO_3 buffer, P^H = 10.2, 0.02ml of brain homogenate and 0.03ml of epinerphrine in 0.005N HCL was used to initiate the reaction. The reference mixture contained 2.95ml buffer, 0.03ml epinerphrine and 0.02ml of water. The absorbance was read at 480nm for 5mins.

Catalase Activity

The method of Aksenes and Njaa, 1981 was used. The reaction mixture contained 0.2ml of brain homogenate and 1.8ml of 30mM of H_2O_2 while the blank contained only the phosphate buffer. Absorbance was read at 240nm at 30secs intervals.

Reduced Gluthaninone Activity

The procedure of Lindsay, 1968 was applied. 10% TCA was added to the brain homogenate and centrifuged. 1ml of the supernatant was treated with 0.5ml of Ellman reagent (19.8g of 5,5-dithiobisnitrobenzoic acid, DTNB in 100ml of 0.1% $NaNO_3$) and 3.0ml of 0.2M phosphate buffer at P^H=8.0. The absorbance was taken at 412nm.

Lipid Peroxidation Activity

Buege and Aust, 1978 method was followed. 1.0ml of the supernantant was added to 2ml of TCA – TBA – HCL reagent (15% TCA, 0.37% TBA and 0.24N HCL) in the ratio 1 : 1 : 1. The mixture was then boiled at 100^{o}C for 15mins and then allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10mins. The supernatant was removed and the absorbance read at 532nm.

Mutagenicity Test using Allium cepa

The protocol of Fiskesjo, 1993 was adopted. Different concentrations of the plant extracts of *Viscum album* were prepared ranging from 2, 4, 6, 8, and 10mg/ml

respectively alongside the control (distilled water). Onion bulbs were then placed on each of the plants concentrations and left for 48hrs. Every 24hrs, the root length were determined using ruler. After 48hrs, the root tips were cut off with scissor and then fixed in a fixative solution (Acetic acid – alcohol mixture in ratio 3: 1). The root tips were placed on a clean, plain glass slide and a drop of 1N HCL was added to dehydrate and soften the tissue for easy marceration for 2mins. A dissection needle was then used to marcerate the root tip after which a drop of acetic orcein stain (dissolving 2.2g of orcein powder in 45cm^3 of glacial acetic acid or propanoic acid and making up to 100cm^3 with distilled water) was added for 15 – 20mins. The slide content was then covered with a cover slide to allow the stain to spread evenly. The slide with the specimen was then squashed and the edge sealed with white transparent nail hardener. The slide was then viewed under the microscope to observe its mitotic stages and chromosomal aberrations.

Stastical Analysis

The data obtained were analysed using Mean± SD. ANOVA and Fisher's test (F-Test) were used to determine the significance level of difference in the treatment at confidence limit of 95% (p=0.05). The mutagenicity data was expressed as percentage mitotic index and chromosomal aberration.

3. RESULTS:

Antioxidant enzyme assay (brain)	SOD (U/mg pro)	CAT (U/mg pro)	GSH (U/mg pro)	MDA (U/mg pro)
Control	7.10± 0.00	47.51± 0.01	0.51± 0.00	0.049± 0.00
Leaf	7.87± 0.46	52.62± 0.50	0.54± 0.17	0.078± 0.02
Stem	8.35± 0.07	55.86± 0.12	0.59± 0.06	0.081± 0.01

Table 1: effects of 250mg/kg plant extracts on anti-oxidant enzymes in albino rats.

Anti-oxidant enzymes assay (serum)	SOD (U/mg pro)	CAT (U/mg pro)	GSH (U/mg pro)	MDA (U/mg pro)
Control	3.73± 0.00	24.96± 0.00	0.70± 0.00	0.042± 0.01
Leaf	6.27± 0.66	41.91± 0.15	0.80± 0.20	0.048± 0.01
Stem	7.75± 0.81	51.82± 0.41	0.91± 0.90	0.047± 0.02

Table 2: effects of 250mg/kg plant extracts on anti-oxidant enzymes in albino rats.

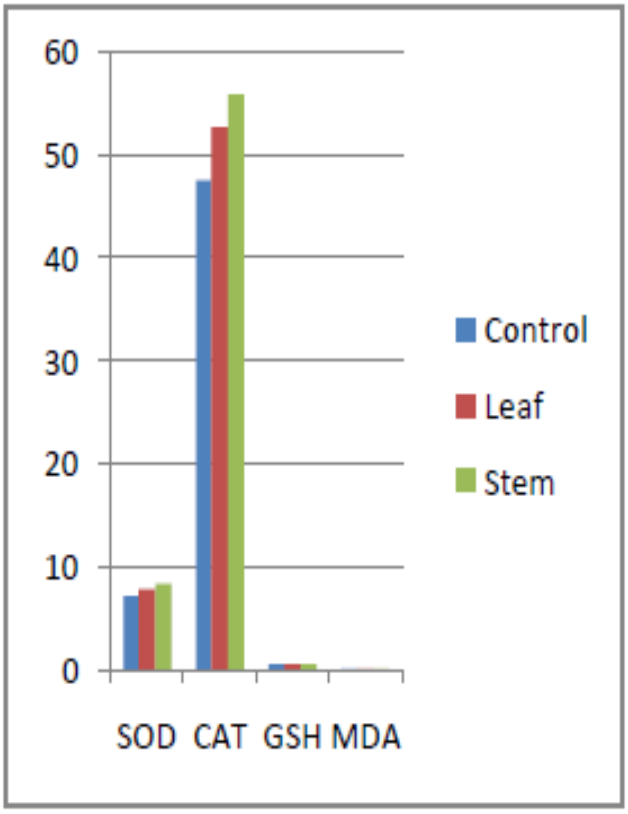

Figure 1: Effect of plant extracts of *Viscum album* on Antioxidant enzyme profile in the brain.

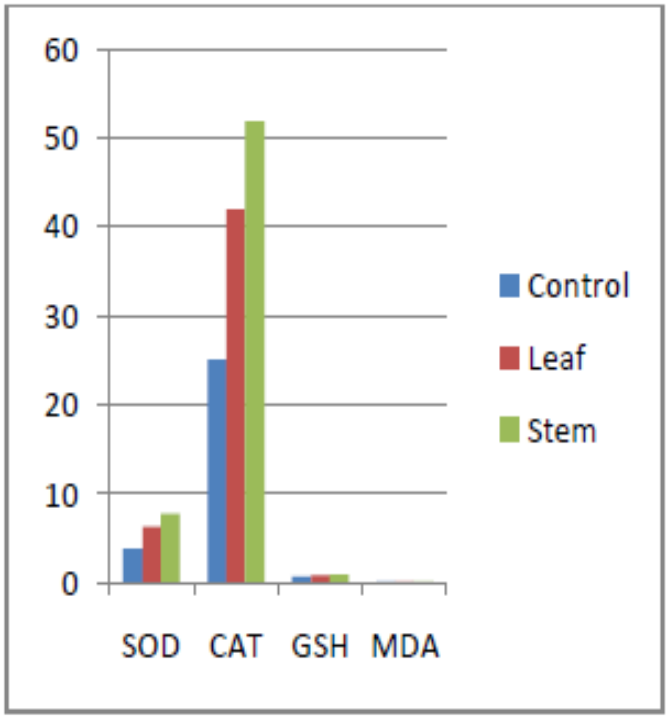

Figure 2: Effect of plant extracts of *Viscum album* on Antioxidant profile in serum.

Concentrations	Control	2.0mg	4.0mg	6.0mg	8.0mg	10.0mg
No of cells	500	306	276	257	243	192
Prophase	8	0	0	3	2	1
Metaphase	9	2	4	2	3	0
Anaphase	8	2	4	6	6	0
Telophase	14	9	12	3	5	0
Dividing cells	39	13	4.35	14	16	1
% Mitotic Index	7.80	4.25	3	5.45	6.58	0.52
Stickness	0	8	1	2	4	0
C- mitosis	0	0	3	0	0	0
Bridge fragments	0	3	2	8	2	0
Vagrant	0	1	2	3	3	0
Binuclei	0	0	2	0	0	0
Multipolar anaphase	0	0	1	0	0	0
Laggard	0	0	1	0	0	0
% CA± SD	0.00± 0.00	3.92± 0.54	4.71± 0.91	5.06± 0.91	5.76± 0.40	0.00± 0.00

Table 3 : Effects Of Leaf Extract Of *Viscum album* On Chromosomal Aberration

Concentrations	Control	2.0mg	4.0mg	6.0mg	8.0mg	10.0mg
No of cells	500	447	432	420	402	387
Prophase	8	4	0	2	5	4
Metaphase	9	8	10	11	7	0
Anaphase	8	7	6	9	7	6
Telophase	14	5	10	6	10	4
Dividing cells	39	24	26	28	29	21
M1% Mitotic Index	7.80	5.37	6.02	6.67	7.21	5.42
Stickness	0	5	4	5	5	3
C- mitosis	0	2	0	0	0	0
Bridge fragments	0	5	5	8	7	6
Vagrant	0	5	5	8	4	6
Binuclei	0	0	0	0	0	0
Multipolar anaphase	0	1	0	0	0	0
Laggard	0	1	0	0	0	0
% CA± SD	0.00± 0.00	3.80± 0.80	3.13± 0.29	4.86± 0.93	3.98± 0.04	3.88± 0.66

Table 4: effect of stem extract of *Viscum album* on chromosomal aberration.

% MI = No of dividing cells / total no of cells x 100

% CA = No of aberration / total no of cells x 100

4. DISCUSSION

Aqueous plant extracts of *Viscum album* was found to exhibit antioxidant properties. It was observed that in both Tables 1 and 2, the aqueous plant extracts of *Viscum album* showed increase in the antioxidant action of Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH) and Malonaldehyde (MDA). Catalase showed the most potent antioxidant action compared to other antioxidant markers as shown in both figures 1 and 2. It was suggested that the increase in the antioxidant activity of catalase would have been due to the high presence of hydroperoxides in the normal albino rats.

Research on medicinal plants with proven pharmacological activities and therapeutic benefits especially in the management of chronic diseases for which their use is prolonged, now focuses on the investigation of their role in human mutagenesis/carcinogenesis. The result of this study as shown in Tables 3 and 4, showed that aqueous plant extracts of *Viscum album* elicits anti-mitotic activity.

Mitotic was considered a parameter that allows one to estimate the frequency of cellular division (Marcano *et al*., 2004). Inhibition of mitotic activity is often use for tracing cytotoxic substances (Linnaimaa *et al*., 1978). The decreased in the rate of mitotic index, the more cytotoxic it becomes (Smaka-Kincl *et al*., 1996). From the result obtained in the cytotoxicity (or genotoxicity) studies carried out on the aqueous plant extract of the stem and leaf of *Viscum album* using the *Allium cepa* test, it showed that the aqueous plant extracts of *Viscum album* increased the rate of chromosomal division (i.e percentage mitotic index) as the concentrations increased.

C-mitosis was observed only at 4mg/ml of the aqueous leaf extract and 2mg/ml of the aqueous stem extract. This indicates a relatively high level of clastogenic constituents at this concentration and this further corroborates the highest mitotic index has been associated with sub-lethal effects on chromosomes of *Allium cepa* (Badr and Ibrahim,1987). The concentration at which highest number of cells with

chromosomal bridges for leaf extract (n=8) while for the stem extract (n=8), but lowest vagrant for leaf extract (n=0) while for the stem extract (n=4). Binucleated cells were not observed in *Allium cepa* for both leaf and stem extracts treated at different concentrations. In this study, both aqueous plant extracts at 8mg/ml were found to induce an increased in mitotic index. The raised MI due to 8mg/ml of aqueous plant extracts may be the consequence of the host response to a mitodepressive agent in the extract at sub-inhibitory concentration. Stickness was highest (n=8) for leaf extract and for stem extract (n=5). The lagging phase for both the leaf and stem extracts was at 4mg/ml and 2mg/ml respectively. Chromosomal aberration was observed at 6mg/ml for both the leaf and stem extracts.

In etiology terms, C-metaphase has been explained to occur due to inhibition of microtubule formation during mitosis and this may lead to aneuploidy and cell death, while stickness is due to inter-chromosomal linkages of sub-chromatid strands coupled with excessive formation of nucleoprotein and inappropriate protein-protein interaction (Odeigah et al., 1997; Turkoglu, 2007; Badr and Ibrahim, 1987). The latter is also believed to have resulted from altered physico-chemical properties of DNA due to interactions with other chemicals *viz-a-viz* mutagens and carcinogens (Badr and Ibrahim, 1987).

CONCLUSION:

Authors conclude from the research findings that aqueous plant extracts (Leaf and Stem) of *Viscum album* possessed both antioxidant and antimutagenic properties.

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