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Anti-Haemolytic Potentials of the Ethanolic Leaf Extract of *Dracaena Arborea* on Zidovudine-Induced Hemolysis in Wistar Albino Rats

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Abstract:

This study was conducted to evaluate the anti-hemolytic impact of the ethanolic leaf extract of *Dracaena arborea* on zidovudine induced hemolysis in Wistar Albino rats. Sixteen rats were randomly separated into four groups; group A served as a control while groups B, C and D were treated with (0.5mg/100g body weight) of the leaf extract, (0.4mg/100g body weight) of zidovudine and a combination of (0.5mg/100g body weight) of the leaf extract and (0.4mg/100g body weight) of zidovudine respectively. The results showed a significant ($p < 0.05$) change in the obtained values of erythrocyte fragility from (0.45 ± 0.1^{ab}) in group A to, (0.53 ± 0.1^a), (0.35 ± 0.1^b) and (0.40 ± 0.1^{ab}) in groups B, C and D respectively. For the packed cell volume, a significant ($p < 0.05$) increase in value when the plant extract was administered was observed; (43.50 ± 3.9^a) as compared to the control (37.67 ± 2.51^b) as well as a significant ($p < 0.05$) decrease when zidovudine alone was administered (27.50 ± 3.4^c). There was no significant ($p > 0.05$) difference in PCV in the group taking both combinations (35.75 ± 3.3^b) when compared to the control. The result equally showed a significant elevation in the obtained values of white blood cell count from (5.30 ± 1.0^b) in group A, to (5.78 ± 0.6^b), (11.63 ± 2.4^a) and (9.40 ± 2.4^a) in groups B, C and D respectively. For RBC, there is a significant increase in its value in group B (5.4750 ± 0.6^a) as compared to the control (4.53 ± 1.1^{ab}). A significant decrease was also observed in groups C (2.98 ± 0.4^c) and D (3.73 ± 0.5^{bc}). For haemoglobin concentration, there was a significant ($p < 0.05$) decrease in the values obtained in groups B (10.53 ± 0.1^b) and C (9.18 ± 1.1^b) when compared to group A (12.57 ± 0.8^a), while group D showed no significant difference from A. These results suggest that the ethanolic leaf extract of *Dracaena arborea* may not only possess anti-hemolytic properties, but also stimulate the haematopoietic system and reduce oxidative damage to red blood cells.

Keywords; Anti-hemolytic, Potentials, Zidovudine, Induced, Hemolysis, *Dracaena arborea*

1. Introduction

The rationale for the utilization of medicinal plants has rested largely on the fact that they contain active constituents (ingredients) which are used in the treatment of many different ailments. Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the on long-term clinical experience. (Zhu, 2002).

Fruits, species, herbs and leafy vegetables used as food and for medicinal purposes are obtained from the wild where there may be many as a thousand species. The implication is that several of these plants could become extinct due to deforestation menace and reluctance of people to venture into the forest to harvest them and little attempt has been made to identify these plants (Ogwuru, 1995).

Dracaena arborea belongs to Agavaceae family which is locally known as dragon tree and enjoy an abundant distribution. In folk medicine, it is widely used as a bioactive agent. It is said to improve testes morphology and restore spermatogenesis in type 1 diabetic rats, without having major anti-hyperglycemic properties. These effects could be attributed to saponins, flavonoids, phenols and sterols revealed in this plant, which could be a useful component in the treatment of diabetes-induced testicular dysfunction. (Tambi and Imran, 2010). The roots of *Dracaena arborea* possess potent sexual stimulant activity and provide some scientific evidence in favour of the claims regarding this action. (Watcho and Wankeu-Nya, 2007).

From a phytochemical perspective the plant evidently has substances that inhibit the growth of bacteria and fungi. It yields medicines to: stop nausea and vomiting (i.e. antiemetics), treat cutaneous and subcutaneous parasitic infections, reduce swelling, oedema and gout, treat oral complaints, expel worms (vermifuges), treat lung problems, reduce pain, used as an arrow-poison. Presently no research has been done on the plant with regards to hemolysis, even though it is believed in folklore to arrest hemolysis. Keeping this in view, the present study was undertaken to investigate the anti-hemolytic activity of the ethanolic leaf extract of the plant on rats administered with zidovudine.

Hemolysis can be described as the breakage of the red blood cells (RBC's) membrane, causing the release of the hemoglobin and other internal components into the surrounding fluid. Hemolysis is visually detected by showing a pink to red tinge in serum or plasma. Hemolysis is a common occurrence seen in serum samples and may compromise the laboratory's test parameters.

(Vermeer et al, 2005). Hemolysis occurs in a variety of pathological conditions such as, mechanical disruption of RBC, malaria/clostridium infection, thalassemia and sickle cell disease. Erythrocytes are exclusive blood cells that deliver oxygen to our body, act as vendor of nutrients and participate in detoxification of a great variety of toxic xenobiotics. They are very susceptible to oxidative stress due to high cellular concentration of oxygen and hemoglobin, as well as high polyunsaturated fatty acid content. (Burns, 2002).

Zidovudine is a Nucleoside Reverse Transcriptase Inhibitor (NRTI), one of the earliest antiretroviral agents used as a combination in the Highly Active Antiretroviral Therapy (HAART) for the treatment of HIV infection. Its use is however not without adverse effect particularly bone marrow aplasia leading to varying degrees of cytopenias [blood cell deficiencies] predominantly anaemia. This calls for adequate evaluation and monitoring of patients on this drug. Its major side effect; which is anaemia limits its use in some patients. (Hassan *et al.*, 2009).

Zidovudine is the preferred nucleoside reverse transcriptase inhibitor in the first line antiretroviral regimen. It is known to be associated with life threatening toxicity like anaemia and hemolysis (Majluf-Cruz A *et al.*,2000).

2. Materials and Method

2.1. Preparation of Plant Extract

The leaves of the *Dracaena arborea* was collected, washed, air dried and ground to powder. The ground leaves (200 g) of *Dracaena arborea* was macerated with ethanol (1000mls) for 48 hours, after which it was filtered using whatman no 1 filter paper and the filtrate was put in a water bath for evaporation of the ethanol to obtain the concentrated extract.

2.2. Phytochemical screening

Qualitative phytochemical evaluation of the leaf extract revealed the presence of; flavonoids, phenolics, tannins, pseudotannins, triterpenes, glycosides, and saponins. (Treaks and Evans.,1989).

2.3. Experimental Animals

A total of 16 albino rats weighing 150-200 g, grown in the Animal House of the Department of veterinary medicine” University of Nigeria, Nsukka, Enugu were acclimatized for one-week in the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike. They were placed in Rubber cages and fed with standard food and water ad libitum. After the acclimatization, the animals were randomly divided into 4 groups of 4 animals each. Group A received food and water only, Group B received 0.5mg/100 body weight of the leaf extract of *Dracaena arborea* Group C received 0.4mg/100g body weight of zidovudine while Group D received a combination of 0.5mg/100g of plant extract and 0.4mg/100g body weight of zidovudine. All experiments were done under conducive environment and lasted for 28 days

2.4. Blood sample collection

At the end of 28 days, blood was collected from each rat via ocular puncture in heparinized lithium bottles. The following haematological parameters were tested for; Erythrocyte fragility, packed cell Volume (PCV), using standard hematological techniques as described by Ochei and Kolhartkar (2008), Hemoglobin estimation and total white blood cell (WBC), (Rammik 1999). Red blood cell (RBC) Jain (1986), Daice and Lewis, (1991).

2.5. Statistical Analysis

Statistical analysis of the data obtained from the experiment was performed using the one way analysis of variance (ANOVA) followed by post HOC LSD test (Fisher 1935). The significance in the difference was accepted at $p > 0.05$. The results are expressed as mean \pm SD (standard deviation).

3. Results

The phytochemical screening revealed the presence of; flavonoid, phenolics, tannins, pseudotannins, triterpenes, glycosides and saponins.

Groups	Erythrocyte fragility	PCV	WBC	RBC	HB Conc
A (control)	0.45 \pm 0.1 ^{ab}	37.67 \pm 2.51 ^b	5.30 \pm 1.0 ^b	4.53 \pm 1.1 ^{ab}	12.57 \pm 0.8 ^a
B (plant extract only)	0.53 \pm 0.1 ^a	43.50 \pm 3.9 ^a	5.78 \pm 0.6 ^b	5.48 \pm 0.6 ^a	10.53 \pm 0.1 ^b
C(zidovudine only)	0.35 \pm 0.1 ^b	27.50 \pm 3.4 ^c	11.63 \pm 2.4 ^a	2.98 \pm 0.4 ^c	9.18 \pm 1.1 ^b
D(zidovudine + plant extract)	0.40 \pm 0.1 ^{ab}	35.75 \pm 3.3 ^b	9.40 \pm 2.4 ^a	3.73 \pm 0.5 ^{bc}	11.93 \pm 1.1 ^a

Table 1: Effects of the Ethanolic Leaf Extract of *Dracaena arborea* and Zidovudine on some Haematological parameters in wistar albino rats

Values are mean \pm SEM, Values with the same superscripts are not statistically significant at 95% confidence level ($p < 0.05$). Erythrocyte fragility curve, showing percentage hemolysis of the different groups at varying salt concentration.

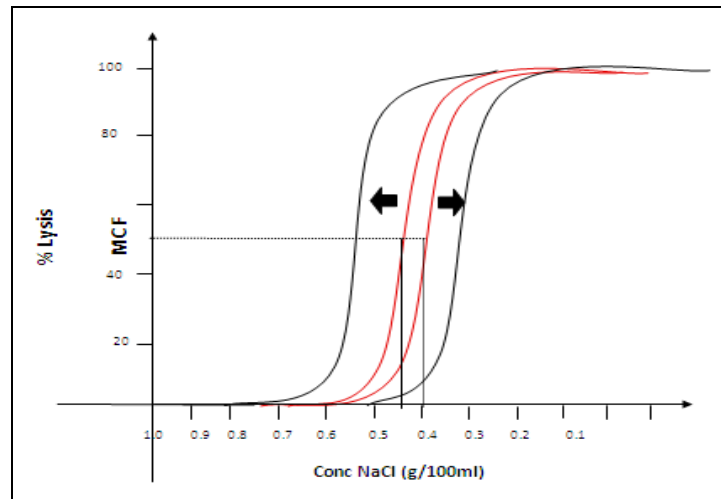


Figure 1

MCF (50% hemolysis)

Mean corpuscular fragility Groups A = 0.45

B = 0.53

C = 0.35

D = 0.40

4. Discussion

The ethanolic leaf extract of *Dracaena arborea* contain high concentrations of flavonoids and phenolic compounds which are potent antioxidants, flavonoids are especially important for protection against human diseases. The absence of steroid and Alkaloids in the leaf extract agrees with the findings of Isman, (1997).

Our literature review revealed that presently no previous research concerning *Dracaena arborea* on haematological parameters have been carried out. However it is believed to be an anti-hemolytic plant in some parts of Nigeria.

Erythrocyte (RBC) membrane composition Varies with factors, including diets, oxidative stress, and genetic variation. (Chaudhuri, 2007). This study revealed obvious alterations in the erythrocyte fragility of the test animals as compared with the control. The increase in erythrocyte fragility observed in group B may mean that the plant extract possesses no anti-hemolytic properties after all. High osmotic fragility occurs in hereditary spherocytosis, severe burns, chemical poisoning, or in hemolytic disease of the newborn (Ochei and Kolkartha, 2007). On the other hand, the decrease observed in groups C as compared to the other groups is an indication that the anaemia induced by zidovudine may not be as a direct result of hemolysis but due to other factors that oppose red blood cell synthesis e.g. Iron deficiency. Low osmotic fragility is characteristics of thalassemia, iron deficiency anemia and other red blood cell disorders in which codocytes (target cells) and leptocytes are found, It can also occur after splenectomy. (Ochei and Kolkartha, 2007).

The increase observed in PCV in group B when compared to the control, suggests an increased production of majority of the cells involved in the immune system which are produced in the stem cells of the bone marrow. Dehydration is not likely the cause of this increase since the animals were exposed to food and water ad libitum. Elevated packed cell volume can reflect decreased plasma level which might be due to dehydration (Rodak, Bernadette F et al 2007). Group C had the lowest PCV, confirming zidovudine as an inducer of anaemia. (Scruggs et al 2008). There was a slight decrease in hemoglobin concentration of the treated rats when compared to the control. This indicates that the plant extract may have little or no effect on the oxygen-carrying protein hemoglobin in the blood.

From this study there was a significant increase in Red Blood Cell Count when the plant extract was administered as compared to the control, this again indicate that the plant may be good in the treatment of anaemia. The rats administered with zidovudine again had the lowest red blood cell count when compared to the other groups. This further implicates zidovudine as an anaemic agent.

For White Blood Cell Count, there was no significant difference in WBC of the rats administered with the plant extract when compared to the control group. However zidovudine possessed the highest WBC count when compared with the other groups. This confirms zidovudine as a strong defensive agent against diseases and infections, and also useful in boosting the immunity of the body system.

The results obtained in group D (zidovudine and plant extract) for all the tested parameters, showed the ameliorating impact of the plant extract of *Dracaena arborea* on zidovudine induced anaemia. The plant extract cushioned the grievous impact of zidovudine on all the tested parameters including the erythrocyte fragility. *Dracaena arborea* should be considered in the diets of both adults and children.

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