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BLOOD STABILITY PARAMETERS AS INDICES OF HEMATOLOGICAL DISORDERS REMAIN PRESERVED ON EXPOSURE TO *CORCHORUS OLITORUS* LEAF CONSUMPTION

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Abstract

This study focused on the integrity retention profile of hematological disorder indices on exposure to ethanol extract of *Corchorus olitorus* leaf.
Seventy two apparently rats were obtained from the animal farm of the Department of Zoology and Environmental Biology University of Nigeria, Nsukka and used for this study. These indices assessed were Red blood cell distribution width standard deviation, Red blood cell distribution width coefficient of variation, Platelet Distribution Width, Mean Platelet Volume and Platelet Large Cell Ratio after oral administration of 50, 100 and 200 mg/kg body weight of the extract to three groups of rat for 30, 90 and 180 days. These indices were analyzed using automated Hematology Sysmex analyzer.
The results showed a non-significant changes in the indices which included red blood cell distribution width standard deviation, red blood cell distribution width coefficient of variation, platelet distribution width, and mean platelet volume for all the groups respectively. The results of this study revealed that oral administration of *C. olitorus* could not cause significant increase of the platelet indices at the doses studied. Suggesting that *C. olitorus* leaf does not put the system at risk of any blood associated disorder or disease.

Keywords: *Blood Stability, Hematological disorders, Corchorus olitorus, leaf, Wistar rat*

Introduction

Red blood cell distribution width standard deviation (RDW-SD), Red blood cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), and Platelet Large Cell Ratio (P-LCR) have become very viable parameters to be measured as indices of blood associated disorders, where conventional diagnostic methods may not have revealed (Michael, 2011). RDW is a new routine parameter that shows the earliest morphologic changes in iron deficiency anemia which increases due to the population of microcytic cells that appear in the blood, with higher concentration indicating greater variation in size (Khan *et al*., 2014). Red cell circulation width (RDW), a conventional parameter used in the differential diagnosis of anemia, has recently been recognized as a marker of long-lasting inflammation and high levels of oxidative stress (OS). Red cell distribution width (RDW) reflects the variation in red blood cell size or red blood cell volume. Increased RDW appears as a consequence of deregulation of red blood cells (RBC) homeostasis, involving both impaired erythropoiesis and RBC degradation (Marks and Glader, 2009). The reference range for RDW is as follows: RDW-CV 11-15% in adult, RDW-SD 39-46 fl (Shehata *et al*., 1998). High RDW value predicts high incidence of stroke and other cardiovascular diseases (Soderholm *et al*., 2015) and heart failure (Bome *et al*., 2011). Platelets (PLT) are membrane bound discoid structures that play a central role in hemostasis (Baig, 2015). Mean platelet volume (MPV) reflects the size of platelets that determines the function of platelet function. Investigations reveal that the concentrations of mean platelet volume (MPV) are associated with diseases such as diabetes (Kaya *et al*., 2013) and hypertension (Ani and Ovbiagele, 2009). Platelet Large Cell Ratio (P-LCR) has been shown to be inversely related to platelet count and directly related to PDW and MPV (Baig, 2015). Plants have contributed significantly to the well-being of man and lower animals. *Corchorus olitorus* is among the common vegetables used as spices for making soup and stew in West African countries, especially Nigeria (Omeje *et al*., 2014a). Qomen and Grudden (1978) reported that it is rich in phytochemicals such as vitamin A, protein, fibre, carotene, calcium, iron and folic acid. In Asia, it is not only used as spices, but also used in the treatment of chronic cystitis, dysuria, and possibly other blood disorders (Pan *et al*., 2000). Also, its ethanol extract had the ability to aggregate blood platelet (Omeje *et al*., 2014b), similarly, *C. olitorus* could be used in the management of *Diabetes mellitus* (Omeje *et al*., 2016). Having proven platelet aggregation effect *in vitro and in vivo* (Omeje *et al*., 2014b), it became important to do a time dependent study of its effect on the blood profile, in consonance with long term consumption.

Materials And Methods

Collection and Identification of Plant Material

Fresh leaf of *C. olitorus* were bought from Eke market in Edem-ani, Nsukka L.G.A, Enugu State of Nigeria. The plant sample was identified and authenticated by a taxonomist at Department of Plant Science and Biotechnology, University of Nigeria.

Preparation of Plant Material

The leaf was dried at room temperature (29 ± 2ºC) for four days and pulverized with an electric blender. The powdered leaf (1000 g) was extracted by cold maceration at room temperature with 2.0 L of 70% ethanol for 48 h with constant agitation. The extract was concentrated *in vacuo* at reduced temperature and pressure to obtain the dry extract.

Animals

Seventy two rats were obtained for the research and divided into three groups of Twenty four wistar rats (160 – 220g) each from the animal farm of the Department of Zoology and Environmental Biology University of Nigeria, Nsukka. They were maintained in Biochemistry Animal house for seven days for acclimatization. They were fed with commercial rat chow. Each group was further divided into four groups of six rats each, with oral administration of the extract for the period of 30, 90 and 180 days.

Group I: (served as normal control and received only distilled water)

Group II (Received 200 mg/kg body weight of the ethanol extract).

Group III (Received 100 mg/kg body weight of the ethanol extract).

Group VI (Received 50 mg/kg body weight of the ethanol extract).

Sample Collection

After oral administration of ethanol extract for thirty (30), ninety (90) and 180 days, the animals were anaesthetized with chloroform and blood sample collected from the retro-bulbar plexus of the medial canthus of the eye. A microhematocrit tube was carefully inserted into the medial canthus of the eye to puncture the retro-bulbar plexus and thus enable outflow of 3 ml of blood into a clean sample container containing 3.8% tri-sodium citrate as anti-coagulant. Each was thoroughly mixed to prevent coagulation of the blood samples.

Platelet Indices Count

Platelet indices (Red cell distribution width standard deviation (RDW-SD), Red cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR) were counted by an automated hematology sysmex analyzer (Coulter Electronics, Bedfordshire, England).

Results

The blood samples were analyzed for Red cell distribution width standard deviation (RDW-SD), Red cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR) after 30 days of oral administration as shown in table 1. There was no significant increase among the varying concentrations of the extract when compared with the control group. A dose dependent decrease of the RDW-CV concentration was observed (Table 1). 200, 100 and 50 mg/kg body weight of the extract gave an RDW-CV concentration of 13.70, 13.10 and 12.60% respectively, which were lower when compared to the control (14.20 %). The concentrations of other indices (PDW, MPV and P-LCR) (Table 1) showed a non-significant increase when compared with the values for the control group. This could be an indication that the ethanol extract of *C. olitorus* does not contain bioactive compound that affects the tested parameters. Contrary to the findings of this study, previous research, has shown that high doses of *O. gratissimum* led to a decrease in the platelet indices (i.e., MPV, P–LCR, and PDW) (Ofem *et al*., 2012) in rats. In addition, Table 2 showed the concentrations of platelet indices such as Red cell distribution width standard deviation (RDW-SD), Red cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR). Among all the indices assayed, RDW-CV concentration decreased concomitantly as the extract concentration decreased when compared to the control group. There was no significant difference observed for other parameters studied.

After 30, 90 and 180 day oral administration, the RDW-CV obtained were 13.37±0.59, 14.83±2.87 and 14.80±1.16 respectively. Increased red cell distribution width (RDW) has been reported as a predictor of cardiovascular mortality in the general population (Söderholm, *et al*., 2015). Hence, it is inferred that the leaf of *C. olitorus* may not predispose a consumer to any cardiovascular illness. It has been reported that increased RDW is independently related to long-term mortality in patients with coronary artery disease (Tonelli et al., 2008). The non-significant increase obtained from this research shows that the vegetable may not predispose its' consumers to coronary artery diseases by increasing this platelet index. It has also been reported that, increased RDW is an indication of iron deficiency anemia (Khan *et al*., 2014). The non-significant increase effect of the extract on RDW is indicative that the extract does not inhibit red blood cell proliferation. Though, Omeje *et al*. (2014b) had earlier reported the ability of *C. olitorus* extract to proliferate red blood cell thereby controlling anemia. The values of PDW were decreasing as the days of administration increased from 30, 90, 180, which yielded 9.94±0.72, 5.17±7.95 and 0.13±8.88 Fl respectively. Muriithi *et al*. (2015) reported a significant increase in platelet indices after oral administration of methanolic extracts of *V. lasiopus,* which they attributed to the presence of tannins. P-LCR is increased in destructive thrombocytopenia than those with hypo-proliferative thrombocytopenia (Baig, 2015). A non-significant increase of the P-LCR was observed among all the groups studied, this is could be an indication of the

absence of bioactive agent capable of destroying thrombocyte.

Comparison among the platelet indices after 180 days of oral administration of the extract at varying concentrations is shown in table 4, and the concentration of RDW-CV was observed to increase with time.

In conclusion, the results of this study reveal that oral administration of *C. olitorus* could not cause significant increase of the platelet indices (RDW, PDW, MPV and P–LCR) at the doses studied after 180 days. The findings revealed as shown in the blood indices that *C. olitorus* does not put the system at risk of any blood associated disorder or disease. This suggests there is no harmful effect of the long term consumption of *Corchorus olitorus* products, hence, making the vegetable an ideal one for consumption. It is also important to add that, products of *Corchorus olitorus* shouldn't be considered as a therapeutic option for hematological disorders to which the parameters tested for in this study were viable indices. Notwithstanding, having established the preservatory potential of the *Corchorus olitorus* product on healthy models, further studies could be carried out on diseased conditions to ascertain if the extract is curative.

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Table 1: Results of the platelet indices after 30 days of oral administration of ethanol extract at varying concentrations

Platelet indices	control	200mg/kg bwt	100mg/kg bwt	50mg/kg bwt
RDW-SD (fL)	-30.40	-31.30	-29.00	-28.50
RDW-CV (%)	14.20	13.70	13.10	12.60
PDW (fL)	10.00	9.20	10.70	9.20
MPV (fL)	-7.80	6.70	-7.70	6.80
P-LCR (%)	13.40	8.90	13.30	9.90

Red cell distribution width standard deviation (RDW-SD), Red cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR)

Table 2: Results of the platelet indices after 90 days of oral administration of ethanol extract at varying concentrations.

Platelet indices	control	200mg/kg bwt	100mg/kg bwt	50mg/kg bwt
RDW-SD (fL)	-29.40	-27.20	-27.90	-29.80
RDW-CV (%)	19.20	12.90	12.10	14.30
PDW (fL)	11.00	9.80	-7.80	7.40
MPV (fL)	-7.60	-7.30	-6.70	6.50
P-LCR (%)	-11.50	-10.70	-7.90	6.90

Red cell distribution width standard deviation (RDW-SD), Red cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR) .

Table 3: Results of the platelet indices after 180 days of oral administration of ethanol extract at

Platelet indices	Control	200mg/kg bwt	100mg/kg bwt	50mg/kg bwt
RDW-SD (fL)	-30.70	-27.90	-29.50	-30.40
RDW-CV (%)	14.80	13.20	16.20	14.20
PDW (fL)	-8.00	8.90	8.10	-8.50
MPV (fL)	-6.70	6.50	7.00	-7.10
P-LCR (%)	7.00	8.30	13.40	9.90

varying concentrations.

Red cell distribution width standard deviation (RDW-SD), Red cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR).

Table 4: Comparison among platelet indices after 180 days of oral administration of ethanol extract at varying concentrations

Platelet indices	30days	90days	180days
RDW-SD (fL)	-29.89±1.16	-28.65±1.14	-29.75±1.19
RDW-CV (%)	13.37±0.59	14.83±2.87	14.80±1.16
PDW (fL)	9.94±0.72	5.17±7.95	0.13±8.88
MPV (fL)	-0.47±7.60	-3.81±6.27	-0.02±7.22
P-LCR (%)	11.42±2.15	-7.31±9.45	1.23±10.41

Red cell distribution width standard deviation (RDW-SD), Red cell distribution width coefficient of variation (RDW-CV), Platelet Distribution Width (PDW), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (P-LCR)