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Original Research Article

Preliminary Investigation on Effect of *Eugenia uniflora* on Some Haematological Parameters of Male Wistar Rats

Constance I. Nkpurukwe[1], Chibuike Obiandu[2*], Bright I. Owhorji[2], Gideon I. Emeghara[2], Karibo Okari[3]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Nigeria
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria
[3]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria

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Abstract: The purpose of this study is to investigate the effects of extract of *Eugenia uniflora* on some haematological parameters of male Wistar rats. A total of 24 male Wistar rats were used in the study. They were divided into four groups of six (6) rats each. Group 1 functioned as the control group and received distilled water while Groups 2, 3 and 4 received 200mg/kg, 400mg/kg and 800mg/kg extract of *Eugenia uniflora* respectively. The administration was done orally once daily for 30 days. At the end, the rats were sacrificed under light chloroform anaesthesia and their blood collected for analysis of haematological parameters. Data was analysed using SPSS version 23 (SPSS incorporated, Chicago, Illinois, USA). Results are expressed as mean±standard error of mean (SEM). Significant differences were determined by one-way analysis of variance (ANOVA). The differences in values were considered to be statistically significant at $p<0.05$. The packed cell volume (PCV) decreased significantly in some test groups when compared to control. The RBC count and Hb concentration also decreased significantly. There was a significant increase in white blood cell count amd neutrophils but reductions in lymphocytes and mean corpuscular haemoglobin concentration. Result of the study has shown that the hydromethanolic extract of *Eugenia uniflora* may possess anti-erythropoietic actions because of the significant reductions in RBC count and Hb concentration as well as leucopoetic and possibly immunomodulatory actions which boosted white blood cell (WBC) production.

Keywords: *Eugenia uniflora*, Wistar rats, haematological parameters, packed cell volume (PCV).

INTRODUCTION

Ethnomedicinal practices has gained much popularity throughout the world with the application of medicinal plants in traditional medicinal practices. Increasing number of Phytopharmaceutical components are been discovered with the sole purpose of extracting its beneficial pharmacological effect on the human or animal body (Namdeo, 2018). Plants were employed to treat diseases by early humans, who were guided by instinct, taste, and experience; thus, medicinal plants have a history as long as mankind (Da-Cheng, 2018). In African traditional medicine, as well as some other methods of treatment throughout the world, medicinal plants have long been utilized to treat a range of ailments. Most successful therapeutic herbs have few dangerous or unpleasant side effects when taken by people, but some may be toxic to both humans and animals, with the ability to injure specific organs in the body. This calls for caution when using medicinal plants, which are becoming increasingly popular due to their easy availability, affordability, accessibility and promising efficacy comparable to established synthetic pharmacological medicines in terms of costs and negative side effects (Okoye, *et al.*, 2014). With relatively few studies carried out on some of these plants, not much is known about the negative effects they may instigate in the animal and human systems.

Eugenia uniflora, a member of the Myrtaceae family, also known as Pitanga cherry is native to South America's tropical and subtropical regions (Heywood *et al.*, 2007; Wilson, 2011). It can be found in Brazil,

***Corresponding Author:** Chibuike Obiandu
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Paraguay, Uruguay, and Argentina (Consolini and Sarubbio, 2002), and also in the Caribbean islands, China, India, Australia, Egypt, and Nigeria (Kanazawa *et al.,* 2000). *Eugenia uniflora* possesses a number of noteworthy pharmacological characteristics (Lim, 2012). Its essential oil possesses antihypertensive, antidiabetic, anticancer, and analgesic properties, as well as antiviral and antifungal properties (Costa *et al.,* 2010). It has shown to be effective against Trichomonas gallinae (in vitro), Trypanosoma cruzi, and Leishmania amazonensis (Rodrigues *et al.,* 2013). It also has anti-inflammatory properties, (Schapoval *et al.,* 1994), and is widely used in South America as a folk cure for stomach ailments (Tabata *et al.,* 2013). But, there is a dearth of scientific report on its possible effect on the haematological system.

The study of the morphology and physiology of blood is known as haematology (Institute of Biomedical Science, 2013). Haematology is a discipline of biology (physiology) dealing with the study of blood, blood-forming organs, and blood illnesses, according to Wikihow (2013). The factors connected to blood and blood-forming organs are known as haematological parameters (Waugh and Grant, 2001; Bamishaiye *et al.,* 2009). Blood parameters alter in response to an animal's physiological state. The haematological examination is one of the approaches that can help detect changes in health and physiological state that aren't visible during a physical examination but have an impact on the animal's fitness (Bamishaiye *et al.,* 2009). Etim (2010) found that haematological measures are good markers of an animal's physiological state. Erythrocytes [Red Blood Cells (RBC)], leucocytes [White Blood Cells, (WBC)], haemoglobin concentration (Hb), Packed Cell Volume (PCV), and values such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) are some of the most commonly used haematological parameters (Carlson, 1996; Chineke, 2006).The haematopoietic system is one of the most sensitive targets for hazardous chemicals, and it may be used to measure the level of blood damage in humans and animals (Omodamiro *et al.,* 2016). It provides vital information on bone marrow activity as well as intravascular complications such as haemolysis and anemia (Adeneye *et al.,* 2006).

Anaemia is a blood disorder in which there are insufficient red blood cells or the cells are not working properly. Anaemia is a condition that can be moderate or severe (very bad). It could be a temporary or long-term issue. It is the most common blood disorder affecting around a third of the global population (Janz *et al.,* 2013). Iron deficiency anemia affects about 1 billion people worldwide (Vos, 2012). The goal of this study is to investigate the effects of hydromethanolic leaf extract of *Eugenia uniflora* on the haematological parameters of male Wistar rats.

MATERIALS AND METHODS

Animal Models

A total of twenty four (24) adult male Wistar rats used for this study were bred in the animal house of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Port Harcourt, Nigeria. They were placed in standard cages and acclimatized in two weeks while maintaining them in environmental conditions with proper ventilation and free access to food and water. Generally, the procedures conformed to the established principles for the care and use of laboratory animals published by the National Institute of Health, USA (National Institutes of Health, 1985). Appropriate institutional approval was obtained for this study.

Preparation of Plant Extract

The *Eugenia uniflora* leaves used in this study were collected from Adanta-Isiokpo community in Ikwerre Local Government Area of Rivers State, Nigeria and identified by a taxonomist at the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The plant's voucher specimen was deposited at the herbarium. Before being pulverized into powder, the leaves were dried for two weeks. In a maceration jar containing 80% methanol and 20% water, the powdered substance was dissolved for 72 hours. The mixture was agitated three times a day for the three days it was allowed to sit. To get a clear filtrate, the substance was filtered with a white handkerchief and then re- filtered with filter paper at the end of the maceration period. The filtrate was concentrated at an appropriate temperature (40–50°C) using a rotary evaporator. The extract-containing filtrate was poured into an evaporating dish and dried in a water bath until it became a pastry form.

Experimental Design/Procedure

The animals were divided into four groups of six rats each. Group 1 functioned as the control group which received distilled water, while Groups 2, 3 and 4 received 200mg/kg, 400mg/kg and 800mg/kg of hydromethanol leaf extract of *Eugenia uniflora* respectively. The entire administration was done orally once daily for 30 days. At the end of the administration, the rats were sacrificed under light chloroform anaesthesia and their blood collected for analysis of haematological parameters in accordance with established methods.

Statistical Analysis

Results are expressed as mean ± standard error of mean (SEM). Significant differences were determined by one-way analysis of variance (ANOVA) using SPSS version 23 (SPSS incorporated, Chicago, Illinois, USA). The differences in values were considered to be statistically significant at $p<0.05$.

RESULT

The result for the study is presented in tables 1-4.

Table 1: Effects of Leaf Extract of *Eugenia uniflora* on Packed Cell Volume, Red Blood Cell count and Haemoglobin concentration

Groups (mg/kg)	Packed cell volume (%)	Red blood cell count (10^{12}/L)	Haemoglobin (g/dL)
Control	44.00 ± 1.22	5.84 ± 0.24	14.68 ± 0.40
200	38.00 ± 1.09*	4.84 ± 0.24*	12.40 ± 0.46*
400	39.00 ± 0.71*	5.14 ± 0.22*	13.00 ± 0.24*
800	42.20 ± 0.86	5.42 ± 0.14	14.06 ± 0.29

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 when compared to the control.

Table 2: Effects of Leaf Extract of *Eugenia uniflora* on Platelets count and White Blood Cell count

Groups (mg/kg)	Platelet count (10^9/L)	White blood cell count (10^{-9}/L)
Control	279.80 ± 19.68	8.22 ± 1.15
200	303.40 ± 15.00	12.34 ± 1.98*
400	242.00 ± 10.63	6.66 ± 0.79
800	244.60 ± 4.53	5.54 ± 0.30

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 when compared to the control.

Table 3: Effects of Leaf Extract of *Eugenia uniflora* on Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, and Mean Corpuscular Volume

GROUPS (mg/kg)	MCH (fl)	MCHC (g/dl)	MCV (pg)
Control	24.80 ± 0.58	33.20 ± 0.20	75.40 ± 1.21
200	25.40 ± 0.68	32.00 ± 0.32*	77.20 ± 1.50
400	25.00 ± 0.71	32.20 ± 0.37*	66.00 ± 9.46
800	24.00 ± 0.32	32.80 ± 0.20	72.00 ± 0.32

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 when compared to the control.

Table 4: Effects of Leaf Extract of *Eugenia uniflora* on Neutrophils, Lymphocytes, Eosinophils, and Monocyte count

GROUPS (mg/kg)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)
Control	28.20 ± 2.15	58.60 ± 3.31	4.20 ± 0.37	9.00 ± 1.70
200	35.00 ± 1.84*	54.00 ± 1.87	3.40 ± 0.51	7.60 ± 0.24
400	37.20 ± 0.97*	52.00 ± 1.22*	3.60 ± 0.40	7.20 ± 0.20
800	28.00 ± 1.41	60.40 ± 1.63	4.40 ± 0.40	7.20 ± 0.37

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 when compared to the control.

DISCUSSION

This study focused on evaluating changes in some haematological parameters following administration of extracts of *Eugenia uniflora* in male Wistar rats. Although a medicinal plant product has been adjudged to be safer compared to synthetic agents, it could however cause undesirable effects when consumed without precautions. Certain medicinal agents have been discovered to cause injury to an organ especially when consumed in overdoses and sometimes even when taken within therapeutic ranges (Omodamiro *et al*, 2016). There was significant (p<0.5) reductions in the red blood cell series in the treated groups when compared to control. The degree of alterations in these parameters often indicates the healthiness of the animal. There was a significant (p<0.5) decrease in RBC count, PCV and Hb concentrations after 30 days of *Eugenia uniflora*.extract administration. The red blood cells also

called erythrocytes are non-nucleated formed elements of blood produced from the red bone marrow in adult life (Sembulingam and Sembulingam, 2016)[a]. They serve as a carrier of haemoglobin. The haemoglobin combine with oxygen in blood to form oxyhaemoglobin during the process of respiration (Chineke *et al.,* 2006); (Etim *et al.,* 2014). Red blood cells are involved in the transport of respiratory gases in the body. A reduced red blood cell count implies a reduction in the level of oxygen that would be transported to the tissues and the level of carbon dioxide returned to the lungs (Martin *et al.,* 1998; Soetan *et al.,* 2013; Isaac *et al.,* 2013). The packed cell volume is the percentage of red blood cells in blood (Sembulingam and Sembulingam, 2016)[b]. In a report, the three parameters (red blood cell, packed cell volume and haemoglobin concentration) are interrelated (Scalm *et al.,* 2016), hence, any change in one affects the other parameters. The reductions in the red blood cells, packed cell volume and haemoglobin concentration in this study show that the extract of *Eugenia uniflora* exhibited a negative effect on erythropoiesis. The inhibitions on erythropoeisis may be due to certain constituents of the extract which has the tendency to impair the processes of red blood cell formation. The constituents of this extract which impacted negatively on erythropoietic mechanisms in this study need to be further investigated.

There was a significant increase in white blood cell count in the group that received the lower dose (200mg/kg) of the extract. The higher doses did not cause significant alterations of the white blood cell. The significant increase in white blood cell count generated by the plant extract reflects leucopoetic and possibly immunomodulatory actions of the extract which has the ability to boost WBC production (Bashir *et al.,* 2015). There was no significant change in platelet count in this study.

There was a significant decrease in MCHC in lower (200mg/kg) and medium (400mg/kg) doses of the extract. Reduced MCHC values in this group could indicate blood osmoregulation failure, high plasma osmolarity or abnormal haemoglobin synthesis (Stookey *et al.,* 2007). Iron deficiency can be detected by a low MCHC. This suggests that extremely high doses of *Eugenia uniflora* could cause iron deficiency anemia in rats. There was no significant difference in MCH and MCV.

A significant increase in neutrophils was observed in the groups that were administered the lower (200mg/kg) and medium (400mg/kg) doses of the extract while the lymphocytes were reduced in the group that took the medium (400mg/kg) dose of the extract. When the body is infected by bacteria, neutrophils are the first line of defense against invading microorganisms (Ganong 2005). The granules of the neutrophil contain many enzymes, making it a powerful and effective killer machine. As a result, a lack of neutrophils in the body causes a variety of problems including chronic granulomatous disease. *Eugenia uniflora* may have also antibacterial properties based on its effect on neutrophil count (Akinyemi *et al.,* 2004). The significant decrease in lymphocytes may negatively impact on the body's defence mechanism. There were no significant changes in the eosinophil and monocyte count. This study has shown that the haematopoietic system is indeed sensitive to different constituents of plant extract which may exert both toxic and nontoxic effects on the bone marrow and other tissues/organs involved in blood cell formation. The various processes including erythropoietic and leucopoietic functions were affected to some degree in this study with a clear negative effect on the red blood cell lines.

CONCLUSION

The extract of *Eugenia uniflora* significantly reduced RBC count, PCV and Hb concentration in male Wistar rats by inhibiting or causing impairment in function of erythropoietic mechanisms of the bone marrow. This may possibly be due to a constituent of the extract which needs to be further investigated. The effects on the white blood cell suggest an improvement in leucopoiesis and immunomodulatory functions.

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