Original Research Article

Haematopoietic Potential of Ethanolic Leaf extract of *Talinum triangulare* in Wistar Rats

ABSTRACT

Aim: This study focuses on assessing the haematopoietic potential of ethanolic extract of *Talinum triangulare* leaves in Wistar rats.

Methods: Fresh plants of *T. triangulare* were purchased from a local market in Orita-Challenge area of Ibadan, Nigeria. They were air dried at room temperature in an open laboratory space for 21 days and milled into powder. The extraction was done using soxhlet apparatus and ethanol as the solvent. The ethanol was evaporated in a rotary evaporator at 35 ^{o}C with a yield of 2.76 g which represents a percentage yield of 11.04%. Ten adult male Wistar rats with body weight between 150 and 170 g were used for this study. They were randomly divided into two groups of five rats each. Animals in group A were administered saline solution while those in group B were administered *T. triangulare* leaf extract. The administration was done 12 hourly for twenty-eight days at 100 mg/kg body weight via oral route since the plant is consumed orally. At the end of the treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture into heparinized bottles. Haematological parameters were determined using standard methods.

Results: A significant increase was observed in the PCV, Hb, RBC, MCV, WBC, lymphocyte and platelet counts of control animals when compared with those treated with leaf extract of *T. triangulare* at $p<0.05$. However, treatment had no significant difference on the neutrophil of animals.

Conclusion: The significant increase observed in the erythrocyte parameters of animals used in this study indicates that *T. triangulare* leaves have haematopoietic properties and can be used to boost blood level especially in menstruating and pregnant women as well as anaemia patients. Its ability to significantly increase white blood cell parameters is an indication that *T. triangulare* leaves can boost the immune system and thus defend the body against xenobiotics.

Keywords: *Talinum triangulare*, Haematopoietic Potential, Ethanolic Leaf-Extract, Anaemia, Immune System

1. INTRODUCTION

Vegetables are the cheapest and most available sources of protein, vitamins, minerals and essential amino acids [1]. They are added as supplements to provide useful and correct proportions of nutrients and some have medicinal properties useful for the invalids and convalescences. Consumable vegetables are those plants whose leaves or aerial parts have been integrated into a food chain for consumption over a large span of time. They are highly recommended because they have a relatively high nutritional value and their consumption gives daily food intake and adding flavour to the diet. Vegetables constitute essential components of the diet, by contributing nutrients and food properties that are important to human health. A healthy heart and circulation system could benefit from a balanced diet with adequate fruits and vegetables [2]. Epidemiological evidences

support a significantly positive correlation between eating fruits and vegetables as well as cardiovascular health [3, 4]. Increased awareness on the usefulness of vegetable inclusions in human food has enhanced their consumption as part of daily diet. Consumption of vegetables has been associated with a reduction in the incidences of chronic diseases of lifestyle such as cardiovascular diseases, coronary heart diseases and various types of cancer.

Talinum triangulare (Jacq.) Willd. (Portulacaceae) is a caulescent, perennial herb growing to a height of 80-100 cm. It is popularly known as Waterleaf because of its high moisture content of almost 90.8 g per 100 gm of edible leaf [5]. In Nigeria, the plant is known by different names: Gbure in Yoruba, Nteoka/inene in Igbo, and Alenyruwa in Hausa language. It is widely grown in most of the humid tropical countries such as West Africa, Asia and South America [6]. It thrives well under shade and in cloudy weather. Waterleaf is relatively tolerant to drought conditions as they tend to adopt a crassulacean acid metabolism (CAM) pathway, thus resulting in efficient utilization of available moisture, carbon dioxide assimilation during night and increased growth [5]. The plant has a rich content of crude protein, total lipids, essential oils, cardiac glycosides, flavonoids and polyphenols [7]. Preliminary phytochemical studies on *T. triangulare* revealed the presence of omega-3-fatty acids and high levels of essential nutrients like minerals (such as calcium, potassium and magnesium), soluble fibres (such as pectin) and vitamins (such as C, α and β tocopherols and β-carotene) which are required for growth and development [6].

The leaf extracts of waterleaf have been proved to possess remarkable antioxidant activity [8] and high kaempferol content [9]. Waterleaf is a mucilaginous vegetable with high oxalate content and is rich in saponins. Cooking or blanching removes nearly most of the soluble oxalate. Furthermore, the leaves serve as sauce, condiment, spice, softening of soups and for flavouring in foods [10]. *T. triangulare* leaves have been implicated medically in the management of cardiovascular diseases like stroke and obesity [11]. According to traditional medicine the leaves of waterleaf are used to treat polyuria [12], internal heat, measles [5], gastrointestinal disorders [13], hepatic ailments and cancer [8]. Furthermore, Airaodion et al. [14] has reported its hypoglycemic and hypolipidaemic activities in Wistar rats. The preventive and therapeutic activities of methanolic extract of *T. triangulare* leaves against ethanol-induced oxidative stress in Wistar rats have also been reported [15]. There is dearth of research on its effect on haematological parameters. This study therefore sought to investigate its haematopoietic potential in Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Fresh plants of *T. triangulare* were purchased from a local market in Orita-Challenge area of Ibadan, Nigeria and were identified by a botanist. The leaves were carefully removed from the stem and washed in running water to remove contaminants. They were air dried at room temperature in an open laboratory space for 21 days and milled into powder using an electronic blender (Moulinex). The extraction was done using soxhlet apparatus and ethanol as the solvent according to the methods described by Airaodion et al [16]. About 25 g of the powder was packed into the thimble of the soxhlet extractor and 250 mL of ethanol was added to a round bottom flask, which was attached to the soxhlet extractor and condenser on a heating mantle. The solvent was heated using the heating mantle and began to evaporate moving through the apparatus to the condenser. The condensate dripped into the reservoir housing the thimble containing the sample. Once the level of the solvent reached the siphon, it poured back into the round bottom flask and the cycle began again. The process was allowed to run for a total of 18 hours. Once the process was completed, the ethanol was evaporated in a rotary evaporator at 35 ^{o}C with a yield of 2.76 g which represents a percentage

yield of 11.04%. The extract was preserved in the refrigerator until when needed.

2.2. Experimental Design

Ten adult male Wistar rats with body weight between 150 and 170 g were purchased from the Central Animal House, College of Medicine, University of Ibadan, Nigeria. They were housed in Imrat animal house, Ibadan. They were acclimatized for seven (7) days during which they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into two groups of five rats each. Animals in group A were administered saline solution while those in group B were administered *T. triangulare* leaf extract. The administration was done 12 hourly for twenty-eight days at 100mg/kg body weight via oral route since the plant is consumed orally. At the end of the treatment, animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture into heparinized bottles. The blood samples were centrifuge for 10 minutes using a bench-top centrifuge (Centromix) and the supernatant plasma was preserved for further analysis.

2.3. Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain [17], using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis [18]. Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells [19]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain [17].

2.4 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean ± standard deviation. One way analysis of variance (ANOVA) was used for comparison of the means followed by Turkey's (HSD) multiple comparison test. Differences between means were considered to be significant at $p<0.05$.

3. RESULTS

Results of this study are presented in tables 1 and 2.

Table 1: Effect of Ethanolic Leaf Extract of *T. triangulare* on Erythrocyte Parameters in Wistar Rats after 28 days of Administration

Parameters	Control	*T. triangulare*
PCV (%)	37.08 ± 3.45^{a}	48.21 ± 2.43^{b}
Hb (g/dL)	9.41 ± 0.47^{a}	13.11 ± 1.84^{b}
RBC ($X10^{12}$/L)	6.79 ± 0.67^{a}	13.74 ± 2.27^{b}
MCV (FL)	54.65 ± 6.80^{a}	44.24 ± 4.33^{b}
MCH (pg)	14.87 ± 1.19^{a}	12.79 ± 1.39^{b}
MCHC (g/dL)	25.38 ± 2.74^{a}	31.31 ± 3.28^{b}

Values are presented as Mean±standard deviation, where n = 5. Values with different superscript along the same row are significantly different at $p<0.05$.

LEGEND: PCV = Packed Cell Volume; Hb = Haemoglobin; RBC = Red Blood Cell; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

Table 2: Effects of Ethanolic Leaf Extract of *T. triangulare* on White Blood Cells Parameters and Platelets in Wistar Rats after 28 days of Administration

Parameters	Control	*T. triangulare*
WBC ($X10^9$/L)	13.02 ± 1.96^a	19.94 ± 1.33^b
Lymphocyte (%)	38.08 ± 6.79^a	53.38 ± 5.44^b
Neutrophil (%)	61.98 ± 6.79^a	58.93 ± 3.48^a
Platelet ($X10^9$/L)	418.02 ± 1.96^a	435.24 ± 3.04^b

Values are presented as Mean±standard deviation, where n = 5. Values with different superscript along the same row are significantly different at $p<0.05$. WBC = White Blood Cell

4. DISCUSSION

The affordability of herbs over expensive pharmaceutical drugs to treat diseases among non-industrialized societies is fast becoming revolutionized. In some countries, it has been integrated into the health scheme despite advances in orthodox medicine. It is believed that the natural products if utilized in the correct form and dosage are less harmful than synthetic products, which most often elicit some side effects [1].

Anaemia increases in prevalence and severity as renal function decreases, it becomes much more common at reduced glomerular filtration rate. Depending on the severity, some of the symptoms of anaemia may include: pale skin, fatigue, weakness, loss of appetite, low haematocrit and hemoglobin in a RBC etc. Factors likely to contribute to anaemia in chronic kidney diseases include blood loss, shortened red cell life span, vitamin deficiencies, the "uremic milieu," erythropoietin (EPO) deficiency, iron deficiency and inflammation [21]. However, the typical "anaemia of chronic renal insufficiency" is a result of a decreased production of red blood cells by the bone marrow. This defect in red blood cell production is largely explained by the inability of the failing kidneys to secrete hormone erythropoietin. This hormone is a necessary stimulus for normal bone marrow to produce red blood cells. Several researchers have reported the beneficial effect of *T. triangulare* leaves but there is dearth information on its effect on haematological parameters. This study is therefore aimed at assessing the haematopoietic potential of its leaves in Wistar rats.

In this study, a significant increase was observed when the blood levels of erythrocyte parameters (packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), Mean Corpuscular Volume (MCV)) of control animals were compared with animals treated with leaf extract of *T. triangulare* at $p<0.05$ as presented in table 1. This is an indication that there may be increased production of red blood cells therefore, suggesting the non-toxic nature of the plant to red blood cells at this period of administration. This might be due to the high phytochemical content of *T. triangulare* leaves reported by Swarna and Ravindhran [22] and antioxidant content reported by Liao *et al.* [23]. Airaodion et al. [15] had previously reported that *T. triangulare* leaves acts as preventive and therapeutic agent against ethanol-induced oxidative stress in Wistar rats due to its antioxidant potential.

The increase in the blood levels of erythrocyte parameters observed in this study might be

suggestive that *T. triangulare* leaves have possible potentials to enhance erythropoietin release from the kidneys, which is the humoral regulator of RBC production and also affect the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and haemoglobin (Hb) are very important in transferring respiratory gases [24, 25]. This may be due to the high content of iron and proteins in the plant. It is therefore possible that consumption of *T. triangulare* by humans can help prevent anaemia especially in menstruating and pregnant women. It has also been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia [26], thus, the 28-day treatment with *T. triangulare* leaves extract may not have the potential to induce anemia nor polycythemia. This is in agreement with the findings of Airaodion et al. [27] who investigated the haematopoietic properties of ethanolic leaf and seed extracts of *Telfairia occidentalis* in Wistar rats but contradicts that of Airaodion et al [28] who reported a significant decrease in erythrocyte parameters when animals were treated with leaf extract of *Vernonia amygdalina*.

The results of this study revealed a significant difference in the white blood cells parameters and platelet of control animals when compared with those treated with leaf extract of *T. triangulare* at $p<0.05$ as presented in table 2. White blood cells, platelet, neutrophil, and lymphocytes are used to provide useful information for diagnosis in routine clinical evaluation of the state of health of a patient. Changes in the haematological system have a higher predicative value for human toxicity [29].

The increase in WBC parameters and platelet counts may be due to the presence of anti-nutritional compounds such as saponins, flavonoids and steroid glucosides in *T. triangulare* leaves [30]. It has been emphasized that the high percentage of WBC especially lymphocytes are associated with the ability of the animals to perform well under very stressful conditions [31]. This increase in the WBC and percentage lymphocyte counts suggests that the phytochemical compounds present in the extracts elicited stress responses. The effect of this plant on the total WBC count could be due to the presence of glycosides. This compound has an anti-inflammatory property and so has vital effect on inflammatory processes of some pathological states such as bacterial infection, malaria and liver diseases [32]. This might also imply that *T. triangulare* leaves may strengthen the immune system through many cytokines regulation.

The nonsignificant difference observed in the level of neutrophil count probably indicates that the body's ability to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) was not compromised. This contradicts the findings of Airaodion et al. [27] who reported a significant difference in the level of neutrophil count when animals were treated with extract of *Telfairia occidentalis* leaves and seed.

The significant increase in platelet count at $p<0.05$ observed in this study may be an indication that leaf extract of *T. triangulare* has stimulates the actions of platelet activating factor (PAF) and thus the blood clotting potentials. It could also be an indication that it has the potential to stimulate thrombopoietin production [33]. This contradicts the findings of Airaodion et al. [27, 28] who reported a nonsignificant difference in the level of platelet count when animals were treated with extract of *Telfairia occidentalis* leaves and seed as well as *Vernonia amygdalina* leaves respectively.

5. CONCLUSION

The significant increase observed in the erythrocyte parameters of animals used in this study indicates that *T. triangulare* leaves have haematopoietic properties and can be used to boost blood level especially in menstruating and pregnant women as well as anaemia patients. Its ability to significantly increase white blood cell parameters is an indication that *T.*

triangulare leaves can boost the immune system and thus defend the body against xenobiotics.

6. ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

7. COMPETING INTERESTS

Authors have declared that no competing interests exist.

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