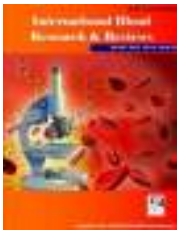

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Effect of Leaf Essential Oil of *Citrus sinensis* on Haematological Parameters of Alloxan-induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSO designed the study, performed the laboratory experiment and wrote the report. Author NOM supervised the study while Author BPO performed the statistical analysis. Author LAU supervised the extraction and analysis of the essential oil. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The aim of this study was to investigate the effect of leaf essential oil of *Citrus sinensis* (Rutaceae) on haematological parameters of alloxan – induced diabetic rats.
Methodology: Diabetes was induced in albino rats by intraperitoneal administration of single dose of alloxan monohydrate (150 mg/kg body weight). The leaf essential oil of *Citrus sinensis* at a dose of 110 mg/kg b.wt was administered every other day to the diabetic rats during 15 days. The effects of leaf essential oil on the erythrocyte and leucocyte indices were then evaluated.
Results: Red Blood Cell, Packed Cell Volume, White blood cell, Neutrophils and Leucocytes were reduced significantly in diabetic animals. However, treatment with leaf oil of *C. sinensis* increased these parameters subsequently.
Conclusion: It is concluded that leaf essential oil of *Citrus sinensis improved the erythrocyte and leucocyte indices of diabetic rats.*

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Keywords: Erythrocyte; Leucocyte; Citrus sinensis; Rutaceae; essential oil; diabetic rats.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Diabetes is divided into two categories namely, Type 1 and type 2 diabetes. Type 1 occurs due to absolute deficiency of insulin and 5-10% of people are affected by this type of diabetes, while type 2 on the other hand occurs mostly due to a combination of insulin resistance and an inadequate compensatory insulin secretory response [1]. The number of people suffering from diabetes worldwide is increasing at an alarming rate. It is predicated that about 366 million people are likely to be diabetic by the year 2030 [2]. Different types of oral hypoglycaemic agents are available along with insulin for the treatment of diabetes. Type 1 is treated with exogenous insulin while type 2 with oral hypoglycaemic agents such as sulphonylureas and biguanides [3]. The effects of diabetes mellitus include dysfunction and failure of various organs [4]. In the short term, hyperglycemia causes symptoms of increased thirst, increased urination, increased hunger, and weight loss and in the long-term, it causes damage to eyes (leading to blindness), kidneys (leading to renal failure) and nerves (leading to impotence and foot disorders/ possibly amputation). As well, it increases the risk of heart disease, stroke and insufficiency in blood flow to legs [4].

The occurrence of anaemia in diabetes mellitus has been reported due to the increased non-enzymatic glycosylation of RBC membrane proteins [5]. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [6]. Platelet aggregation ability has been shown in diabetic patient with long term poor glycaemic control due to lack or deficiency of insulin [7]. The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, Platelets known as thrombocytes help to mediate blood clotting, which is a meshwork of fibrin fibres. The fibres adhere to any vascular opening and thus prevent further blood clot. It plays a crucial role in reducing blood loss and repairing of vascular injury [8].

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition [9]. *Citrus sinensis* also known as sweet orange belong to the citrus family *Rutaceae*. Oranges are grown commercially worldwide in tropical, semi-tropical and some warm temperate regions and have become the most widely planted fruit tree in the world. They are eaten fresh and used for juice. Citrus species contain a wide range of active ingredients and research is still underway in finding uses for them. They are rich in vitamin C, flavonoids, acids and volatile oils. Studies have documented the use of essential oil from the peel as food flavourant, used in making perfumes and medicines [10,11]. The essential oils of *Citrus* are placed within the glands in the outer layer of the fruit skin. This oil is composed of many constituents, including monoterpenes, sesquiterpenes, alcohols, esters and aldehydes. Chemical composition, antioxidant as well as the hypoglycemic properties of the oil has been investigated [10,11]. This study was carried out to investigate the effect of the leaf essential oil of *Citrus sinensis* on the haematological parameters of diabetic animals.

2. MATERIALS AND METHODS

2.1 Materials

Alloxan monohydrate and dimethylsulfoxide (Sigma Chemical Company, St. Louis, Mo, USA), Accu-chek active glucometer and strips (Roche Diagnostic, Mannheim, Germany) and OHAUS analytical balance (Ohaus Corporation, NJ, USA), were used. Fresh leaves of *Citrus sinensis* were obtained from the Junior Staff quarters of the University of Ilorin, Nigeria where a voucher specimen of the plant was deposited. Identification of the leaf was carried out at the Plant Biology Department of the University of Ilorin. Albino rats (*Rattus norvegicus*) were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria.

2.2 Methods

2.2.1 Essential oil extraction

Pulverished leaves of *Citrus sinenis* (800 g) were hydrodistilled for 3 hours in a Clevenger type apparatus. According to the British Pharmacopeia Specification [12]. Five (5) percent v/v of the resulting oil was prepared using saline solution of dimethylsulphoxide (DMSO) [12].

2.2.2 Experimental animals

Forty (40) male albino rats of *norvegicus* strain (150–200 g) were housed in standard cages and allowed to acclimatize to animal house for 14 days. All rats were maintained under standard laboratory conditions (12-h light/dark cycle, 25±2ºC). They were fed with standard rat chow and tap water *ad libitum.* Animals were then randomly selected into 4 groups (i.e. A, B, C and D) of 10 rats each representing (respectively) the Normal Control, Diabetic Control, Diabetic treated with 14.2 mg/kg b. wt. Metformin (reference drug) and Diabetic treated with 110 mg/kg b.wt. leaf essential oil of *Citrus sinensis.*

2.2.3 Induction of experimental diabetes

After fasting for 18 h, animals in the diabetic groups were subjected to a single intraperitoneal injection of freshly prepared 150 mg/kg body weight alloxan monohydrate freshly dissolved in sterile distilled water. 48 h after alloxan injection, fasting blood glucose (FBG) was determined using AccuChek active glucometer and compatible strips. Rats showing glucose concentration above 110 mg/dl were considered diabetic.

2.2.4 Administration of oil

All treatments were intraperitoneally (IP) administered to rats once daily as shown below:

Group A (Normal control) received distilled water
Group B (Diabetic control) received no treatment
Group C (Diabetic + reference drug) was treated with 14.2 mg/kg body weight of metformin
Group D (Diabetic + essential oil) was treated with 110 mg/kg body weight of leaf essential oil of *Citrus sinensis*

2.2.5 Estimation of fasting blood glucose concentration

Fasting blood glucose (FBG) concentration of all experimental groups were determined using a glucose oxidase-based commercial glucometer (AccuChek active, Roche Diagnostic) by withdrawing blood from the caudal vein of rat tail.

2.2.6 Collection and Treatment of Blood Samples

Animals were sacrificed 24 hrs after the last day treatment. They were anaesthetized with ethyl ether and sacrificed by simply incising the jugular vein. Blood samples were collected into EDTA sample bottles for haematological analysis.

2.2.7 Haematological Parameters

The following haematological parameters were analysed using automated hematologic analyzer: Hemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC), Neutrophils and Lymphocytes employing the methods of Dacie and Lewis [13]. The analyzer uses whole blood samples to produce values for the parameter. It uses two fields, the cell packs (which functions as a detergent and self rinses the system to avoid introduction of errors) and the stromatolyzer (which works on the cells).

Principle: The red cells are counted and lysed by the stromatolyser releasing the haemoglobin and estimates its concentration photometrically. The machine assumes that all nucleated cells are white and counts them into their different forms, i.e. lymphocytes and neutrophils.

2.2.8 Statistical analysis

All data were expressed as the mean of five replicates ± standard error of mean (S.E.M). Statistical evaluation of data was performed by SPSS version 16.0 using one way analysis of variance (ANOVA), followed by Duncan's multiple range test for multiple comparison.

3. RESULTS AND DISCUSSION

3.1 Results

Results are reported in Table 1 for the effect of Administration of Leaf Essential Oil of *Citrus sinensis* on Erythrocyte Indices of Alloxan-Induced Diabetic Rats and in Table 2 for the effect of Administration of Leaf Essential Oil of *Citrus sinensis* on Leukocyte Indices of Alloxan-Induced Diabetic Rats.

Table 1. Effect of administration of leaf essential oil of *Citrus sinensis* on erythrocyte indices of alloxan-induced diabetic rats

Treatment	RBC (x10^{12}µ/L)	PCV %	Hb (g/dL)
NC	5.26±0.09^{a}	47.33±1.76^{a}	14.80±1.06^{a}
DC	4.59±0.40^{b}	43.33±1.33^{b}	13.67±1.22^{a}
DR	4.97±0.61^{ab}	45.00±1.15^{ab}	14.03±0.78^{a}
DO	5.08±0.60^{ab}	44.67±1.45^{ab}	14.27±0.46^{a}

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different (P < 0.05).

NC – Normal control; DC – Diabetic control; DR – Diabetic treated with reference drug; DO – Diabetic treated with leaf essential oil of Citrus sinensis; RBC – Red Blood Cell; PCV – Packed Cell Volume; Hb – Hemoglobin.

Reference drug used was metformin

Table 2. Effect of Administration of Leaf Essential Oil of *Citrus sinensis* on Leukocyte Indices of Alloxan-Induced Diabetic Rats

Treatment	WBC (x10^{12}µ/L)	Neutrophils%	Lymphocytes%
NC	12.27±0.15^{a}	42.00±0.58^{a}	68.33±1.71^{a}
DC	7.13±1.42^{b}	31.33±1.96^{a}	57.33±1.48^{b}
DR	7.53±0.45^{b}	42.67±1.48^{a}	65.33±1.33^{a}
DO	7.47±0.64^{b}	31.67±1.71^{a}	58.00±1.15^{b}

Values are expressed as mean of five replicates ± S.E.M and those with different superscripts along a column are statistically different (p < 0.05)

NC – Normal control; DC – Diabetic control; DR – Diabetic treated with reference drug; DO – Diabetic treated with leaf essential oil of Citrus sinensis; WBC – White Blood Cell

Reference drug used was metformin

3.2 Discussion

This study investigated the effect of leaf essential oil of *C. sinensis* on haematological parameters of diabetic rats. Basically, the Erythrocyte and Leucocycte indices were checked in the diabetic and control animals. Results showed a significant reduction in the Erythrocyte indices; Red Blood Cell (RBC), Packed Cell Volume (PCV) and a non significant decrease in the Hemoglobin (Hb) content. A similar finding was reported in a study investigating the antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats, the levels of RBC, Hb, in the diabetic animals were drastically reduced [14]. Similarly, some authors have also reported reduction in haemoglobin, RBC, Hct and PCV level of diabetic animals [15-18]. Reduction in the level of the erythrocyte indices observed in this study might be due to the effect of autooxidation and lipid peroxidation in diabetic condition [19]. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS) [20]. Possible sources of elevated free radicals in type2 diabetes include increased production of radical oxygen species, especially from glycation or lipoxidation processes, autooxidation of glucose/oxidizing of glucose and decreased antioxidant defense systems [21]. Free radicals are produced as a result of glycosylation of several proteins including hemoglobins by non enzymatic mechanisms. Carbohydrates such as glucose can bind non enzymatically to proteins such as hemoglobins in a process known as glycation. The human erythrocytes are freely permeable to glucose and within each erythrocyte; glycated hemoglobin is formed continuously from hemoglobin. Individuals with higher level of blood glucose will have higher levels of glycated hemoglobin [22]. The increased non-enzymatic glycosylation of RBC

membrane proteins has been associated with the occurrence of anaemia in diabetes mellitus [23]. Studies have also shown that Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC [24]. Lipid peroxidation is a free radical-related process that causes disruption of membranes, lipids and other cell components. The free radicals steal electrons from the lipids in the cell membrane, resulting in cell damage [19].

Following the treatment of the diabetic rats with the leaf essential oil of *C. sinensis*, there was a significant increase in the level of RBC, PCV and Hb of the animals. This ameliorative effect observed may be due to the presence of its phenolic content. Essential oil of *C. sinensis* contains mixtures of numerous compounds rich in polyphenols, terpenes and hydrocarbons [25]. These compounds have been reported to possess strong antioxidant capacity [26], therefore, could inhibit peroxidation of polyunsaturated fatty acids in the cell membrane and haemolysis of red blood cells in the diabetic animals [23,27]. Correlations have also been established between the phenolic contents and antioxidant activities of various food sources [20,21]. Reports from previous studies also revealed the ability of the orange volatile oil to chelate Fe^{2+} which might serve to prevent the metal from initiating lipid peroxidation [25]. The OH* scavenging ability of the volatile oil was attributed to the ability of the phenolic compounds in the oil to donate hydrogen as the radical can be neutralized by the hydrogen atom [28,29]. Fe-catalyzed hydrogen peroxide decomposition which has been implicated in the formation of various deleterious lipid peroxidation products has been described as a very important reaction in biological systems.

A significant decrease was observed in the Leucocytes indices of the experimental animals. The reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection [29]. White blood cells (WBC) or leukocytes are the mobile units of the body's protective system. There are six types of WBC normally found in the blood. These are polymorphonuclear neutrophils, eosinophils, basophils, monocytes, lymphocytes and occasionally plasma cells. A very low WBC can be caused by problems in the bone marrow. This condition, called cytopenia or leucopenia means the body is less able to fight of infections [5].

Administration of the treatment oil however brought about a slight non significant increase in the leucocytes indices when compared to the diabetic control. The treatment could not bring about a total amelioration of these indices. This finding is contrary to the reports of some studies that have studied the effect of plants extracts on haematological parameters of diabetic rats. Plants extracts used were able to restore the white blood cell count and related indices to near normal and this have been attributed to the presence of some phytochemicals with ability to stimulate the production of white blood cell in the extract [14,29].

4. CONCLUSION

This work follows our publication in the journal of Annual Review and Research in Biology [25]. Leaf essential oil of citrus sinensis was able to ameliorate the effect of diabetes on the haematological parameters of the diabetic rats. However, the increase in the leucocyte indices was not statistically significant. Hence, there is a need for further experimental investigations to ascertain potential therapeutic benefits of the treatment plant.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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