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Effects of Ethanol Extract of *Moringa oleifera (Lam)* Leaves on Nicotine-induced Changes in Haematological Parameters in Male Wistar Rats (*Rattus norvegicus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author OB participated in the experimental design of the study, literature review, interpretation of the data and wrote the manuscript. Author EOO carried out the experimental procedures, participated in the literature review and the data analysis. Authors AOA, LDB and GSA participated in literature review and data interpretation. Author DSA participated in the experimental design of the study, literature review and interpretation of the data. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This research was conducted to determine the potential role of *Moringa oleifera* leaves extract in attenuating the toxic effects that may be caused by nicotine on haematological parameters in rat.
Study Design: Experimental animal study of ameliorative effect of *Moringa oleifera* on nicotine-induced changes in haematological parameters.

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Place and Duration of the Study: Department of Physiology, Faculty of Basic Medical and Health Sciences, College of Health Sciences, Bowen University, Iwo, Nigeria. Between October 2014 to June 2015.

Methodology: Thirty adult male wistar rats were randomly divided into six groups of 5 each. Group A served as the normal control. Group B served as the nicotine control and the rats were injected intraperitoneally (i.p) with nicotine (3 mg/kg bw). Group C rats were pre-treated with the moringa extract (150 mg/kg bw), and then later given i.p nicotine (3 mg/kg bw). Group D rats received i.p. nicotine (3 mg/kg bw) and the moringa extract (150 mg/kg body weight) concurrently. Group E rats received i.p. nicotine (3 mg/kg bw) and were then post-treated with the moringa extract (150 mg/kg bw). Group F rats received only the moringa extract (150 mg/kg bw).

Results: The results revealed a significant increase ($p < 0.05$) the RBC, PCV, Hb, MCV and MCH of the group that was pre-treated with the moringa extract and the group that was administered solely with the extract. In the groups treated with nicotine, the PCV, RBC, Hb, MCV and MCH were significantly decreased ($p < 0.05$) when compared with the untreated control group. The group that received concurrent administration of nicotine and moringa extract had significantly lower ($p < 0.05$) red blood cell count when compared to both the untreated and nicotine control groups. In the group treated with nicotine, followed by post-treatment with the moringa extract there was a significant increase ($p < 0.05$) in the RBC, PCV, Hb and other red cell indices when compared to the nicotine control group, following the administration of the extract. No significant change ($p > 0.05$) in the leukocyte levels was observed in all treated groups. There was however a significant increase ($p < 0.05$) in the platelet level of all treated groups except the group that received concurrent administration of moringa extract and nicotine, which showed a significant decrease ($p < 0.05$) instead, when compared with the untreated control group.

Conclusion: The results obtained suggest that *Moringa oleifera* leaves have positive haematological effect and that post-treatment with moringa is protective against nicotine induced changes in haematological parameters.

Keywords: Moringa oleifera; nicotine; haematological parameters, wistar rat.

1. INTRODUCTION

The highly addictive alkaloid, nicotine and its metabolites are among the most well characterized chemicals found in tobacco and tobacco smoke [1]. Although approximately 4000 components occur in the cigarette, nicotine is the most active alkaloid in the tobacco. Nicotine is commonly self-administered by the inhalation of tobacco smoke and by chewing of tobacco. Previous studies have shown that nicotine administration induces changes in certain haematological parameters. It has been shown to cause a decrease in red blood cells count, haemoglobin concentration and haematocrit, but an increase in white blood cells and platelet count [2].

The invasion of the RBC membrane by peroxidants, which occurs with the consumption of nicotine and the decreased function of antioxidant systems, can lead to RBC haemolysis [3]. In addition to causing lipid peroxidation, peroxidants can cause the oxidation of -SH groups in proteins and RBC membranes. The –SH groups are highly reactive and can be a target during oxidative stress. Glutathione (GSH) directly protects membrane proteins and preserves their stability. Decreased levels of glutathione lead to a decrease in -SH groups and can result in the oxidization of membrane -SH groups and loss of membrane stability [4]. Cotinine has been shown to increase RBC haemolysis in tobacco users [1]. It has also been shown that nicotine treatment at a dose of 2 $mgkg^{-1}$ induced oxidative damage in both liver and kidney which were attenuated by the glutathione supplementation [5].

Moringa oleifera Lam is a tropical plant species that is an indigenous tree in northern India and Pakistan. It has been introduced throughout the tropics and subtropics and has become popular in many African countries. It is commonly known as Ben oil tree or Horseradish tree in English language, *'Okwe oyibo'* in Igbo, *'Gawara' or 'Habiwal'* in Hausa and *'Adagba maloye'* or '*Ewe Igbale*' in Yoruba, and it grows rapidly in most regions and climatic conditions of Nigeria. *M. oleifera* is the best known and most widely distributed species of *Moringaceae* family, having an impressive range of medicinal uses with high nutritional value throughout the world [6]. A number of medicinal properties have been

ascribed to various parts of this tree. In Asia, the fruits are the most important part of *Moringa oleifera* while the leaves are preferred in Africa [7].

Besides culinary and other domestic uses, several biological properties ascribed to various parts of this tree have been reviewed in the past [6,8]. The leaves of *M. oleifera* have been reported to be a valuable source of both macro- and micronutrients, rich source of β-carotene, protein, vitamin C, calcium, and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat containing foods [9,10]. Fruit (pod)/drum sticks and leaves have been used to combat malnutrition, especially among infants and nursing mothers for enhancing milk production [9,11] and also regulate thyroid hormone imbalance [12]. Most uses of this plant for medicinal purposes have been correlated to their possession of antioxidant activity [13,14]. Previous studies have shown that aqueous and ethanolic extract of *Moringa* leaf and fruit have protective effects on erythrocyte glutathione concentration, which may be attributed to the presence of phytoconstituents (polyphenols, tannins, anthocyanin, glycosides, thiocarbamates) that scavenge free radicals, activate the antioxidant enzymes, and inhibit oxidases [15].

The high nutritional value of *Moringa oleifera* coupled with its possession of antioxidant activity is likely to have beneficial effects on the nicotine-induced changes in haematological parameters. This however has not been proven and the aim of this research work is to investigate the effects of ethanolic extract of *moringa oleifera (lam)* leaves on nicotine-induced changes in haematological parameters in male wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

30 male *Rattus norvegicus* weighing between 180 and 220 g were obtained from a private animal farm in Ibadan, Nigeria. They were brought to the Animal house of the Department of Physiology, Bowen University, Iwo. The animals were kept in cages made of wooden material and wire meshes, at room temperature for about four weeks before the commencement of the experiment, in order to allow them acclimatize to the new environment. They were fed with rat pellet diet and water ad libitum. The use and care of laboratory animals were conducted in accordance with the internationally accepted best practices as contained in the European Commission guidelines (EEC Directive of 1986; 86/609/EEC). The study was approved to be conducted by College of Health Sciences Ethical Committee, Bowen University, Iwo, Osun State, Nigeria.

2.2 Chemicals

The nicotine used in this study was obtained from the department of Physiology, University of Ibadan. The nicotine was in the form of nicotine dihydrogen tartrate (Sigma Aldrich, USA).

2.3 Collection of Plant Material and Preparation of Extract

Fresh *Moringa oleifera* leaves were purchased at a market in Iwo, Osun State, Nigeria. The herbarium specimens were deposited in department of Botany, University of Ibadan (Voucher Specimen no. UIH-22387). The plant material was authenticated by Esimekhuai, D. The leaves were dried for about 12 days in an airy room, away from direct sunlight to avoid possible damage to their phytoconstituents. The dried leaves were ground into powder and then 100 g of the powder was soaked in 1000 mls of 70% ethanol, shaken for 10 minutes and allowed to stay in refrigerator for 72 hours at 4℃. The mixtures were first filtered with cheese cloth, then with Whatman No 1 filter paper (24 cm). The filtrates were concentrated to complete dryness in water bath in order to obtain the crude extract [16].

2.4 Experimental Design

A total of 30 animals were used in this study and the administration was done for 24 days. These were divided randomly into 6 groups consisting of five rats each. The 6 experimental groups were as follows;

2.4.1 Untreated control group (A)

The rats in this group were given nothing except food and water ad libitum.

2.4.2 Nicotine control group (B)

The rats in this group were given 3 mg/kg body weight of intraperitoneal nicotine daily and were allowed access to food and water ad-libitum.

2.4.3 Moringa pre-treatment group (C)

The rats in this group were given moringa extract orally at a dose of 150 mg/kg body weight, with the aid of an oropharyngeal cannula for the first 12 days. They were given 3 mg/kg body weight of intraperitoneal nicotine daily for the following 12 days and were allowed access to food and water ad-libitum.

2.4.4 Concurrent moringa and nicotine group (D)

The rats in this group were given moringa extract orally at a dose of 150 mg/kg body weight, with the aid of an oropharyngeal cannula, and 3 mg/kg body weight of intraperitoneal nicotine concurrently throughout the duration of the experiment. They were also allowed access to food and water ad-libitum.

2.4.5 Moringa post-treatment group (E)

The rats in this group were given 3 mg/kg body weight of intraperitoneal nicotine daily for the first 12 days. They were then given moringa extract orally at a dose of 150 mg/kg body weight, with the aid of an oropharyngeal cannula for the following 12 days, and were allowed access to food and water ad-libitum.

2.4.6 Moringa only group (F)

The rats in this group were given moringa extract orally at a dose of 150 mg/kg body weight, with the aid of an oropharyngeal cannula for 24 days, and were allowed access to food and water ad-libitum.

2.5 Blood Collection

Blood samples were obtained from the tail vein on the 12th day of administration, and on the 24th day of administration the animals were anaesthesized using chloroform and blood was obtained via cardiac puncture. The whole blood samples were put in Ethylene di-amine tetra acetate (EDTA) treated sample tubes.

2.6 Analysis of Blood Samples

2.6.1 Determination of hematological parameter

RBC count was done using the method of Dacie and Lewis [17]. Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with a Neubauer counting chamber under a light microscope. The counting of total white blood cells was done after the method of Brown (1974) using a diluting fluid (Turk's fluid) in a ratio of 1:20. Sahli's haemoglobinometer was employed for estimation of haemoglobin (Hb) content of the blood, and packed cell volume (PCV) was done using the macrohaematocrit method [17]. Differential cell counts were done on a thin slide, prepared with a smearing blood sample, using Wright-Giemsa's stain. According to staining and morphological criteria, differential cell analysis was carried out under a light microscope by counting 100 cells, and the percentage of each cell type was calculated.

Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated from values of RBC, PCV and Hb as follows:

MCV (fL) = PCV (%) x 10 / RBC count;

MCH (pg) = Hb (g/dl) x 10 / RBC count

and

MCHC (g/dl) = Hb (g/dl) x 100 / PCV (%)

2.7 Statistical Analysis

The statistical method applied in this study expressed the values in form of their mean ± SEM (Standard Error of Mean). The difference of the mean between the groups was analyzed with one way Analysis of Variance (ANOVA) using Duncan Method at a 0.05 significance level.

3. RESULTS

The results of the study of the effect of ethanolic extract of *Moringa oleifera* leaves on nicotine-induced haematological changes in rats are shown in the Table 1.

3.1 Effect of Ethanolic Extract of *Moringa oleifera* Leaves on Nicotine-induced Changes in Red Blood Cell Count, Packed Cell Volume and Haemoglobin Concentration

Statistical analysis of the results obtained from the estimation of the red blood cell count, packed cell volume and haemoglobin concentration as

expressed in Table 1 reveals that after two weeks of treatment, the red blood cell counts of groups B (4.17 ± 0.159 ($x10^6$) /mm3), D (3.58 ± 0.250 ($x10^6$) /mm3) and E (4.04 ± 0.183 ($x10^6$) /mm3) showed a significant decrease ($p < 0.05$) when compared to the untreated control group (A) (5.28 ± 0.102 ($x10^6$) /mm3). Also, a significant increase ($p < 0.05$) was recorded in groups C (5.38 ± 0.172 ($x10^6$) /mm3) and F (5.37 ± 0.190 ($x10^6$) /mm3) when compared to the nicotine control group (B) (4.17 ± 0.159 ($x10^6$) /mm3).

The packed cell volume of groups B (30.80 ± 2.222%) and E (29.40 ± 2.657%) showed a significant decrease ($p < 0.05$) when compared to the untreated control (40.40 ± 1.600%). Groups C (41.60 ± 1.860%) and F (41.00 ± 2.073%) however showed a significant increase ($p < 0.05$) when compared to the nicotine control group (30.80 ± 2.222%). The packed cell volume of group D (34.33 ± 2.729%) showed no significant ($p>0.05$) change when compared to both the nicotine and untreated control groups.

With respect to the haemoglobin concentration, there was a significant decrease ($p < 0.05$) in group B (10.32 ± 0.628 g/dl) and D (10.60 ± 0.693 g/dl) when compared to the untreated control group (13.12 ± 0.488 g/dl). No significant ($p>0.05$) change was observed in group E (11.68 ± 0.554 g/dl). However, there was a significant increase ($p < 0.05$) in groups C (13.60 ± 0.856 g/dl) and F (13.28 ± 0.731 g/dl) when compared to the nicotine control (10.32 ± 0.628 g/dl).

Following 4 weeks of treatment, statistical evaluation also reveals that no significant changes ($p < 0.05$) were observed in the red blood cell count of group B (4.35 ± 0.168($x10^6$) /mm3) and C (4.73 ± 0.263($x10^6$) /mm3) when compared to the untreated control group (4.94 ± 0.192($x10^6$) /mm3). However there was a significant increase ($p < 0.05$) in that of group E (5.32 ± 0.158($x10^6$) /mm3) and F (5.47 ± 0.244($x10^6$) /mm3).

The packed cell volume of groups E (43.80 ± 2.200%) and F (47.20 ± 1.772%) showed a significant increase ($p < 0.05$) when compared to the untreated (35.40 ± 0.812%) and nicotine control (34.80 ± 2.154%) groups. With respect to the haemoglobin concentration, a significant increase ($p < 0.05$) was observed in group F (15.32 ± 0.609 g/dl) when compared to both the nicotine (11.88 ± 0.745 g/dl) and untreated control (12.08 ± 0.644 g/dl) groups. No significant ($p>0.05$) change was observed in group B (11.88 ± 0.745 g/dl), C (13.36 ± 0.699 g/dl) and D (11.47 ± 0.751 g/dl). The haemoglobin concentration of group E (14.13 ± 0.418 g/dl) also showed a significant increase ($p < 0.05$) when compared to that of the nicotine control.

3.2 Effect of Ethanolic Extract of *Moringa oleifera* Leaves on Nicotine-induced Changes in Mean Cell Volume, Mean Cell Haemoglobin and Mean Cell Haemoglobin Concentration

After two weeks of treatment, statistical analysis of the results obtained (Table 2) reveals that the mean cell volume of groups B (73.70 ± 3.728 cu.µ), C (77.21 ± 1.217 cu.µ), E (72.88 ± 6.307 cu.µ) and F (76.21 ± 1.224 cu.µ) showed no significant ($p > 0.05$) change when compared to the untreated control group (76.42 ± 2.193 cu.µ). There was, however, a significant increase ($p < 0.05$) in group D (95.79 ± 1.587 cu.µ) when compared to both the untreated and nicotine control groups.

The mean cell haemoglobin of groups D (29.93 ± 2.909 pg) and E (28.98 ± 1.033 pg) showed a significant increase ($p < 0.05$) when compared to both the untreated and nicotine control groups. No significant ($p > 0.05$) changes were observed in the remaining groups when compared to both the untreated (24.81 ± 0.577 pg) and nicotine control (24.81 ± 1.544 pg) groups.

With respect to the mean cell haemoglobin concentration, there was a significant increase ($p < 0.05$) in group E (40.93 ± 3.945%), but no significant changes in the remaining groups when compared to both the untreated (32.56 ± 1.106%) and nicotine control (33.75 ± 1.540%) groups.

Following 4 weeks of treatment, a significant increase ($p < 0.05$) was observed in the mean cell volume of groups B (79.73 ± 2.187 cu.µ), C (84.30 ± 2.925 cu.µ), E (82.24 ± 2.372 cu.µ) and F (86.47 ± 1.595 cu.µ) when compared to the untreated control group (71.88 ± 2.185 cu.µ). Group D (96.99 ± 4.728 cu.µ) showed a significant increase ($p < 0.05$) when compared to both the untreated (71.88 ± 2.185 cu.µ) and nicotine control (79.73 ± 2.187 cu.µ) groups. The mean cell haemoglobin of groups C (28.48 ± 1.781 pg) and E (26.56 ± 0.355 pg) showed a significant increase ($p < 0.05$) when compared to the untreated control (24.42 ± 0.842 pg) group.

However, groups B (27.22 ± 0.719 pg) and E (26.56 ± 0.355 pg) showed no significant ($p > 0.05$) change when compared to the untreated control group (24.42 ± 0.842 pg). No significant ($p > 0.05$) change was observed in the mean cell haemoglobin concentration of groups B (34.15 ± 0.536%), C (33.72 ± 1.314%), D (32.65 ± 2.965%), E (32.38 ± 0.871%) and F (34.12 ± 1.668%) when compared to the untreated control group (34.12 ± 1.668%).

3.3 Effect of Ethanolic Extract of *Moringa oleifera* Leaves on Nicotine-induced Changes in Eosinophils, Basophils and Neutrophils Levels

Statistical analysis of the results obtained from the estimation of the eosinophils, basophils and neutrophils levels as expressed in Table 3 reveals that after two weeks of treatment, the eosinophil levels of groups C (2.98 ± 0.460%), D (3.48 ± 0.405%), E (3.26 ± 0.480%) and F (2.60 ± 0.341%) showed no significant ($p>0.05$) changes when compared to both the untreated control group (A) (2.40 ± 0.278%) and the nicotine control group (B) (3.03 ± 0.346%). After four weeks, there was still no significant difference between the treated groups and the control groups. The basophil levels of group D (0.60 ± 0.102%) showed a significant increase ($p < 0.05$) when compared to the nicotine control group (B) (0.25 ± 0.060%). No changes were however observed when compared with the untreated control group (A) (0.36 ± 0.055%). Groups C (0.29 ± 0.033%), E (0.54 ± 0.182%) and F (0.38 ± 0.110%) on the other hand, showed no significant ($p>0.05$) changes when compared to both the untreated control group (A) (0.36 ± 0.055%) and the nicotine control group (B) (0.25 ± 0.060%). After four weeks, there was no significant ($p>0.05$) change in all treated groups when compared to both the untreated control group (A) (0.56 ± 0.171%) and the nicotine control group (B) (0.53 ± 0.155%).

With respect to the neutrophil levels, statistical evaluation reveals that after two weeks of treatment, the neutrophil levels of groups C (57.26 ± 1.439%), D (60.63 ± 1.261%), E (59.61 ± 2.382%) and F (56.28 ± 2.218%) showed no significant ($p>0.05$) changes when compared to both the untreated control group (A) (54.94 ± 1.700%) and the nicotine control group (B) (57.51 ± 1.620%). After four weeks, there was still no significant difference between the treated groups and the control groups (A) (58.49 ± 2.78%) and B (55.55 ± 1.17%).

3.4 Effect of Ethanolic Extract of M*oringa oleifera* Leaves on Nicotine-induced Changes in Monocytes, Lymphocytes and Platelets Levels

Statistical analysis of the results obtained from the estimation of the monocyte, lymphocyte and platelet levels as expressed in Table 4 reveals that after two weeks of treatment, the monocyte levels of groups C (2.50 ± 0.467%), D (2.97 ± 0.655%), E (59.61 ± 2.382%) and F (2.80 ± 0.400%) showed no significant ($p>0.05$%) changes when compared to both the untreated control group (A) (3.04 ± 0.161%) and the nicotine control group (B) (1.96 ± 0.377%). After four weeks, there was still no significant difference between the treated groups and the control groups (A) (1.96 ± 0.377%) and B (2.47 ± 0.362%).

The lymphocyte levels of group D (32.32 ± 2.111%) showed a significant decrease ($p < 0.05$) when compared to the nicotine control group (B) (37.24 ± 1.569%). No changes were however observed when compared with the untreated control group (A) (39.26 ± 1.760%). Groups C (36.97 ± 0.831%), E (33.79 ± 2.099%) and F (37.49 ± 1.604%) on the other hand, showed no significant ($p>0.05$) changes when compared to both the untreated control group (A) (39.26 ± 1.760%) and the nicotine control group (B) (37.24 ± 1.569%). After four weeks, there was no significant ($p>0.05$) change in all treated groups when compared to both the untreated control group (A) (35.49 ± 2.894%) and the nicotine control group (B) (38.78 ± 1.193%).

After two weeks of treatment, the platelet levels of the untreated control group (A) (2.16 ± 0.205($x10^5$)/µL), and groups C (2.91 ± 0.215($x10^5$)/µL), D (1.90 ± 0.132($x10^5$)/µL) and F (3.18 ± 0.234($x10^5$)/µL) showed a significant decrease ($p < 0.05$) when compared with that of the nicotine control group (B) (4.17 ± 0.203($x10^5$)/µL). A significant increase ($p < 0.05$) was however observed in the nicotine control group (B) (4.17 ± 0.203($x10^5$)/µL), and groups C (2.91 ± 0.215($x10^5$)/µL), E (3.81 ± 0.199($x10^5$)/µL) and F (3.18 ± 0.234($x10^5$)/µL) when compared to the untreated control group (A) (2.16 ± 0.205($x10^5$)/µL). After four weeks, the platelet levels of the untreated control group (A) (2.58 ± 0.502($x10^5$)/µL), and groups C (3.15 ± 0.750($x10^5$)/µL), D (1.90 ± 0.606($x10^5$)/µL), E (3.15 ± 0.382($x10^5$)/µL) and F (3.24 ± 0.596($x10^5$)/µL) showed a significant decrease when compared to the nicotine control group (B)

(4.23 ± 0.502(x10^5)/µL), which revealed a significant increase when compared with the untreated control group (A) (2.58 ± 0.502(x10^5)/µL).

4. DISCUSSION

The effect of ethanolic extract of *M. oleifera* leaves on nicotine-induced changes in haematological parameters was evaluated, analyzed and interpreted. The assessment of haematological parameters is a biomarker for evaluating the haematotoxic potential of the extract in the area of pharmacognosy [18].

A significant increase was observed in the RBC, PCV, Hb, MCV and MCH of the group that was pre-treated with the moringa extract and the group that was administered solely with the extract. This observation correlates with that of Ujah et al. [19] that moringa increases red blood cell count and its related indices. This indicates that moringa has a positive haematological effect.

The possible mechanism by which *Moringa oleifera* increased the red blood cell count may be due to the fact that *M. oleifera* is a rich source of antioxidant [20,21] and it contains some bioactive constituents or phytoconstituents which should have imposed or boosted haematopoietic activities. It is also supported by the fact that *M. Oleifera* leaf is rich in terms of vitamin A, B, C and also among the best plant source of minerals like iron and also an excellent source of protein [19].

The aqueous extract of Moringa leaves contains certain non-phenolic, biologically active components such as selenium, thiocarbamates, glucosinolates, and its hydrolysis products as glucoraphanin, isothiocyanate sulforaphane, nitriles [22], in addition to the phenolics such as quercetin and kaempferol, which could serve as antioxidants and may effectively scavenge various reactive oxygen species/free radicals under in vivo conditions.

In the groups treated with nicotine, the PCV, RBC, Hb, MCV and MCH were significantly decreased when compared with the untreated control group. This correlates with the findings of Mosbah et al. [2] that nicotine has negative haematologic effects. An explanation for this is that nicotine produces peroxidants when metabolized in the body, and these peroxidants cause oxidative damage of the erythrocyte membrane leading to haemolysis of the cell. The recurring haemolysis that occurs with continuous use of nicotine and subsequent peroxidation explains the cause of the decrease in haemoglobin concentration, packed cell volume and other red blood cell indices.

The group that received concurrent administration of nicotine and moringa extract had significantly lower red blood cell count when compared to both the untreated and nicotine control groups. This may possibly be due to interactions between certain components of the moringa extract and nicotine. However, for definite answer to this is beyond the scope of this work. Further research work will be required to confirm this finding.

In the group treated with nicotine, followed by post-treatment with the moringa extract there was a significant increase in the RBC, PCV, Hb and other red cell indices when compared to the nicotine control group, following the administration of the extract. This indicates the potency of moringa in alleviating the reduction in these parameters that occurred following the initial treatment with nicotine. On the other hand, the group that was pre-treated with the moringa extract followed by the administration of nicotine, showed a slight decrease in the red blood cells, however this decrease was not statistically significant when compared with the untreated and nicotine control groups. This is an indication that moringa may be helpful as part of management or rehabilitatory measures for past users of tobacco products.

In contrast to the findings of Mosbah et al. [2], no significant change in the leukocyte levels, was observed in all treated groups. This may be as a result of environmental factors or the duration of study involved in both studies. There was, however, a significant increase in the platelet level of all treated groups except the group that received concurrent administration of moringa extract and nicotine, which showed a significant decrease instead, when compared with the untreated control group. Nicotine may affect platelets by releasing epinephrine, which is known to enhance platelet reactivity; by inhibiting prostacyclin, an antiaggregatory hormone that is secreted by endothelial cells; or perhaps directly. Finally, by increasing the heart rate and cardiac output, nicotine increases blood turbulence and may promote endothelial injury [23]. While the increase in the group treated with moringa extract, correlates with the findings of Hisham et al. [24].

Table 1. Results of the effect of ethanolic extract of *Moringa oleifera* leaves on nicotine-induced changes in red blood cell count, packed cell volume and haemoglobin concentration

Group	RBC (x10^6) /mm^3		PCV (%)		HB (g/dl)	
	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
A (untreated control)	5.28 ± 0.102	4.94 ± 0.192	40.40± 1.600	35.40 ± 0.812	13.12± 0.488	12.08± 0.644
B (nicotine control)	4.17 ± 0.159[a]	4.35 ± 0.168	30.80± 2.222[a]	34.80 ± 2.154	10.32± 0.628[a]	11.88± 0.745
C (moringa pre-treatment)	5.38 ± 0.172[b]	4.73 ± 0.263	41.60± 1.860[b]	39.80 ± 2.417	13.60± 0.856[b]	13.36± 0.699
D (concurrent moringa and nicotine)	3.58± 0.250[ab]	3.65± 0.171[ab]	34.33± 2.729	35.33 ± 1.453	10.60± 0.693[a]	11.47± 0.751
E (moringa post-treatment)	4.04 ± 0.183[a]	5.32 ± 0.158[b]	29.40± 2.657[a]	43.80± 2.200[ab]	11.68± 0.554	14.13± 0.418[b]
F (moringa only)	5.37 ± 0.190[b]	5.47 ± 0.244[b]	41.00± 2.073[b]	47.20± 1.772[ab]	13.28± 0.731[b]	15.32± 0.609[ab]

Values are expressed as the mean ± standard error of the mean.
a -Significantly different from the untreated control group at p < 0.05
b -Significantly different from the nicotine control group at p < 0.05
**RBC- Red blood cell *PCV- Packed Cell Volume *HB- Haemoglobin concentration*

Table 2. Results of the effect of ethanolic extract of *Moringa oleifera* leaves on nicotine-induced changes in mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration

Group	MCV (cu.µ)		MCH (pg)		MCHC (%)	
	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
A (untreated control)	76.42± 2.193	71.88 ± 2.185	24.81± 0.577	24.42± 0.842	32.56± 1.106	34.12± 1.668
B (nicotine control)	73.70± 3.728	79.73± 2.187[a]	24.81± 1.544	27.22± 0.719	33.75± 1.540	34.15± 0.536
C (moringa pre-treatment)	77.21± 1.217	84.30± 2.925[a]	25.18± 0.881	28.48± 1.781[a]	32.61± 1.014	33.72± 1.314
D (concurrent moringa and nicotine)	95.79± 1.587[ab]	96.99± 4.728[ab]	29.93± 2.909[ab]	31.42± 1.715[ab]	31.36± 3.483	32.65± 2.965
E (moringa post-treatment)	72.88 ± 6.307	82.24 ± 2.372[a]	28.98± 1.033[ab]	26.56 ± 0.355	40.93± 3.945[ab]	32.38± 0.871
F (moringa only)	76.21 ± 1.224	86.47 ± 1.595[a]	24.69 ± 0.727	28.04± 0.326[a]	32.38 ± 0.727	32.46± 0.579

Values are expressed as the mean ± standard error of the mean.
a -Significantly different from the untreated control group @ p < 0.05
b -Significantly different from the nicotine control group @ p < 0.05
**MCV- Mean Cell Volume *MCHC- Mean Cell Haemoglobin Concentration *MCH- Mean Cell Haemoglobin*

Table 3. Results of the effect of ethanolic extract of *Moringa oleifera* leaves on nicotine-induced changes in eosinophils, basophils and neutrophils levels

Group	Eosinophils (%)		Basophils (%)		Neutrophils (%)	
	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
A (untreated control)	2.40 ± 0.278	2.76 ± 0.386	0.36 ± 0.055	0.56 ± 0.171	54.94 ± 1.700	58.49 ± 2.78
B (nicotine control)	3.03 ± 0.346	2.67 ± 0.436	0.25 ± 0.060	0.53 ± 0.155	57.51 ± 1.620	55.55 ± 1.17
C (moringa pre-treatment)	2.98 ± 0.460	2.06 ± 0.286	0.29 ± 0.033	0.40 ± 0.071	57.26 ± 1.439	58. 27 ± 1.64
D (concurrent moringa and nicotine)	3.48 ± 0.405	2.75 ± 0.203	0.60 ± 0.102[b]	0.39 ± 0.129	60.63 ± 1.261	58.24 ± 2.67
E (moringa post-treatment)	3.26 ± 0.480	1.86 ± 0.213	0.54 ± 0.182	0.22 ± 0.038	59.61 ± 2.382	57.76 ± 1.56
F (moringa only)	2.60 ± 0.341	2.21 ± 0.340	0.38 ± 0.110	0.40 ± 0.038	56.28 ± 2.218	55.65 ± 2.08

Values are expressed as the mean ± standard error of the mean.
b -Significantly different from the nicotine control group at $p < 0.05$

Table 4. Results of the effect of ethanolic extract of *Moringa oleifera* leaves on nicotine-induced changes in monocytes, lymphocytes and platelets levels

Group	Monocytes (%)		Lymphocytes (%)		Platelets (x10^5)/µL	
	Week 2	Week 4	Week 2	Week 4	Week 2	Week 4
A (untreated control)	3.04 ± 0.161	2.72 ± 0.419	39.26 ±1.760	35.49 ± 2.894	2.16 ± 0.205[b]	2.58 ± 0.502[b]
B (nicotine control)	1.96 ± 0.377	2.47 ± 0.362	37.24 ±1.569	38.78 ± 1.193	4.17 ± 0.203[a]	4.23 ± 0.502[a]
C (moringa pre-treatment)	2.50 ± 0.467	2.42 ± 0.361	36.97 ±0.831	36.85 ± 1.635	2.91 ±0.215[ab]	3.15 ± 0.750[b]
D (concurrent moringa and nicotine)	2.97 ± 0.655	2.37 ± 0.500	32.32 ±2.111[a]	36.24 ± 2.502	1.90 ± 0.132[b]	1.90 ± 0.606[b]
E (moringa post-treatment)	2.80 ± 0.400	2.62 ± 0.241	33.79 ±2.099	37.54 ± 1.798	3.81 ± 0.199[a]	3.15 ± 0.382[b]
F (moringa only)	3.25 ± 0.471	2.83 ± 0.338	37.49 ±1.604	38.90 ± 2.287	3.18 ±0.234[ab]	3.24 ± 0.596[b]

Values are expressed as the mean ± standard error of the mean.
a -Significantly different from the untreated control group @ $p < 0.05$
b -Significantly different from the nicotine control group @ $p < 0.05$

5. CONCLUSION

From the available findings in this study, it can be concluded that administration of *Moringa oleifera* to Wistar rats attenuated the nicotine-induced changes in the red blood cell count, haemoglobin concentration and other red blood cell indices; however, post-treatment with *moringa* produced a protective effect against these changes in haematological parameters, hence, this may be recommended to consumers of tobacco products as part of the management or rehabilitatory measures for such individuals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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