**Assessment of the influence of Extracts of Kigelia Africana Fruit and Sorghum Bicolor on the Haematological Indices in Rats with** Alloxan-Induced Diabetes.

ABSTRACT

*Diabetes mellitus (DM), a metabolic disorder characterized by an excessive increase in blood glucose levels when poorly managed, has been associated with functional and structural changes in the haemoglobin molecule, cytoplasmic viscosity and osmotic disturbances within the red cell with changes reflected in the red cell and other haematological parameters. With recent breakthroughs in the development of herbal drugs in the treatment of diabetes, there are still limitations in areas of their toxic side effects with derangements in haematological parameters associated with the progression and pathophysiology of the disease. The study is therefore aimed at evaluating the beneficial effect of extract of K. africana and S. bicolor on the haematological parameters of diabetic-induced rats.*

*A total of eleven groups, each containing five rats, were randomly selected from Alloxan-induced diabetic Wistar rats of both sexes for the study. One group served as the control, another as the glibenclamide-treated group, and the remaining nine groups were treated with extracts. After an overnight fast the control group received a dose of 0.5 ml of 2 % w/v acacia solution, the glibenclamide treated group received 600 μg/kg bwt glibenclamide while the other nine groups received specified doses (125, 250 and 500mg/kg bwt) of K. africana and S. bicolor extracts singly and in mixture of ratio 1:1 respectively.* *After receiving the specified doses once a day orally for 30 days, the rats were fasted overnight, and 5 ml of blood collected via cardiac puncture in fluoride and EDTA bottles with the fluoride, spun and separated for fasting plasma glucose using the commercially prepared kit (COBAS, Mumbai, India) following standard method while the EDTA is use for various haematological parameters using hematology automated analyzer (Beckman Coulter (5-part autoanalyzer).*

 *Result showed that the rats treated singly with extracts of K. Africana and S. bicolor as well as their mixture had lower glucose levels when compared to the control group. Although their Hb, PCV, RBC, MCV, MCH, MCHC and WBC were not statistically different from the control at all doses, they had a decrease (p < 0.05) in PCV and RBC levels with an increase in WBC when compared with the diabetes untreated group.*

*Extracts from K. africana and S. bicolor are glucose lowering agents and protects against haematological changes that are detrimental to diabetic patients. There is need to exploit further therapeutic effects to substantiate their ethnomedicinal usage.*

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by excessive blood glucose levels1. Elevation of blood glucose levels without intervention results in macro- and microvascular complications that could lead to disability and, ultimately, death if not checked.2  Furthermore, poorly managed diabetes, as seen in the consistent elevation of HbA1c, can be associated with functional and structural changes in the haemoglobin molecule, cytoplasmic viscosity, and osmotic disturbances within the red cell. These changes may be reflected in the red cell and other haematological parameters.Thus, Haematological parameters, which are used to establish the body's functional status, play a key role in determining the progress of diabetic management.4 They also serve as good pathological mirrors of the entire body. Hence, the cellular component of blood is valuable in immunotoxicology to evaluate the immunotoxic potential of a compound. Also, Haematological parameters are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems.5 Some of such Medicinal plants have been used in several countries to manage metabolic diseases like diabetes mellitus and are thought to be cheaper than allopathic drugs.6 They are available and affordable to many, especially in developing countries like Nigeria.7,8 One of such plant which is Kigelia africana (Lam.) Benth. Kigelia africana (Family: Bignoniaceae), with a wide-spread distribution in the West, South and Central Africa and known as the sausage or cucumber tree is used traditionally in diabetes management.9 Similarly, Sorghum, which is very important in the world's human diet, has been known to provide natural antioxidants and essential fatty acids that could fight cardiovascular-related diseases.10 Previous studies have reported the anti-inflammatory and analgesic bioactivities of this plant.11 Although widely used, the explanation of their blood-related functions and effects is scarce. Especially with recent breakthroughs in the development of drugs used in the treatment of diabetes, limitations in areas of their toxic side effects with derangements in haematological parameters associated with the progression together with the generation of free radicals implicated in the pathophysiology of the disease is still a very common phenomenon. The study is therefore aimed at evaluating the beneficial effect of the extract of K africana fruit and S. bicolor stalk on the haematological parameters of diabetic-induced rats.

Material and methods

 PLANT MATERIALS

The fresh fruits of K. africana (Lam.) Benth (Fam. Bignoniaceae) and S. bicolor stalks (L) Moench (Fam. Poaceae) were bought from Mushin market in Lagos suburb, Nigeria in November 2014. The fruits and stalks were identified and authenticated by a taxonomist, Mr. O.O Oyebanji of the Department of Botany, Faculty of Science, University of Lagos, Nigeria. The specimens were given voucher no LUH 6487 (K. africana) and no LUH 6488 (S. bicolor) respectively and were deposited in the Department’s Herbarium. The plant materials were washed with copious amount of clean tap water and spread to drain, then cut into small pieces and dried in an oven at a temperature of 450C for a period of seven days.

PREPARATION OF EXTRACTS

The dried materials were pulverized to a coarse powder with an electric grinder. The powdered materials of K. africana fruits (3200 g) and S. bicolor stalks (3150 g) were macerated with 25 litres of hydroethanolic (2:8), respectively, and allowed to stand for seven days, with regular stirring. The extracts were clarified by filtration (Sofowora, 1993) using Whatman no.4 filter paper. They were then concentrated using a rotary evaporator then dried in a laboratory oven (450C) to a dry weight of 243.12 g (7.60 %w/w yield) for K. africana and 174.94 g (5.60 %w/w yield) for S. bicolor respectively.

ANIMALS

Male and female Wistar rats (150 ± 20 g) and Swiss albino mice (22.50 ± 2.50 g) obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria, were used. They were kept under standard environmental conditions (23-25oC, 12 h/12 h light/dark cycle), housed in plastic cages (5 rats/mice per cage), maintained on animal pellets (Livestock Feed PLC, Lagos, Nigeria) and allowed access to water ad libitum. The rodents were allowed to acclimatize for 7 days to laboratory conditions before any experiment. The use and care of the rodents were in strict compliance with the National Research Council (NRC) Guidelines on the Care and Use of Laboratory Animals (NRC, 2011).12

ETHICAL APPROVAL

The College of Medicine of the University of Lagos Health Research Ethics Committee approved the study (Ethical Approval No: CM/HREC/10/16/101).

EXPERIMENTAL DESIGN

Methodology

Healthy Wistar rats of both sexes were used. Diabetes was experimentally induced after an overnight fast by administering 150 mg/kg body weight (bwt) of Alloxan monohydrate dissolved in normal saline through the intraperitoneal route (i.p.). (Ogbonnia et al., 2014) (Ogbonnia et al., 2014) 13

After 72 hours, blood glucose levels were monitored using a glucometer (Accu-Chek, Roche Diagnostics, Mannheim, Germany). Rats with plasma glucose levels≥ 200 mg/dL were classified as diabetic and included in the study. Eleven groups, each containing five rats, were randomly selected.

Treatment was done daily for 30 days as follows:

Group I: Alloxan-induced diabetic rats treated with 125 mg/kg bwt K. africana

Group II: Alloxan-induced diabetic rats treated with 250 mg/kg bwt K. africana

Group III: Alloxan-induced diabetic rats treated with 500 mg/kg bwt K. africana

Group IV: Alloxan-induced diabetic rats treated with 125 mg/kg bwt S. bicolor

Group V: Alloxan-induced diabetic rats treated with 250 mg/kg bwt S. bicolor

Group VI: Alloxan-induced diabetic rats treated with 500 mg/kg bwt S. bicolor

Group VII Alloxan-induced diabetic rats treated with 250 mg/kg bwt of mixture (1:1)

Group VIII Alloxan-induced diabetic rats treated with 500 mg/kg bwt of mixture (1:1)

Group IX: Alloxan-induced diabetic rats treated with glibenclamide 600 μg/kg bwt

Group X: Alloxan-induced diabetic rats treated with 2% acacia solution

Group XI Normal rats not induced with diabetes but received acacia 2% w/v solution.

The rats were initially weighed and subsequently weighed every seven days from the beginning to the end of the treatment to observe variations in the body weights. On the 31st day, after an overnight fast, the animals were sacrificed, and blood was obtained via cardiac puncture. The blood was then distributed into EDTA and fluoride containers for haematological and plasma glucose determinations, respectively.

The blood was centrifuged within 5 minutes of collection at 4000 rpm for 10 minutes to obtain plasma, which was used for plasma glucose assay.

Hematological Parameters Studies

Hematological studies were done using a haematology automated analyzer (Beckman Coulter (5-part autoanalyzer).14

Principle

The coulter determines the number and size of particles suspended in an electrically conductive liquid by forcing the suspension to flow through a small aperture with an immersed electrode on either side. As particles pass through the aperture, the resistance between the electrodes changes, producing a voltage pulse of short duration with a magnitude proportional to particle size. The pulse height and instrument responses are essentially proportional to particle volume and fluid receptivity. The series of pulses is then electronically scaled and counted.

* The samples were placed on the electronic blood mixer.
* They were allowed to mix well for about 30 minutes before analysis.
* Each sample was then picked and aspirated into the coulter counter by the probe.
* The complete blood count results were displayed after the processing.

Various haematological parameters, such as WBCs (× 10^3 μL^-1), lymphocyte (%), monocyte (%), RBCs (× 10^6 μL^-1), haemoglobin concentration (g/dL), mean corpuscular haemoglobin (pg), mean corpuscular volume (fL), mean corpuscular haemoglobin concentration (g/dL), and packed cell volume (%), were therefore analysed.

 Statistical analysis

The statistical program ‘XLSTAT’ and ‘GraphPad Prism’ were employed for the analyses. Results were expressed as Mean ± SEM for 5 animals per group and evaluated by one-way analysis of variance (ANOVA). ANOVA was followed by Tukey's as post hoc multiple comparison test with significance taken at p ≤ 0.05. Pattern recognition method: hierarchical cluster analysis (an unsupervised learning method) was employed for data collection as described by Patras et al.15

Results

**Table 1:** Shows the effect of *K. africana* and glibenclamide on hematological test values in the diabetes study. The results showed that Hb, PCV, RBC, MCV, MCH, MCHC, and WBC were not statistically different from the control at any dose. The Platelet values increased at p < 0.05 for different doses of *K. africana* and glibenclamide but at p < 0.01 in the diabetes group. The diabetes untreated also showed a decrease (p < 0.05) in PCV and RBC levels with an increase in WBC.

**Table 2:** Shows the effect of *S. bicolor* and glibenclamide on hematological test values in the diabetes study. The results showed that the Hb, PCV, RBC, MCV, MCH, MCHC, and WBC values were not statistically different from the control at any dose. The Platelet values increased at p < 0.05 for different doses of *S. bicolor* and glibenclamide but at p < 0.01 in the diabetes group. The diabetes untreated also showed a decrease (p < 0.05) in PCV and RBC levels with an increase in WBC when compared to control.

**Table 3:** Shows the effect of extracts mixtureand glibenclamide on haematological test values in the diabetes study. The results showed that Hb, PCV, RBC, MCV, MCH, MCHC, and WBC were not statistically different from the control at any dose. The Platelet values increased (p < 0.05) for the two doses of extract mixtures and glibenclamide but at p < 0.01 in the diabetes group. The untreated diabetes group also showed a decrease (p < 0.05) in PCV and RBC levels, with an increase in WBC, when compared to the control.

**Table 1: Effect of *K. africana* and glibenclamide on haematological test values in the diabetes study.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Cont.**  | **Diab. Untreat.** | **Glib.** | **GPIV** | **GPV** | **GPVI** | **F** | **P value** |
| **Hb (g/dl)** | 13.08±1.45 | 10.42±1.83 | 13.03±1.90 | 11.78±2.06 | 15.40±2.68 | 12.45±0.16 | 3.987 | 0.009 |
| **PCV (%)** | 42.42±2.28 | 30.50±4.72\* | 34.04±2.95\* | 39.96±3.40 | 48.80±4.70 | 40.91±4.74 | 13.53 | <0.0001 |
| **PLTx 103** | 549.50±7.85 | 843.40±10.06\*\* | 665.80±7.92\* | 789.60±7.85\* | 770.50±9.41\* | 737.70±10.08\* | 687.7 | <0.0001 |
| **RBC x 106 cells/ml** | 7.42±1.61 | 5.06±0.56\* | 6.51±0.11 | 7.36±1.12 | 8.27±0.13 | 7.47±0.11 | 8.78 | <0.0001 |
| **MCV(fL)** | 57.21±5.64 | 57.42±3.91 | 61.81±6.04 | 56.62±4.56 | 59.10±3.80 | 60.23±6.78 | 0.7415 | 0.6 |
| **MCH(pg)** | 18.54±3.35 | 18.91±2.39 | 19.92±2.37 | 18.34±2.73 | 18.62±1.23 | 19.11±1.68 | 0.2804 | 0.9193 |
| **MCHC(g/dl)** | 32.51±4.36 | 33.03±5.19 | 32.20±3.85 | 32.53±2.44 | 31.51±2.35 | 31.80±3.62 | 0.1059 | 0.9899 |
| **WBC x 103 cells/ml** | 9.04±1.68 | 12.76±1.36\* | 8.01±1.01 | 8.03±1.39 | 7.91±0.47 | 7.32±1.16 | 17.33 | <0.0001 |
| **Glu (m mol/L)** | 4.95±0.56 | 21.60±0.83\*\* | 3.53±0.40\* | 4.57±0.07 | 4.45±0.07 | 3.48±0.04\* | 1303 | <0.0001 |

Mean ± SD (n = 5) \*p<0.05, \*\*p<0.01 (Significant difference at 95 and 99% confidence limit respectively) vs control group

Cont.: Control (0.5 ml of acacia solution (2%w/v))

Diab. Untreat.: Diabetes untreated

Glib.: Glibenclamide 600 µg/kg bwt

GPIV: 125 mg/kg bwt of *K. africana*

GPV: 250 mg/kg bwt of *K. africana*

GPVI: 500 mg/kg bwt of *K. africana*

**Table 2: Effect of *S. bicolor* and glibenclamide on hematological test values in the diabetes study**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Cont.** | **Diab. Untreat.** | **Glib.** | **GPVII** | **GPVIII** | **GPIX** | **F** | **P value** |
| **Hb (g/dl)** | 13.08±1.45 | 10.42±1.83\* | 13.03±1.90 | 14.31±0.11 | 14.31±0.11 | 15.30±2.37 | 5.86 | 0.0011 |
| **PCV (%)** | 42.42±2.28 | 30.50±4.72\* | 34.04±2.95\* | 45.81±5.84 | 46.50±4.83 | 48.50±5.81 | 12.71 | <0.0001 |
| **PLT x 103** | 549.50±7.85 | 843.40±10.06\*\* | 665.80±7.92\* | 733.00±10.31\* | 726.00±8.07\* | 630.10±9.30\* | 2507 | <0.0001 |
| **RBC x 106cells/ml** | 7.42±1.61 | 5.06±0.56\* | 6.51±0.11 | 7.63±1.43 | 8.41±0.76 | 8.43±1.36 | 6.69 | 0.0005 |
| **MCV(fL)** | 57.21±5.64 | 57.42±3.91 | 61.81±6.04 | 60.11±3.73 | 60.12±5.26 | 59.91±3.58 | 0.6877 | 0.6375 |
| **MCH (pg)** | 18.54±3.35 | 18.91±2.39 | 19.92±2.37 | 18.72±2.80 | 18.82±3.80 | 19.11±6.04 | 0.08774 | 0.9935 |
| **MCHC (g/dl)** | 32.51±4.36 | 33.03±5.19 | 32.20±3.85 | 31.20±5.93 | 31.32±3.58 | 31.94±4.61 | 0.1137 | 0.9882 |
| **Glu (m mol/L)** | 4.95±0.56 | 21.60±0.83\*\* | 3.53±0.40\* | 3.90±0.42\* | 3.86±0.16\* | 3.71±0.11\* | 1132 | <0.0001 |

Mean ± SD (n = 5), \*p<0.05, \*\*p<0.01 (Significant difference at 95 and 99% confidence limit respectively) vs control group.

Cont.: Control (0.5 ml of acacia solution (2%w/v)).

Diab. Untreat. : Diabetes untreated

Glib. : Glibenclamide 600 µg/kg bwt

GPVII: 125 mg/kg bwt of *S. bicolor*

GPVIII: 250 mg/kg bwt of *S. bicolor*

GPIX: 500 mg/kg bwt of *S. bicolor*

**Table 3: Effect of extracts mixtureand glibenclamide on hematological test values in the diabetes study**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Cont.** | **Diab. Untreat.** | **Glib.** | **GPX** |  **GPXI** | **F** | **P value** |
| **Hb (g/dl)** | 13.08±1.45 | 10.42±1.83\* | 13.03±1.90 | 14.63±3.40 | 14.84±5.88 | 1.415 | 0.2651 |
| **PCV (%)** | 42.42±2.28 | 30.50±4.72\* | 34.04±2.95\* | 46.30±4.99 | 47.01±7.18 | 12.24 | <0.0001 |
| **PLT x 103** | 549.50±7.85 | 843.40±10.06\*\* | 665.80±7.92\* | 655.00±10.38\* | 686.00±10.38\* | 632 | <0.0001 |
| **RBC x 106 cells/ml** | 7.42±1.61 | 5.06±0.56\* | 6.51±0.11 | 7.74±0.16 | 7.74±1.34 | 6.88 | 0.0012 |
| **MCV(fL)** | 57.21±5.64 | 57.42±3.91 | 61.81±6.04 | 59.91±10.33 | 60.93±5.59 | 0.4828 | 0.7481 |
| **MCH (pg)** | 18.54±3.35 | 18.91±2.39 | 19.92±2.37 | 18.82±3.62 | 19.12±1.43 | 0.181 | 0.9456 |
| **MCHC (g/dl)** | 32.51±4.36 | 33.03±5.19 | 32.20±3.85 | 31.53±5.59 | 31.43±5.08 | 0.09602 | 0.9826 |
| **WBC x 103cells/ml** | 9.04±1.68 | 12.76±1.36\* | 8.01±1.01 | 10.18±1.14 | 9.43±3.40 | 9.524 | 0.0002 |
| **Glu (m mol/L)** | 4.95±0.56 | 21.60±0.83\*\* | 3.53±0.40\* | 3.45±0.18\* | 3.28±0.13\* | 1281 | < 0.0001 |

Mean ± SD (n = 5), \*p<0.05, \*\*p<0.01 (Significant difference at 95 and 99% confidence limit respectively) vs control group.

Cont.: Control (0.5 ml of acacia solution (2%w/v)).

Diab. Untreat. : Diabetes untreated

Glib. : Glibenclamide 600 µg/kg bwt

GPX: 250 mg/kg bwt of mixture

GPXI: 500 mg/kg bwt of mixture

Discussion

In this study, we observed that the extracts of K. Africana and S. bicolor demonstrated a potent plasma glucose lowering effect hence could be useful in the control of diabetes mellitus. Previous studies by Fagbohun *et al.* 202016 have reported bioactive compounds present in these plants that may be responsible for the hypoglycemic effect observed. Thus, application of tradomedical plants is proving to be the solution to the research of Scientists in recent times.

However, Rashid et al 201917 in their report stated that the high prevalence of morbidity and mortality seen in diabetic patients is as a result of the complications associated with the disease as evident by the derangements in the hematological, biochemical, and other parameters. Hence the present study assessed the effects of the hexane fraction of Kigelia Africana fruit, S. bicolor stalk, their mixture and glibenclamide on the hematological parameters. The findings were that the diabetes untreated group showed a decrease (p < 0.05) in PCV and RBC levels with an increase in WBC when compared to the treated groups and control. The decrease in the key red blood indices observed in the diabetes untreated group is an indication of diabetes-induced anaemia, which may be attributed to the oxidation of proteins and hyperglycaemia in diabetes mellitus, resulting in an increase in the production of lipid peroxides that lead to hemolysis of RBC.18 The alterations of these parameters are well known to cause anemic condition in man with persistent hyperglycemia progressing the pathology to cardiac damage.19

We also observed that the Hb, PCV, RBC, MCV, MCH, MCHC and WBC of the treated rats were not statistically different from the control at all doses when compared to the untreated diabetes group. This could be a reflection of the plant extract administration to all the treated groups which would have appreciably improved their levels. This gives an indication that the plant extract may contain some phytochemicals that can stimulate the formation or secretion of erythropoietin a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells.20 The stimulation of this hormone enhances the rapid synthesis of RBC, which is supported by the improved level of MCH and MCHC parameters that are used mathematically to define the concentration of haemoglobin and to suggest the restoration of oxygen-carrying capacity of the blood.21 it may be attributed to the ability of plant extract to lower lipid peroxidation level that causes hemolysis of erythrocytes.22 Previous studies23,24 on this plants showed that they contained flavonoids, proanthocyanidins, tannins, phenols and flavonols; compounds that have been reported to possess strong antioxidant capacity.

Furthermore, the increase in the levels of WBC when compared to the control seen in this study is very significant because of the crucial role of WBC in defending the body against infection and tissue damage. This supports previous reports that some commonly prescribed medicinal plants contains agents that stimulate the production of leucocytes.25,26 This suggests that the extract may have immune boosting effect on the animals. Such effects may also be due to increase in vascular permeability. Administration of the extract appears to exhibit stimulatory effect on the effectors cells of the immune system. Immune boosters are usually recommended to strengthen and harmonize degenerative body systems and assist the immune system to fight invading agents such as bacteria and viruses.27

Similarly, the Platelet values however, increased at p < 0.05 for different doses of K. africana and glibenclamide but at p < 0.01 in diabetes untreated group especially after plant extract administration. This effect indicated the ability of the plant extract to stimulate the biosynthesis of clotting factors due to the presence of active compounds that might help to precipitate blood coagulation or clotting, especially during severe bleeding or haemorrhage. This report, however, contradicts Ladokun *et al, 2015* 5, which recorded a reduction in platelet counts obtained from their study. This difference may be due to the herbal plant used for their study as mistletoe extract was used, which they explained may disrupt the oxygen-carrying capacity of the blood as well as thrombopoietin.

Conclusion

In conclusion, the extract of K. africana fruit and S. bicolor stalk possess antihyperglycemic properties and protective effects on some hematological parameters. Consequently, this highlights some beneficial properties of K. africana and S. bicolor extract administration, which can further help in recommending its consumption. Further experimental investigation is needed to exploit its relevant therapeutic effect to corroborate its medicinal usage.

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