ETHANOL PULP EXTRACT OF DATE PALM (*Phoenix dactylifera*) MODULATES HEMATINIC INDICES IN DIABETIC RATS

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Abstract
In Nigeria and indeed most parts of Africa, Phoenix dactylifera *fruit is often consumed between meals as snacks and for treatment of diabetes. This study evaluated the haematinic indices as well as the hypoglycaemic effects. Ethanolic extract of* Phoenix dactylifera *fruit pulp on normal and alloxan-induced diabetic rats were investigated following 14 days of oral administration. A total of thirty five (35) adult male albino rats weighing between 100 to 200 g were used for the study. Diabetes was induced intraperitoneally in animals from groups 2 to 6 while groups 1 and 7 were not induced. Acute toxicity studies showed no signs of toxicity up to a dose of 5000mg/kg b.w. Administration of 100, 300 and 500mg/kg body weight of the extract led to significant decrease ($p<0.05$) in serum glucose concentration of diabetic rats compared to normal control and diabetic untreated rats. However, there was a significant increase ($p<0.05$) in the white blood cells of groups treated with these doses when compared to that of diabetic control. Additionally, the extract resulted in an increase in packed cell volume, haemoglobin and red blood cells of the treatment groups, albeit significantly ($P<0.05$) when compared to the controls. Our results allude to the hypoglycemic properties of the extract as well as demonstrate for the first time, its modulatory effects on the hematopoiesis of diabetic rats.*

Keywords: hematinic indices, diabetes, *Phoenix dactylifera*, erythropoeisis, acute toxicity

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1. INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2006 and Wild *et al*., 2004). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2007). Thus, to prevent complications, good control of diabetes is essential and the management of diabetes should therefore aim to improve glycaemic control beyond that required to control its symptoms. Traditional societies have always exploited edible wild plants to provide adequate nutrition, food security and income generation (Antia *et al*., 2006 and Dhellot *et al*., 2006). These wild plants such as Date fruits serve as an indispensable constituent of human diet supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy (Fleuret, 1979 and Edmonds and Chweya, 1997). *Date*s are sugar-packed, many varieties are low glycemic index diets and refute the dogma that *Date*s are similar to candies, and regular consumption would develop chronic diseases (Liv, 2003).

In folk-lore, *Date* fruits have been ascribed to have many medicinal properties when consumed either alone or in combination with other herbs. The phytochemical compositions, nutritional significance, and potential health benefits of *Date* fruit consumption cannot be over-emphasized. For instance, it is said to have great potential as a medicinal food for a number of diseases including diabetes mellitus, ulcer, cancer, and gastro-intestinal infections. In an *in vitro* study, its aqueous pulp extract was found to significantly inhibit lipid peroxidation and protein oxidation, and also exhibited potent superoxide and hydroxyl radical scavenging activity in a dose-dependent manner (Conn, 1995). *Date* fruit pulp significantly reduced the elevated levels of plasma lipids indicating its possible beneficial effects in atherosclerosis development in

humans (Murugan and Reddy, 2009). However, the pharmacological and hematological properties of these inexpensive and highly nutritious wild plants (***Phoenix dactylifera***) are yet to be exhaustively investigated. This study is focused on understanding the effects of the ethanol fruit extract of ***Phoenix dactylifera*** on some hematinic indices on diabetic rats.

2. MATERIALS AND METHOD

Animals

Thirty five (35) male albino rats weighing 100 – 200g were purchased and kept under room temperature in the Department of Biochemistry, University of Nigeria, Nsukka and were acclimatized for a period of two weeks with adequate feed and clean water.

Experiment design

After acclimatization, experimental animals were evenly distributed into seven (7) groups of five (5) rats each. The route of administration of extract was via oral route with the aid of an oral intubation tube once daily for a period of fourteen (14) days. The baseline blood glucose levels were determined before the induction of diabetes. The rats were fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150 mg/kg body weight and the route of administration was intraperitoneal. After 3 days, rats with blood glucose levels greater than 200 mg/dl were considered diabetic and used for the investigation (Frode and Medeiros, 2008). Diabetes was induced in animals from groups 2 through 6 and no induction was done in animals in groups 1 and 7. After induction, animals in group 1 received only normal saline, group 2 received 2.5 mg/kg b.w of glibenclamide (standard control), group 3 received only normal saline (diabetic control), while groups 4, 5 and 6 received 100, 300 and 500 mg/kg b.w of the plant extract, respectively. Group 7 animals were not induced with diabetes and received 500 mg/kg b.w of extract**.** The animals were handled according to the guidelines of the ethical committee on the use and care of experimental animals of the University of Nigeria, Nsukka. The animals were anesthetized and blood samples collected once through ocular puncture for hematological analyses.

Plant materials

Date fruits (*Phoenixdactylifera*) were bought from Minna in Niger State, Nigeria and identified by the Department of Botany, University of Nigeria Nsukka. A voucher specimen was deposited in the Department's Herbarium.

Preparation of plant extracts

Sun dried *Phoenix dactylifera* pulp was pulverized into fine powder with a grinding machine. A quantity, 250g of pulverized pulp was extracted with 500ml ethanol for 72 h. The mixture was later transferred to a bottle plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 72 hours. The supernatant was collected and concentrated to obtain slurry of the extract by slowly evaporating the solvent in wide mouthed bowls at room temperature for 3 days to make the final volume one fourth of the original volume of solvent used. The semi-pastry extract was stored in a refrigerator until when needed.

Acute Toxicity Test (LD_{50})

Seventy male albino mice weighing 18 – 23g were randomly assigned to seven (7) cages of 10 animals per cage. Each group respectively received the following doses: 100, 200, 400, 800, 1600, 3200 and 5000mg/kg body weight of the extract intraperitoneally (i.p). The maximum volume injected was 0.2ml and the control group received 0.2ml of normal saline i.p. The number of deaths in each group within 24 hours was recorded. The LD50 was estimated from the graph of percentage mortality (converted to probit) against $\log_{10}$ of the dose of the extract (Eno *et al*., 2001).

Qualitative and quantitative phytochemical analysis of methanol seed extract of *Phoenix dactylifera*

The phytochemical analysis of ethanol pulp extract of *Phoenix dactylifera* was carried out on samples according to the method of Harborne (1973) to identify its active constituents.

Determination of Serum Glucose Level

One Touch Ultra Glucometer (LifeScan Inc., USA) and test strips were used for the assay. This method is based on the reaction of gluconic acid and hydrogen peroxide. Hydrogen peroxidase oxidizes the dyes in a reaction producing a blue product. The intensity of the colour, which is proportional to the glucose concentration of the sample, was read from the One Touch Ultra Glucometer. The treatment and blood glucose monitoring was done through the tail vein of the rat before induction, after induction and after treatment.

Full blood count assays

Full blood counts including PCV, Hb, RBC, WBC, differential WBC (lymphocytes and mixed), and red cell indices (MCHC, MCH and MCV), were estimated using the Sysmsex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan. The whole sample method was used where blood was mixed manually, and then fed into the transducers. The transducer chamber has a minute hole called the aperture. On both sides of the aperture, there are electrodes between which flows direct current. Blood cells pass through the aperture, causing direct current resistance to change between the electrodes. As direct current changes, the blood cell size is detected as electric pulses. Blood cell count is then calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data including differential whole blood count, red cell indices and derived values.

Statistical analysis

The data obtained were analyzed using statistical package for social sciences (SPSS) version 16.0 and the result expressed as mean ± standard error of mean. Significant differences of the result were established by one-way and two-way ANOVA and the acceptance level of the significant was $p<0.05$ for all the result.

3. RESULTS AND DISCUSSION

Acute Toxicity Studies

Table 1**:** From the result of the acute toxicity test of the extract of *Phoenix dactylifera* pulp in mice, no symptom or deaths were recorded after 24 h at up to 5000mg/kg body weight. This is an indication of the safety of the extract (plant) for both human and animal nutrition. However, further studies are needed to ascertain the effects of prolonged feeding of high doses of the extract on the organs and tissues of experimental animals.

Tab. 1 Acute Toxicity Studies

Dose in mg/Kg b w	No of deaths recorded with *Phoenix dactylifera*	Symptoms recorded with extract of extract of *Phoenix dactylifera*
Phase 1		
100	0/3	0/3
200	0/3	0/3
400	0/3	0/3
800	0/3	0/3
Phase 2		
1600	0/3	0/3
3200	0/3	0/3
5000	0/3	0/3

Qualitative Phytochemical Analysis of *Phoenix dactylifera*

Table 2: The table below shows that ethanol extract of *Date* fruit pulp contains high concentration of carbohydrates, moderate concentration of flavonoids, tannins, saponins, terpenoids, steroids and glycosides, little amount of protein and has no alkaloids and reducing sugars.

Tab 2. Qualitative Phytochemical Analysis of *Phoenix dactylifera*

Phytochemical Test	Result
Alkaloids	—
Flavonoids	++
Glycosides	++
Proteins	+
Carbohydrates	+++
Reducing sugars	—
Saponins	++
Tannins	++
Terpenoids	++
Steroids	++

Key: - Absent, + Low amounts, ++ moderate amounts, +++ High amounts

Quantitative Phytochemical Analysis Result of *Phoenix dactylifera*

Table 3: The table shows that ethanol extract of *Phoenix dactylifera* quantitatively contain more flavonoids and terpenoids than steroid

Tab. 3. Quantitative Phytochemical Analysis Result of *Phoenix dactylifera*

Phytochemicals	Quantity
Flavonoids (mg/g)	0.90 ± 0.12
Terpenoids (mg/g)	0.78 ± 1.09
Steroids (mg/g)	0.52 ± 0.62

Glucose concentration after treatment was found to be 122.00mg/dl for normal control, 264.33mg/dl for standard control, 348.00mg/dl for diabetic control, 169.69 mg/dl, 194.00 mg/dl, and 76.67mg/dl for the test group administration of 100mg/kg, 300mg/kg and 500mg/kg respectively; and 87.33mg/dl for the uninduced group.

These results suggest a significant decrease ($p<0.05$) in glucose concentration of the rats treated with 100mg/kg, 300mg/kg and 500mg/kg of the extract compared to untreated diabetic rats, normal rats, standard and no induction rats.

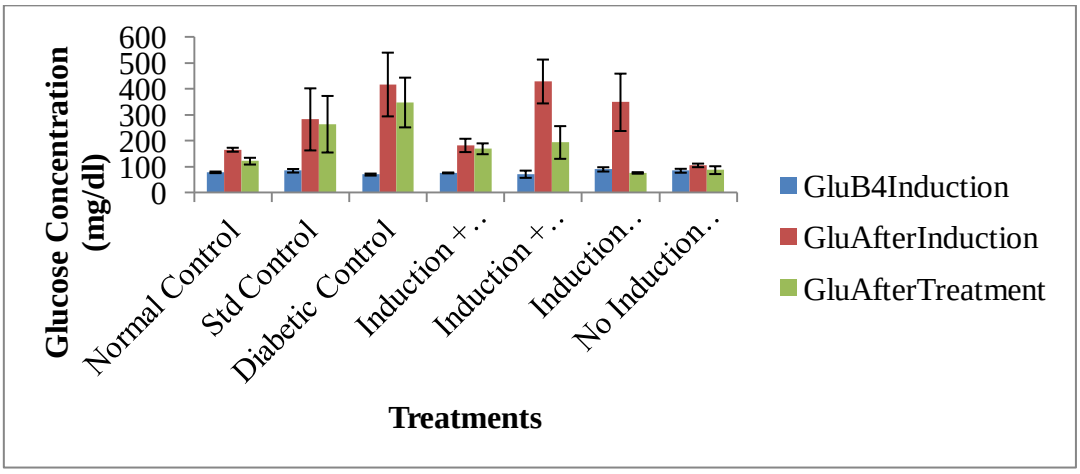

Fig 1: Effect of ethanol pulp extract of *Phoenix dactylifera* on the glucose concentration in normal and diabetic rats

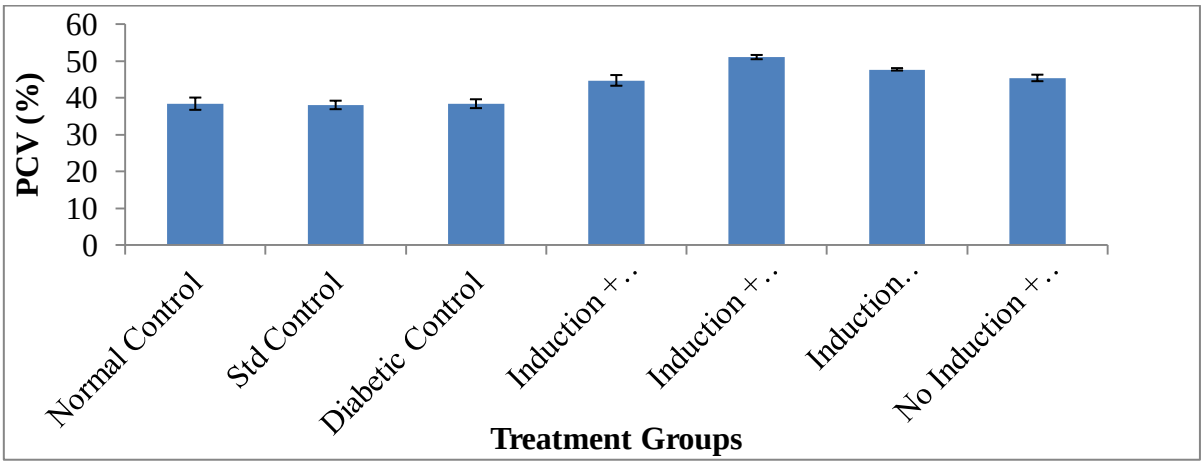

Fig 2: Effects of the extract of *Phoenix dactylifera* pulp on Packed Cell Volume (PCV) Concentration in normal and diabetic rats

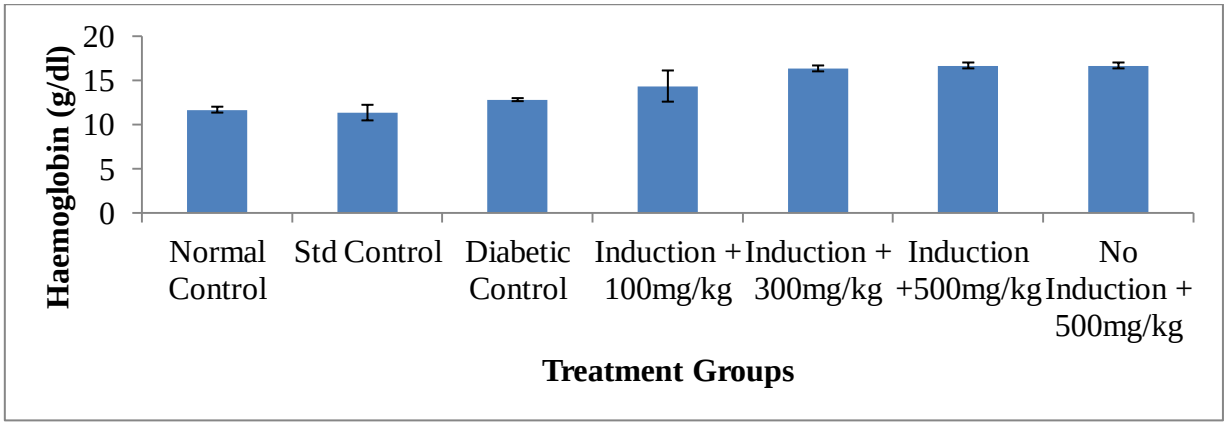

Fig 3: Effects of the extract of *Phoenix dactylifera* pulp on Haemoglobin Concentration in normal and diabetic rats

The result revealed that mean value for the pack cell volume (PCV) concentration after treatment was found to be 38.33% for normal control, 38.00% for standard control, 38.33% for diabetic control, 44.66%, 51.00%, 47.66% for the test group administration of 100mg/kg, 300mg/kg and 500mg/kg respectively and 45.33% for no induction with treatment rats.

This however suggest that there is a significant increase ($p<0.05$) in pack cell volume of the rats treated with 100mg/kg, 300mg/kg and 500mg/kg of induced and non-induced rats compared to untreated diabetic rats, normal rats, standard control rats.

The result revealed that mean value for the Haemoglobin concentration after treatment was found to be 11.66g/dl for normal control, 11.33g/dl for standard control, 12.80g/dl for diabetic control, 14.33g/dl, and 16.33g/dl, 16.66g/dl for the test group administration of 100mg/kg, 300mg/kg and 500mg/kg respectively and 16.66g/dl for no induction rats.

This however suggest that a significant increase ($p<0.05$) in the haemoglobin concentration of the rats treated with 100mg/kg, 300mg/kg and 500mg/kg of extract and the non-induced rats compared to untreated diabetic rats, normal rats and standard control.

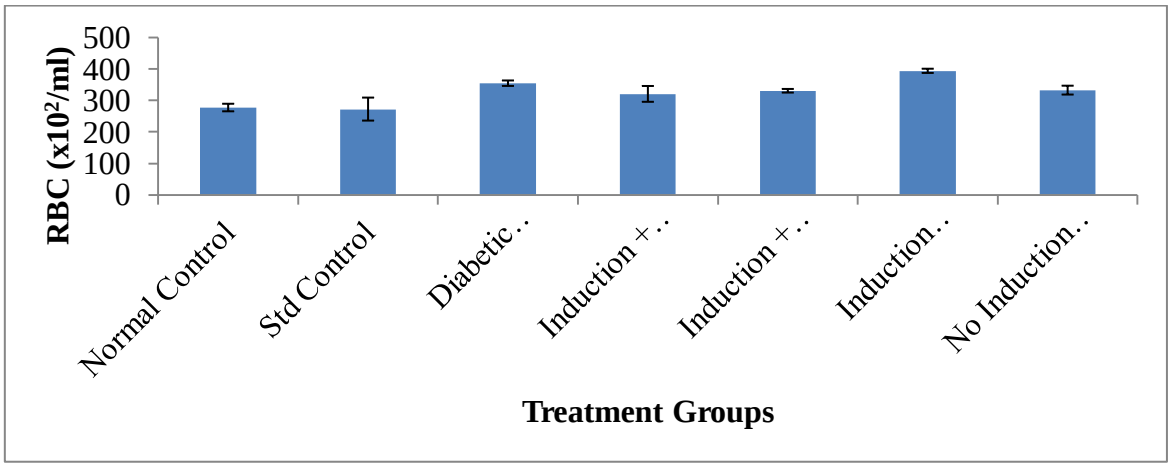

Fig 4: Effects of the extract of *Phoenix dactylifera* pulp on Red Blood Cell (RBCs) Concentration in normal and diabetic rats

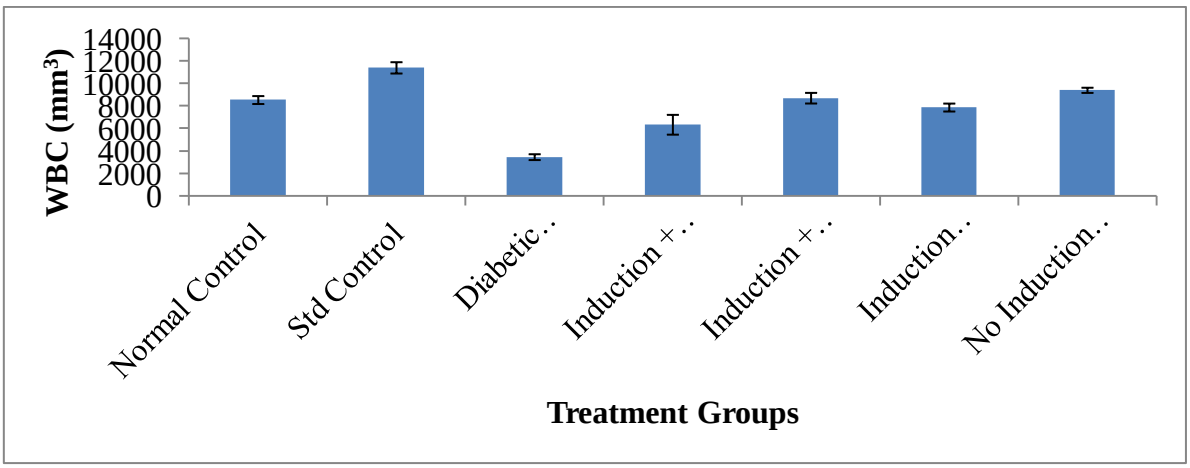

Fig 5: Effect of the extract of *Phoenix dactylifera* on White Blood Cells (WBCs) Concentration in normal and diabetic rats

The result revealed that mean value for the Red blood cell (RBCs) concentration after treatment was found to be $2.76x10^2$/ml for normal control, $2.71x10^2$/ml for standard control, $3.54x10^2$/ml for diabetic control, $3.20x10^2$/ml, $3.30x10^2$/ml, $3.93x10^2$/ml for the test groups administered with of 100mg/kg, 300mg/kg and 500mg/kg respectively and $3.32x10^2$/ml for no induction rats.

This however suggest that there was a significant increase ($p<0.05$) in the red blood cell concentration of the rats treated with 300mg/kg and 500mg/kg of extract compared to diabetic control group.

The result revealed that mean value for the White blood cell (WBCs) concentration after treatment was found to be $8.53mm^3$for normal control, $11.14mm^3$ for standard control, $3.45mm^3$ for diabetic control, $6.33mm^3$, $8.70mm^3$, $7.86mm^3$ for the test group administration of 100mg/kg, 300mg/kg and 500mg/kg respectively and $9.40mm^3$ for no induction rats.

This however suggest that there was a significant increase ($p<0.05$) observed in the white blood cell concentration of the rats treated with 100mg/kg, 300mg/kg and 500mg/kg of extract and 500mg/kg of extract+ no induction when compared to that of diabetic control rats.

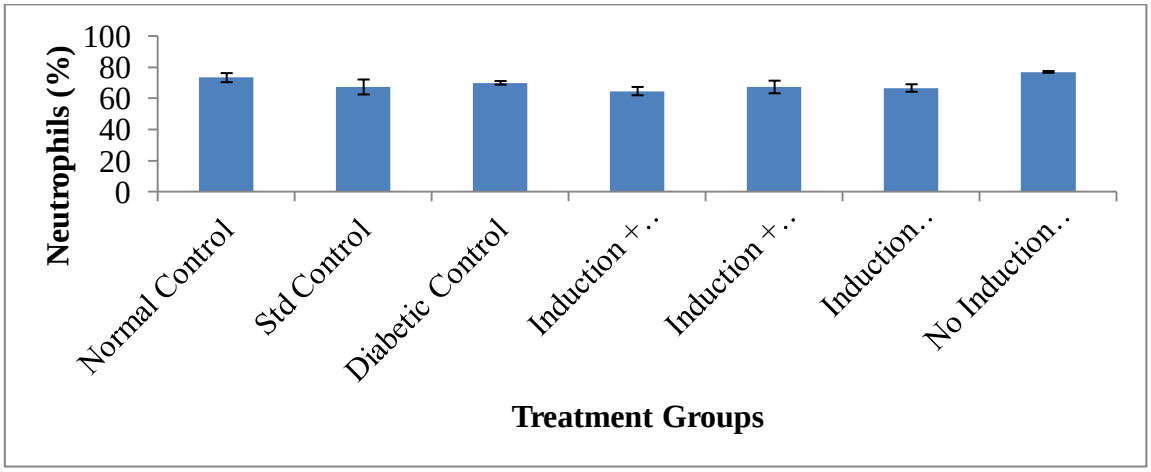

Fig 6: Effect of the extract of *Phoenix dactylifera* on Differential Count (Neutrophils) Concentration in normal and diabetic rats

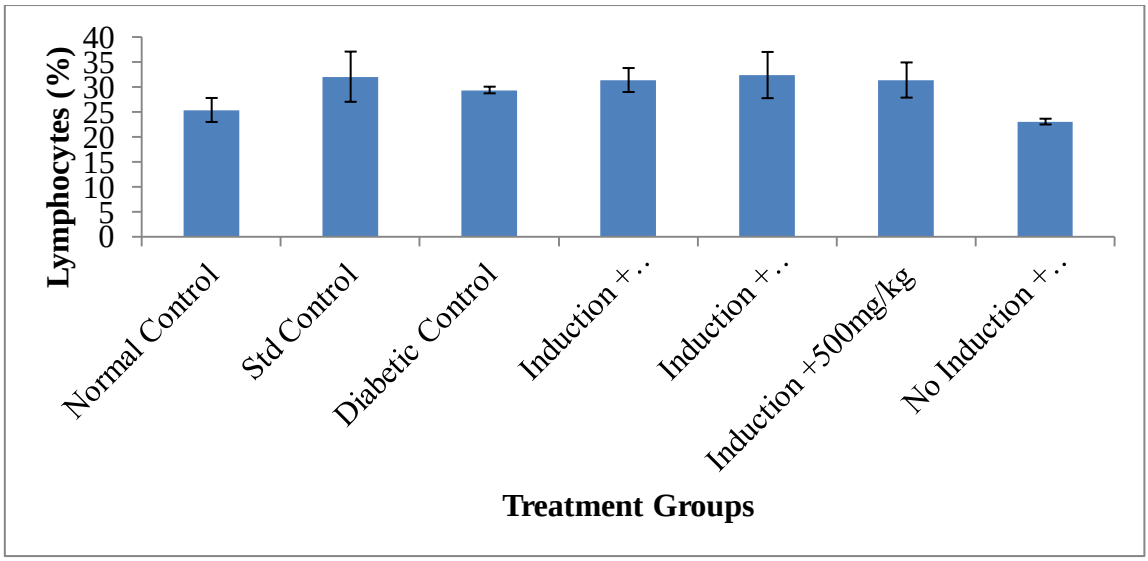

Fig 7: Effect of the extract of *Phoenix dactylifera* on Differential Count (Lymphocytes) Concentration in normal and diabetic rats

The result revealed that mean value for the Neutrophils concentration after treatment was found to be 73.33% for normal control, 67.33% for standard control, 70.00% for diabetic control, 64.66%, 67.33%, 66.66% for the test group administration of 100mg/kg, 300mg/kg and 500mg/kg respectively and 77.00% for no induction rats.

This however suggest that there was no significant decrease ($p>0.05$) observed in the neutrophils concentration of the rats treated with 100mg/kg, 300mg/kg and 500mg/kg of extract compared to untreated diabetic rats, normal rats, standard and no induction rats.

The result revealed that mean value for the Lymphocytes concentration after treatment was found to be 25.33% for normal control, 32.00% for standard control, 29.33% for diabetic control, 31.33%, 32.33%, 31.33% for the test group administration of 100mg/kg, 300mg/kg and 500mg/kg respectively and 23.00% for no induction rats.

This however suggest that there was no significant increase ($p>0.05$) observed in the lymphocytes concentration of the rats treated

with 100mg/kg, 300mg/kg and 500mg/kg of extract and standard control compared to untreated diabetic rats, normal rats and non-induced rats.

DISCUSSION

Ethanol pulp extract of *Phoenix dactylifera* was investigated to ascertain its anti hypergycaemic effect as well as its effect on haematinic parameters in alloxan-induced diabetic rats.

Acute toxicity study (LD_{50}) showed that consumption of up to 5000 mg/kg b.w. was safe. Alloxan has been shown to produce hyperglycemia (a diabetic condition) which may be due to the partial or complete destruction of the beta cells leading to insulin deficiency which leads to various metabolic alterations in the animals via increased blood glucose levels (Murugan and Reddy, 2009).

However, insulin concentration was not determined in this study to ascertain the degree of insufficiency. Glucose concentration was significantly reduced ($p<0.05$) in all the treated groups (100mg/kg, 300mg/kg and 500mg/kg of extract) compared to that of the untreated diabetic group. This could be attributed to the rich presence of dietary fibre in *Phoenix dactylifera* (Alkaabi *et al*., 2001). Consumption of soluble dietary fiber (DF) reduces postprandial glucose responses after carbohydrate-rich meals, as well as lowering total and LDL cholesterol levels (Jenkins *et al*., 2000). These effects likely explained the viscous and/or gel-forming properties of soluble DF, which allows slow gastric emptying and macronutrient absorption from the gut (Weickert and Andreas, 2008). Analysis of the extract revealed the presence flavonoid in the extract, which might have influenced insulin secretion thus reducing the blood glucose concentration. Presence of flavonoids has been shown to increase glucose-stimulated insulin secretion in pancreatic cell line MIN6 (mouse-derived) (Ohno *et al*., 1993). Alkaabi et al. (2001) has demonstrated that consumption of Date palm did not result in postprandial of blood glucose incursion, suggesting that it does not cause an undesirable effect on postprandial blood glucose that this could be as result of low glycaemic index and not result in postprandial glucose spike.

A wide range of haematological values change significantly in patients with diabetes (Pilsczek *et al*., 2008 and Farhana *et al*., 2009).In this present study, we observed that the packed cell volume (PCV), hemoglobin (Hb) and red blood cell (RBC) which serve as indices of anaemia were lowered among diabetic subjects compared to non-diabetic controls. There was significant increase ($p<0.05$) in PCV, Hb and RBC in groups treated with 100mg/kg, 300mg/kg and 500mg/kg of extract. Hemoglobin is an oxygen-binding compound that transports oxygen to the cells, and the hemoglobin test measures how much of this compound is present. The hematocrit test determines how much of the total blood volume contains red blood cells. Red blood cells are basically vessels for hemoglobin, so there is a very direct relationship between hemoglobin and hematocrit (Thomas *et al*., 2005).Previous reports indicated that the occurrence of anaemia in diabetes mellitus is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia (Bilous, 2002 and Belguendouz *et al*., 1997). Alloxan has been shown to induce reactive oxygen species (ROS) which interferes with the integrity of red blood cell thus reducing the concentration of red blood cell. The presence of polyphenolic (table 2&3) could have enhanced the protection of lipid bilayer of red blood cell membrane from ROS. Numerous studies have reported their capacity to reduce general markers of oxidative stress Frémont *et al*. (1998) including protection of red blood cells (RBC) against free radicals Halder *et al*. (1998). Dates are rich in both vitamins and iron which have been demonstrated to enhance red blood cell proliferation and oxygen-carrying function of haemoglobin, accounting for the improved effects of treatment on diabetes (Okonkwo *et al*., 2015 and Afolayan andYakubu, 2009).

Alloxan-induced diabetic untreated animals showed significantly reduced ($p<0.05$ blood levels of total white blood cell count,

neutrophils and lymphocytes when compared to the normal control group. The reduction of these parameters could be attributed to suppression of leucocytosis from the bone marrow. This may imply a reduced immune function in diabetic. Oral administration of 100,300 and 500mg/kg b.w doses of the fruit extract significantly improved the level of total white blood cell count level when compared with that of diabetic group (Fig.5). However, the level of lymphocyte and neutrophil count was not significantly elevated in the diabetic animals treated with all doses of the plant extract. This increase in lymphocyte level as shown in fig.7 though not significant, suggests that the plant might help boost the immune response (Apeh *et al*., 2015). Basophils, monocytes and Eosinophils were produced in insignificant proportion and were therefore not included in the results.

4. CONCLUSION

The study suggests that ethanol extract of *Date medjool* pulp has anti-hyperglycemic activity and could activate erythropoiesis in rats. It also suggests that the extract (plant) is safe for both human and animal nutrition. However, further studies are needed to ascertain the effects of prolonged feeding of higher doses of the extract on the organs and tissues of experimental animals.

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