

Anti-diabetic and Haematological Effects of n-butanol fraction of *Alchornea cordifolia* Leaf Extract in Streptozotocin-induced Diabetic Wistar Rats

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ABSTRACT

The study investigated the anti-diabetic and haematological effects of n-butanol fraction of *Alchornea cordifolia* in streptozotocin-induced diabetic wistar rats. In this work, thirty six adult strain of albino wistar rats were used, which included six normal, diabetic untreated and twenty four diabetic treated rats. Diabetes mellitus was induced by single intraperitoneally injection of 60 mg/kg body weight dose of streptozotocin dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats. Diabetic rats were randomly divided as follows: Group I served as normal control, Group II served as diabetic untreated rats, while Group III to Group VI received 200, 400 and 800mg/kg b w of the extract and glibenclamide 10mg/kg b w respectively by orally by gavage for a period of 28 days. The animals were weighed weekly to determine the change in body weight. Fasting blood glucose was measured after every seven days. Blood was collected from the animals from each group on the 29th day by cardiac puncture after rats were fasted for 12 hours for the determination of haematological parameters. The results of the present study showed that the blood glucose level was significantly ($p < 0.05$) decreased with 200mg/kg b w with a higher decrease ($p < 0.01$) observed with 400 and 800mg/kg b w. However, glibenclamide 10mg/kg b w gave a better activity ($p < 0.001$) when compared to the diabetic control group. After 14th, 21st and 28th day treatment of diabetic rats with all doses of the plant extract and glibenclamide, there was a significantly ($p < 0.01$) increased body weight of the diabetic rats. There was a statistically significant increase ($p < 0.05$) in packed cell volume, red cell count, haemoglobin concentration and total protein in the extract and glibenclamide treated diabetic. Total white blood cell count and lymphocyte revealed a significantly ($p < 0.05$) increased the levels after treatment with the n-butanol fraction of *Alchornea cordifolia* leaf extract after 28th days. In conclusion, the plant extract showed a significant hypoglycemic as well as erythropoietic effects in the diabetic animals, justifying its use traditionally in the management of diabetes mellitus.

Key words: *Alchornea cordifolia*, blood glucose, erythrocytes leucocytes diabetes mellitus, streptozotocin,

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects of insulin secretion and/or increased cellular resistance to insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the blood stream into the cells so they can break it down and use it for fuel (ADA, 2007; Samreen, 2009). It is the most common endocrine disorder and by the year 2010, it is estimated that more than 200 million people worldwide will have diabetes mellitus and 300 million will subsequently have the disease by 2025 (Salim, 2005). Chronic hyperglycemia and other metabolic disturbances of diabetes mellitus lead to long-term tissue and organ damage as well as dysfunction involving the eyes, kidneys, and nervous and vascular systems (ADA, 1998). Meanwhile, medicinal plants that are effective in controlling plasma glucose level with minimal side effects are commonly used in under developed countries as alternative therapy. In Africa, particularly in Nigeria, hundreds of plants are used traditionally for the management and/or control of diabetes mellitus but unfortunately, only a few of such medicinal plants have been scientifically validated (Tanko *et al.*, 2007). Also the fact that none of the anti-diabetic drugs could give a long term glycaemic control without causing any adverse side effects such as : gastrointestinal (GI) disturbances with metformin, weight gain, gastrointestinal disturbances and liver injury with thiazolidinediones, gastrointestinal disturbances, weight gain and hypersensitivity reactions with meglitinides and flatulence, diarrhoea and abdominal bloating with alpha-glucosidase inhibitors. (Salim, 2005; Singh *et al.*, 2007). Hence, the search for drugs with low cost, more potential and with fewer side effects has become very imperative. Medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical as well as dietary supplement to existing therapies (Bailey and Day, 1989). One of the plants commonly used in Africa traditional medicine for the management of diabetes mellitus is *Alchornea cordifolia* and also the fact that there is paucity of information on the use of *Alchornea cordifolia* in the management of diabetes mellitus, hence the need to investigate the anti-diabetic potential of this plant. *Alchornea cordifolia*

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(Schum. & Thonn.) Mull. Arg. (*Euphorbiaceae*) is also known as Ewe ipa, Ubobo and Bambami in Nigeria. It is geographically distributed in secondary forest usually near water, moist or marshy places and it grows to a considerable height but is always of a shrubby or scrambling habit. *Alchornea cordifolia* is an erect and bushy perennial shrub or small tree up to 4 meters high reproducing from seeds (Lamikanra *et al.*, 1990). *Alchornea cordifolia* is commonly used as a medicinal plant throughout its area of distribution. The leaves are mostly used, but also the stem bark, stem pith, leafy stems, root bark, roots and fruits enter in local medicine. In Nigeria, the plant is widely used in traditional medicine, for example, the dried and powdered leaves mixed with palm wine are used for the treatment of gonorrhoea. The leaves are used internally for the management of gastrointestinal disorders, respiratory and urinary tract infections, rheumatic pain and cough (Gbile and Adeshina, 1986 ; Ogunbamila and Samuelson, 1990). A decoction of the leafy twigs is applied on the body to treat feverish chills and sores, and application for sore feet as a lotion (Lovet and Marigold, 2012). The present study aimed at investigating the anti-diabetic and haematological effects of n-butanol fraction of *Alchornea cordifolia* in streptozotocin-induced diabetic Wistar rats.

Materials and Methods

Chemicals:

Streptozocin (STZ) was purchased from Sigma chemicals (St Louis U.S.A). Glibenclamide was obtained from pharmaceutical store in Zaria, Kaduna state, while Accu-chek Advantage a digital glucometer was used for the determination of blood glucose levels.

Plant Material:

Fresh leaves of *Alchornea cordifolia* were collected from old Karu village, Karu Local government of Nasarawa State, Nigeria in the month January 2011. The plant was then taken to the herbarium unit of Biological Science Department Ahmadu Bello University, Zaria, Kaduna state, where the plant was identified by Mal. M. Musa and a voucher specimen (Number 401) deposited.

Extract Preparation:

The fresh leaves *Alchornea cordifolia* collected were air dried under the shade and ground into fine powder. The powder (400g) was macerated in 2.0 L of distilled water at room temperature for 24 h. It was then filtered using a filter paper (Whatmann size 1). The filtrate was then partitioned with n-Butanol to get an n-Butanol fraction which was then evaporated to dryness in an oven at 30°C. A brownish residue weighing 8g was obtained and kept in a sealed container refrigerated until it was reconstituted.

Acute Toxicity Study:

The lethal doses (LD₅₀) of fresh leaves of *Alchornea cordifolia* was carried out by method of Lorke (1983) using 12 rats. The method is biphasic, in the first phase, rats were divided into 3 groups of 3 rats each and were treated with the n-butanol fraction of *Alchornea cordifolia* leaf extract at doses of 10, 100 and 1000 mg/kg body weight orally. They were observed for 24 h for signs of toxicity including death. In the second phase, 3 rats were divided into 3 groups of 1 rat each and were also treated with the n-butanol fraction extract at doses of 1600, 2900 and 5000 mg/kg body weight orally. The median lethal dose (LD₅₀) was calculated using the second phase as the product of highest non-lethal and lowest lethal.

Phytochemical Screening:

The methods of analysis employed were those described by Brain and Turner (1975). The n-butanol fraction of *Alchornea cordifolia* leaf extract obtained were subjected to preliminary phytochemical screening to identify the presence or absence of chemical constituents.

Animals Care:

Strains of albino wistar rats of both sexes that weighed between 150 – 200 g were obtained from the Department of Human Physiology, Animal House, Ahmadu Bello University, Zaria. The animals were kept and maintained under laboratory condition of temperature, humidity and light. The animals allowed to acclimatized for two weeks, but were allowed free access to water, before commencement of the experiments.

Induction of Experimental Diabetes Mellitus:

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats. Three days after Streptozotocin injection, blood was taken from tail artery of the rats (Burcelin *et al.*, 1995). Rats having blood glucose levels greater than 200mg/dl were considered diabetic and included in the study. The diabetic rats were then divided randomly in the different groups.

Experimental design:

In the experiment, a total of 36 rats were used, the animals were divided into 6 groups of 6 rats each as follows:

- Group 1: Normal control received distilled water
- Groups 2: Diabetic control received distilled water
- Group 3: Diabetic received 200mg/kg b w n-butanol fraction of *Alchornea cordifolia* leaf extract
- Group 4: Diabetic received 400mg/kg b w n-butanol fraction of *Alchornea cordifolia* leaf extract
- Group 5: Diabetic received 800mg/kg b w n-butanol fraction of *Alchornea cordifolia* leaf extract
- Group 6: Diabetic received Glibenclamide 10mg/kg b w

Determination of Blood Glucose Levels:

All blood samples were collected from the tail artery of the rats after every seven day. Blood glucose levels was determined by the glucose-oxidase principle (Beach and Turner, 1958) using a digital glucometer (Accu-chek Advantage) and expressed in the unit of mg/dl.

Determination of Haematological Parameters:

This was carried out by method described by Dacie and Lewis (1991). Four weeks after the last treatment with different doses of the extract and standard drug, each animal from different groups were sacrificed and blood samples obtained through cardiac puncture for the determination of haematological parameters: Red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), white blood cell count (WBC) and its differential counts.

Determination of Change on Body Weight:

This was determined after every seven days of treatment with the extract of *Alchornea cordifolia* and glibenclamide change on body weights recorded respectively.

Statistical analysis:

Data for blood glucose, haematological parameters and change on body weight from each group were expressed as mean \pm SEM. The data were statistically analyzed using ANOVA with multiply comparisons versus control group. The values of $p < 0.05$ were considered as significant (Duncan *et al.*, 1997).

Results and Discussion*Results:**Acute Toxicity Study:*

In this study, administration various doses of n-butanol fraction of *Alchornea cordifolia* leaf extract to the animals showed no signs of toxicity and no deaths were recorded. Therefore, the LD₅₀ of n-butanol fraction of leaf extract *Alchornea cordifolia* was safe at least up to 5000mg/kg b w.

Phytochemical Screening:

Preliminary phytochemical screening of the extract revealed the presence of cyanogenetic glycosides, saponins, carbohydrate, flavonoids, tannins, cardiac glycosides and steroids and Triterpenoids.

Effect of n-butanol fraction of Alchornea cordifolia on blood glucose level of streptozotocin-induced diabetic Wistar rats:

The blood glucose levels did not differ significantly ($p > 0.05$) within and between groups after day 1 and when compared to the diabetic control group. But there was a statistically significant ($p < 0.05$) decrease in the blood glucose level in the group treated with 200mg/kg b w with a higher decrease ($p < 0.01$) observed with 400 and 800mg/kg b w. However, glibenclamide (10mg/kg b w) gave a better effect with ($p < 0.001$) after day 7 when compared to the control group. However, after day 14, 21 and 28, the blood glucose level was highly significantly ($p < 0.01$) reduced in all extract and glibenclamide treated groups when compared to the diabetic control group as shown in table 1.

Effect of n-butanol fraction of Alchornea cordifolia on body weight of streptozotocin-induced diabetic Wistar rats:

The body weight of diabetic extract and glibenclamide treated groups did not statistically differ ($p > 0.05$) between and within groups after day 1 and when compared to the diabetic control group. However, there was a non significant ($p > 0.05$) increased body weight in all extract and glibenclamide treated groups after day 7 when compared to the diabetic control group. After day 14, 21 and 28 treatment of diabetic rats with all doses of the extract and glibenclamide, there was a significantly ($p < 0.01$) increased body weight of the animals when compared to the diabetic control group (Table 2).

Effect of n-butanol fraction of Alchornea cordifolia on erythrocyte indices and total protein of streptozotocin-induced diabetic Wistar rats:

There was a statistically significant increase ($p < 0.05$) in PCV in the extract and glibenclamide treated diabetic groups with 200 and 800mg/kg b w having a higher significant increase ($p < 0.01$) when compared to the diabetic control group. There was a statistically significant increase ($p < 0.001$) in Hb concentration all diabetic extract and glibenclamide treated groups when compared to the diabetic control group. The significantly ($p < 0.05$) depleted total protein in diabetic untreated group was elevated after treatment with all doses of the extract and glibenclamide, with 400mg/kg b w having better effect when compared to the diabetic control group (Table 3). The significant decrease ($p < 0.05$) in RBC count in diabetic untreated group was elevated almost near normal when treated with different doses of the plant extract and glibenclamide when compared to the diabetic control group.

Effect of n-butanol fraction of Alchornea cordifolia on leucocyte indices of streptozotocin-induced diabetic Wistar rats:

Table 4 shows the blood levels of total white blood cell count, neutrophils, lymphocytes, monocytes and eosinophils. There was a significant depletion ($p < 0.05$) in the level of total WBC in the diabetic untreated group when compared to the normal control group. However, treatment with 400mg/kg b w showed no statistically significant increase ($p > 0.05$) in total white blood cell count, unlike 200 and 800mg/kg b w and glibenclamide (10mg/kg b w) which significantly ($p < 0.01$) elevated the total WBC count when compared to the diabetic control group. There was no significant ($p > 0.05$) increase in the neutrophils count in extract and glibenclamide treated group when compared to the control diabetic control group. The plant extract at all doses and glibenclamide significantly ($p < 0.05$) increased the level of lymphocytes, with no significant ($p > 0.05$) increase in the levels of eosinophils, monocytes in the diabetic extract and glibenclamide treated when compared to the diabetic control group.

Table 1: Effect of n-butanol fraction of *Alchornea cordifolia* on blood glucose level of streptozotocin-induced diabetic Wistar rats

Treatment Given	Blood glucose levels (mg/dl)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control + distilled water	91.4±3.07	88.6±3.61	90.0±4.11	87.8±3.85	88.6±2.03
Diabetic control + Distilled	381.0±55.75	383.4±29.85	384.0±26.93	363.8±18.03	364.4±17.79
n-But 200 mg/kg b w	382.4±42.01 ^{ns}	282.2±29.03 ^a	203.0±15.55 ^c	154.2±18.47 ^a	114.0±12.87 ^c
n-But 400 mg/kg b w	372.6±20.29 ^{ns}	246.6±19.07 ^b	185.8±20.21 ^c	140.0±13.11 ^b	102.2±5.03 ^c
n-But 800 mg/kg b w	382.6±32.57 ^{ns}	260.8±24.95 ^b	175.8±16.73 ^c	120.8±6.29 ^c	104.8±3.43 ^c
Glibenclamide 10mg/kg b w	358.6±13.8 ^{ns}	187.0±19.57 ^c	129.0±3.87 ^c	103.0±1.95 ^c	95.8±1.75 ^c

Values are presented as mean ± SEM

Values are statistically significant compared to control group at ^a $p < 0.05$ ^b $p < 0.01$ and ^c $p < 0.001$ while ns =not significant

Table 2: Effect of n-butanol fraction of *Alchornea cordifolia* on body weight of streptozotocin-induced diabetic Wistar rats

Treatment Given	Body weight (g)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control + distilled water	163.4±3.67	174.0±2.69	183.0±2.07	191.8±1.65	192.4±1.77
Diabetic control + Distilled	167.8±5.23	153.8±2.27	146.6±2.59	137.6±2.47	135.0±2.03
n-But 200 mg/kg b w	164.8±4.85 ^{ns}	159.2±4.93 ^{ns}	161.4±4.75 ^a	164.8±4.27 ^c	171.4±2.89 ^c
n-But 400 mg/kg b w	163.0±4.85 ^{ns}	155.8±4.21 ^{ns}	161.2±3.43 ^a	166.6±3.41 ^c	172.8±2.97 ^c
n-But 800 mg/kg b w	167.4±5.64 ^{ns}	156.4±4.77 ^{ns}	165.0±3.67 ^b	169.2±4.13 ^c	174.6±3.71 ^c
Glibenclamide 10mg/kg b w	166.4±5.87 ^{ns}	154.2±4.13 ^{ns}	165.2±2.81 ^b	177.0±2.81 ^c	178.2±3.43 ^c

Values are presented as mean ± SEM

Values are statistically significant compared to control group at ^ap < 0.05 ^bp < 0.01 and ^cp < 0.001 while ns = not significant**Table 3:** Effect of n-butanol fraction of *Alchornea cordifolia* on erythrocyte indices and total protein of streptozotocin-induced diabetic Wistar rats

Treatment	PCV (%)	HB (g/dl)	Total protein (g/dl)	RBC (10 ¹² /L)
Normal control + distilled Water	49.60±2.22	16.32±0.88	7.52±0.40	5.40±0.30
Diabetic control + distilled water	33.00±1.30	10.48±0.68	3.78±0.34	3.46±0.22
n-But 200 mg/kg b w	52.20±3.42 ^c	17.34±1.14 ^c	7.88±0.38 ^c	5.76±0.39 ^c
n-But 400 mg/kg b w	48.40±1.93 ^b	16.72±0.58 ^c	8.24±0.42 ^c	5.54±0.19 ^b
n-But 800 mg/kg b w	51.80±3.77 ^c	16.82±0.43 ^c	7.76±0.58 ^c	5.56±0.13 ^b
Glibenclamide 10 mg/kg b w	50.40±1.32 ^b	16.54±0.85 ^c	7.92±0.42 ^c	5.48±0.11 ^b

Values are presented as mean ± SEM

Values are statistically significant compared to control group at ^ap < 0.05 ^bp < 0.01 and ^cp < 0.001 while ns = not significant**Table 4:** Effect of daily oral doses of n-butanol fraction of *Alchornea cordifolia* on leucocyte indices of streptozotocin-induced diabetic Wistar rats

Group (n=5)	Treatment Given	WBC ×10 ⁹ /L	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)
1	Normal + distilled Water	11.40±0.92	22.20±2.76	84.40±1.32	3.00±0.31	1.40±0.67
2	Diabetic control + distilled water	4.00±1.14	12.60±1.53	59.60±3.95	1.40±0.24	0.40±0.24
3	n-But 200 mg/kg b w	8.60±0.50 ^a	18.80±0.73 ^{ns}	79.40±2.94 ^b	0.40±0.24 ^{ns}	0.20±0.20 ^{ns}
4	n-But 400 mg/kg b w	6.60±0.74 ^{ns}	20.00±1.51 ^{ns}	80.20±1.49 ^b	1.20±0.58 ^{ns}	0.40±0.24 ^{ns}
5	n-But 800 mg/kg b w	11.20±1.28 ^c	15.80±2.65 ^{ns}	81.00±2.86 ^b	2.00±0.70 ^{ns}	1.40±0.74 ^{ns}
6	Glibenclamide 10mg/kg b w	9.20±0.58 ^a	14.00±2.88 ^{ns}	82.80±3.48 ^b	2.60±0.87 ^{ns}	0.60±0.40 ^{ns}

Values are presented as mean ± SEM

Values are statistically significant compared to control group at ^ap < 0.05 ^bp < 0.01 and ^cp < 0.001 while ns = not significant

Discussion:

Streptozotocin is a compound commonly used for the induction of type 1 diabetes mellitus in experimental rats (Tomlinson *et al.*, 1992; Subbiah *et al.*, 2006). Streptozotocin selectively destroys the pancreatic cells and induce hyperglycemia (Kurup and Bhonde, 2000). However, nitric oxide has been implicated/demonstrated to participate in the beta cell destruction during streptozotocin-induced diabetes mellitus (Duran Reges *et al.*, 2004). The reduction the serum insulin levels in the streptozotocin treated animals as result of the destruction of pancreatic beta cells which was not determined in this present study might be responsible for the increased blood glucose levels as was observed in the diabetic untreated rats. This is consistent with the studies of Yoo and Ray, (1985) and Chanda *et al.*, (2006) who have reported a significantly reduced serum insulin in streptozotocin-induced hyperglycemia. The findings of this present study showed that administration all doses of *Alchornea cordifolia* leaf extract to the diabetic animals significantly ($p < 0.05$) decreased the blood glucose level in the group treated with 200mg/kg b w with a higher decrease ($p < 0.01$) observed with 400 and 800mg/kg b w. However, glibenclamide (10mg/kg b w) gave a better effect with than the plant extract in decreasing the blood glucose level when compared to the diabetic control group. It is well established that glibenclamide produces hypoglycemia by increasing the secretion of insulin from the existing pancreatic β -cells (Proks *et al.*, 2002; Subbiah *et al.*, 2006). Therefore, the possible mechanisms of action of plant extract in reducing the blood glucose in diabetic animals may be by stimulating existing β -cells or by increasing β -cell regeneration or by modulating intracellular glucose utilization Chhanda *et al.*, 2006; Edwin *et al.*, 2008). Preliminary phytochemical screening of n-butanol fraction of leaf extract of *Alchornea cordifolia* revealed the presence of flavonoids and saponins among other secondary metabolites. Several works have demonstrated that flavonoids may reduce hyperglycaemia and exert protective effect against non-enzymatic glycation of proteins in animals (Ghosh and Konishi, 2007). Flavonoids, especially quercetin, has been reported to possess anti-diabetic activity. Tapas *et al.*, (2008) and Vessal *et al.*, (2003) reported that quercetin brings about the regeneration of pancreatic islets and probably increases insulin release in strptozotocin-induced diabetic rats. This is also in agreement with the work of Hif and Howell (1985) that have reported that quercetin stimulate

insulin release and enhanced Ca^{2+} uptake from isolated islets cell which suggest a place for flavonoids in non-insulin- dependent diabetes. There was a significantly decreased body weight in the diabetic animals from 14th to 28th day after induction of diabetes when compared to the normal control animals. This findings are consistent with the works of Chhanda *et al.*, (2006) and Oyedemi *et al.*, (2011a) that have reported decreased body weight in streptozotocin-induced hyperglycemia. It has been shown that gastrointestinal autonomic neuropathy usually associated with disordered gastrointestinal motor and sensory function occurs frequently in diabetes (Schmidt, 2002; Rotimi *et al.*, 2011). This probably may lead to reduction in the food intake and coupled with degradation of structural proteins and muscle wasting that occurs in diabetes and consequent weight loss as was recorded in this current work (Rotimi *et al.*, 2011; Oyedemi *et al.*, 2011). However, oral administration of extract of *Alchornea cordifolia* to the animals significantly improved the body weight of diabetic rats after 14th, 21st and 28th day respectively when compared to the control group. The results showed that the plant extract of *Alchornea cordifolia* possessed the ability of managing glucose level as well as controlling muscle wasting and induce adipogenesis. This is in agreement with Oyedemi *et al.*, (2011) that reported that aqueous extract of stem bark of *Afzelia africana* (Smith) resulted in increased body weight of diabetic animals on streptozotocin-induced diabetic wistar rats and Chhanda *et al.*, (2006) who showed that separate and composite extract of *Eugenia jambolana* and root of *Musa paradisiaca* administration improved body weight of diabetic animals in streptozotocin-induced diabetic male albino rat. The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds toxins, chemicals and 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, normal functioning and histomorphology of the organs (Magalhaes *et al.*, 2008; Oyedemi *et al.*, 2011). In the present study, the effects of the plant extract in haematological parameters such as packed cell volume, haemoglobin concentration and red cell count, including total protein of streptozotocin-induced diabetic animals was assessed. The results showed a significantly decreased PCV, Hb, total protein and red blood cell count in diabetic untreated animals when compared with the normal control rats. Reactive oxygen species has also been implicated in the mechanism of red cells damage (Rao *et al.*, 2003; Mohammed *et al.*, 2009). Diabetes mellitus is usually accompanied by increased production of free radicals (Young *et al.*, 1995; Baynes, 1999). Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. The anaemic condition that occurs in diabetes mellitus has been reported to be due to the increased non-enzymatic glycosylation of red blood cell membrane proteins (Oyedemi *et al.*, 2011). Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides, a marker of oxidative stress in diabetes, which consequently have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals (Halliwell and Gutteridge, 1990; Tsai *et al.*, 1994; Kawamura *et al.*, 1994), that lead to haemolysis of red blood cell (Arun and Ramesh, 2002; Oyedemi *et al.*, 2011). The study showed that there was a significantly depleted total protein in the diabetic untreated animals when compared to the normal control. However, treatment of diabetic animals with all doses of the *Alchornea cordifolia* extract improved the levels of packed cell volume, haemoglobin concentration, red cell count and total protein. This suggest that the plant extract may contain some phytochemicals that can stimulate increased protein synthesis or mobilization which are largely produced in the liver and also secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2006; Oyedemi *et al.*, 2011). The stimulation of this hormone enhances rapid synthesis of red blood cell which is reflected by increased levels red blood cell and packed cell volume. Streptozotocin-induced diabetic untreated animals showed significantly reduced blood levels of total white blood cell count, neutrophils, lymphocytes, monocytes and eosinophils. when compared to the normal control group. Streptozotocin is a well known chemical has been reported to suppress the immune system by destroying white blood cells and certain organs in the body (Oyedemi *et al.*, 2011) as was observed in this present study. The reduction of these parameters could be attributed to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection, thus may have consequential effects on the immune system and phagocytic activity of the animals (Afolayan and Yakubu, 2009; Oyedemi *et al.*, 2010). Oral administration of 200 and 800mg/kg b w doses of the plant extract significantly improved the level of total white blood cell, but 400mg/kg b w did not have any significant change on the WBC level. However, the level of lymphocyte count was significantly elevated in the diabetic animals treated with all doses of the plant extract, but did not show any significant effects on neutrophils, monocytes and eosinophils when compared to the diabetic control. This observation of increase in the levels of these parameters by this plant extract suggest that the principal function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes of the animals, will be enhanced (Swenson and Reece, 1993; Adedapo *et al.*, 2005)

In conclusion:

The results of this study showed that n-butanol fraction of *Alchornea cordifolia* leaf extract possess anti-diabetic activity and improved the levels of erythrocyte indices and total protein in streptozotocin-induced treated diabetic animals at all doses. In addition, the plant extract significantly elevated the levels of total leucocyte and lymphocyte counts in the diabetic animals, but did not have any significant effects on neutrophils, monocytes and eosinophils.

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