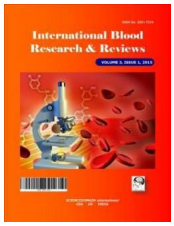

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Haematological Profile of Alloxan-induced Diabetic Rats Treated with Methanol Root Bark Extract of *Cussonia arborea*

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Authors' contributions

This work was carried out in collaboration between the two authors. Author IUA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author PEA managed the literature searches, analyses of the study and the experimental process. Both authors read and approved the final manuscript.

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ABSTRACT

The study was tailored to investigate changes in all haematological parameters of diabetic rats treated with methanol root bark extract of *Cussonia arborea*. A total of 72 male albino wistar rats assigned into 6 groups of 12 rats per group were used. Groups 1-5 were made diabetic by single intraperitoneal administration of alloxan monohydrate at the dose of 160 mg/kg while group 6 rats were not made diabetic. Diabetes was established by determination of fasting blood sugar above 126 mg/dl after which the rats in groups 1-4 were treated with 62.5, 125, 250 mg/kg of the extract and 2 mg/kg glibenclamide respectively while groups 5 and 6 rats received 10 ml/kg distilled water each to serve as negative and positive controls respectively. All treatments were through the oral route daily for 84 days. Red blood cell (RBC),white blood cell (WBC), differential white blood cell counts, packed cell volume (PCV) haemoglobin (Hb), total, conjugated and unconjugated bilirubin values were determined on days 28, 56 and 84 post treatment while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. The results indicate that administration of 125 mg/kg bw of the extract to

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the diabetic rats, significantly ($p<0.05$) increased RBC, PCV, Hb, MCV and conjugated bilirubin levels and significantly ($p<0.05$) decreased, total bilirubin and unconjugated bilirubin values when compared to the diabetic untreated group. There were no significant changes in the total white blood cell counts across the groups. It was therefore concluded that treatment of diabetic rats with methanol root bark extract of *C. arborea* at 125 mg/kg improved red blood cell values with no adverse effects on white blood cell parameters.

Keywords: Cussonia arborea; haematologic profile; diabetic rats.

1. INTRODUCTION

Diabetes is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and protein [1,2] characterized by increased fasting and postprandial blood sugar levels. It is a disease characterized by inability to regulate blood glucose as a result of relative or absolute deficiency in insulin. This results to hyperglycemia often accompanied by glycosuria, ploydipsia and polyuria [3]. Besides hyperglycaemia, several other factors like hyperlipidaemia and enhanced oxidative stress play a major role in diabetes pathogenesis. The disease is progressive and is associated with high risk of complication [4]. It is one of the most common endocrine diseases and has a prevalence rate varying from 1- 50% [5]. All forms of diabetes increase the risk of long term complications. The major long term complications are relative to blood vessel damage [6]. The damage to small blood vessels leads to microangiopathy which has been incriminated in other chronic complications such as diabetic retinopathy, diabetic neuropathy, diabetic nephropathy and diabetic cardiomyopathy, while damage to the large vessels (Macrovascular disease) is sequel to cardiovascular disease such as coronary artery disease, and diabetic myonecrosis (Muscle wasting) [7]. In diabetes, reduced haemoglobin has been reported [8] which may be accompanied by a fall in the red blood cell count and packed cell volume [9]. The evaluation of medicinal plants used traditionally in treating diabetes is of growing interest [10]. Alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed because of the inability of existing therapies to control all the pathological aspects of the disorder, as well as the enomous cost and poor availability for many rural populations in developing countries [11]. Literature search revealed that *Cussonia arborea* is folklorically used to treat symptoms of diabetes [12].

This study aims to investigate the haematological changes associated with treatment of diabetic rats with graded doses of methanol extract of *Cussonia arborea.*

2. MATERIALS AND METHODS

2.1 Experimental Animals

Male albino Wistar rats weighing between 100 g and 105 g were obtained from the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka laboratory animal house. The rats were acclimatized for two weeks. The environmental temperature where the animals were housed varied between 28-32℃. The animals were kept in stainless wire mesh cages and provided with good clean water *ad libitum*. They were fed with standard commercial feed (Guinea^R growers).

2.2 Plant Material

The root bark of the plant material (*Cussonia arborea*) used in this study was collected from Orukpa Local Government Area of Benue state and identified by a plant taxonomist, at the International Centre for Ethnomedicine and Drug Development, Echara, Aku Road, Nsukka.

2.3 Preparation of the Plant Extract

Cold maceration method of extraction was employed. The root bark of *C. arborea* was air dried at a very low intensity of sunlight to avoid denaturation of the active ingredient. It was pulverized and stored in an air tight container pending its usage. About 2 kg of the powdered stem bark was soaked in 10 liters of 80% methanol with intermittent shaking every 2 h for 48 h. The mixture was filtered using Whatmann No 1 filter paper. The filtrate was concentrated using rotary evaporator and the extract stored at 4℃.

2.4 Treatment

Seventy two (72) male albino rats weighing between 100- 105 g were assigned into six groups of 12 rats per group. Diabetes as described by [13] was induced in 60 rats while the remaining 12 rats served as normal control. The rats were treated as follows:

Group 1: Diabetic rats treated with 62.5 mg/kg CAE
Group 2: Diabetic rats treated with 125 mg/kg CAE
Group 3: Diabetic rats treated with 250 mg/kg CAE
Group 4: Diabetic rats treated with 2 mg/kg Glibenclamide
Group 5: Diabetic rats treated with 10 ml/kg Distilled water
Group 6: Undiabetic rats treated with 10 ml/kg Distilled water

The rats were treated daily for eighty four (84) days. Haematological parameters (RBC, WBC, Hb, PCV, MCV, MCH, MCHC differential leucocytes), total bilirubin, conjugated bilirubin and unconjugated bilirubin were determined on days 28, 56 and 84.

2.5 Haematological Determinations

2.5.1 Erythrocyte count

The erythrocyte count was determined by the haemocytometer method [14]. Blood sample (0.02 ml) was added to 4 ml of red blood cell diluting fluid (sodium citrate, formaldehyde solution and distilled water) in a clean test tube, to make a 1:200 dilution. A drop of the diluted blood was charged onto the Neubaeur counting chamber and allowed to settle for 2-3 minutes. The high dry objective (x 40) of the light microscope was used in carrying out the erythrocyte count, in the five groups of 16 small squares. The number of erythrocytes enumerated for each sample was multiplied by 10,000 to obtain the erythrocyte count per microlitre of blood [14].

2.5.2 Determination of packed cell volume

The packed cell volume (PCV) was determined by the microhaematocrit method [14]. Micro-capillary tubes was almost filled with the anti-coagulated blood samples and one end sealed with plasticine. The filled tubes were centrifuged at 10,000 revolutions per minute for 5 minutes using a microhaematocrit centrifuge. The PCV was read as a percentage on the microhaematocrit reader [14].

2.5.3 Determination of haemoglobin concentration

The haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method [15]. The blood sample (0.02 ml) was added to 5 ml of Drabkins reagent in a clean test tube. This was mixed gently and kept at room temperature for 20 minutes to react. The absorbances of both sample and standard were read, against a working reagent blank at a wavelength of 540 nm using a spectrophotometer (Lab-tech, India). The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with the factor derived from the absorbance and concentration of the standard.

2.5.4 Total leukocyte count

The total leukocyte count was determined by the haemocytometer method [14]. Blood sample (0.02 ml) of blood was added to 0.38 ml of white blood cell diluting fluid (glacial acetic acid tinged with gential violet) in a clean test tube, to make a 1:20 dilution. A drop of the diluted blood was charged onto the Neubaeur chamber and allowed to settle for 2 minutes. The x10 objective lens of the light microscope was used in making a total count of white blood cells on the four corner squares. The number of cells counted for each blood sample was multiplied by 50 to obtain the total leukocyte count per microlitre of blood [14].

2.5.5 Differential leukocyte count

Smears for differential leukocyte counts was prepared on clean slides and stained by the Leishman technique [14]. The differential leukocyte count was enumerated by the battlement counting method [16]. The x100 (oil immersion) objective lens of the light microscope was used in making a differential leukocyte count and the different cells of the leukocytic series was identified and scored using the differential cell counter [14].

2.5.6 Determination of mean corpuscular values

The mean corpuscular values – mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular

haemoglobin concentration (MCHC) was calculated using the standard formulae [16].

2.5.7 Bilirubin

Both total and conjugated bilirubin were assayed according to the method of [17]. Direct bilirubin (conjugated) reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the prescence of caffeine, which releases albumin bound bilirubin by the reaction with diazotized sulphanilic acid.

2.5.8 Procedure

2.5.8.1 Sample blank

The serum sample (0.2 ml) was added to 0.2 ml of R1 (sulphanilic acid) and 1ml of R3 (caffeine), mixed and incubated for 10 mins at room temperature. Thereafter, R4 (Tartrate) was added and incubated for further 5-30 mins.

2.5.8.2 Sample

To 0.2 ml of serum sample was added 0.2 ml of R1, 1 drop (50 µL) of R2 (Nitrite) and 1000 µL of R3 , mixed and incubated for 10 mins at room temperature. Thereafter, 1000 uL of R4 was added to the mixture and incubated for further 5-30 mins.

The absorbance of sample A_{TB} was read against sample blank at wavelength 578 nm.

2.5.9 Calculation

Total Bilirubin (mg/dl) = 10.8x A_{TB}.

2.5.10 Direct bilirubin assay

2.5.10.1 Sample blank

To 0.2 ml of serum sample was added 0.2 ml of R1, 2 ml of 0.9 percent of NaCL and incubated for 10 mins at room temperature.

2.5.10.2 Sample

To 0.2 ml of serum sample was added 0.2 ml of R1, 2 ml of 0.9 percent of NaCL, 1 drop (50 µL) of R2 and incubated for 10 mins at room temperature.

The absorbance of sample A_{DB} was read against sample blank at wavelength 546 nm.

2.5.11 Calculation

Direct bilirubin (mg/dl) = 14.4x A_{DB}

Unconjugated bilirubin = Total bilirubin-conjugated bilirubin [17].

2.6 Statistical Analysis

Statistical package for social sciences version 20 was employed. One-way Analysis of Variance (ANOVA) was used to compare the means and their differences separated using Duncans Multiple Range test. p values ≤ 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Haematological evaluations revealed significant ($p<0.05$) reductions in the red blood cell (RBC), total haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and packed cell volume (PCV), values of the diabetic untreated rats compared to the normal control rats (Tables 1-6). These observations agree with existing literature that anaemia is a common pathophysiology associated with diabetes mellitus [18]. The occurrence of anaemia in diabetes mellitus has been reported due to the increased non enzymatic glycosylation of RBC membrane proteins which correlates with hyperglycemia [19]. Oxidation of these proteins and hyperglycemia in Diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBCs [20]. Significant ($p<0.05$) increases in the RBC, haemoglobin and PCV values of the extract-treated rats observed towards the end of the experiment (Tables 1-3) indicates that the plant may have anti-anaemic properties. No significant ($p>0.05$) changes were observed in mean corpuscular haemoglobin concentration, basophil, eosinophil, monocyte, lymphocyte and total white blood cell count (Tables 5-12). This is also in agreement with the findings of [18] who did not also report significant changes in the above parameters in a study similar to this present study. This finding may indicate that diabetes mellitus does not primarily interfere with the said parameters

The negative control rats have significantly ($p<0.05$) elevated levels of both total and unconjugated bilirubin when compared to the normal control rats (Tables 13 and 15). This observation may be consequent upon hepatocellular injuries induced by diabetes thus impairment of hepatocyte function such as bilirubin conjugation. The hepatocytes are

Table 1. Effects of chronic administration of *Cussonia arborea* root bark extract on red blood cell count (X 10^6 cells/µL) in alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	5.81±0.03^{b}	5.18±0.52^{b}	6.93±0.03^{c}
2	6.61±0.15^{c}	6.96±0.03c	7.53±0.03^{d}
3	4.92±0.03^{a}	5.31±0.28^{b}	6.41±0.26^{b}
4	6.72±0.03^{c}	6.96±0.03^{c}	7.46±0.03^{d}
5	4.83±0.08^{a}	3.86±0.06^{a}	3.60±0.10^{a}
6	7.45±0.03^{d}	7.76±0.08^{d}	7.63±0.03^{d}

The red blood cell counts of the normal control rats (group 6) were significantly (p< 0.05) higher than those of the other groups on days 28 and 56 post treatment. On day 84 however, the RBC counts of rats in groups 2 and 4 were statistically comparable (p> 0.05) to that of the normal control rats

Table 2. Effects of chronic administration of *Cussonia arborea* root bark extract on total haemoglobin concentration (g/dL) in alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	11.62±0.10^{b}	11.80±0.17^{c}	13.46±0.15^{bc}
2	13.03±0.26^{c}	13.93±0.03^{d}	14.10±0.15b^{c}
3	10.99±0.34^{ab}	10.99±0.40^{b}	13.26±0.31^{b}
4	12.59±0.21^{c}	13.49±0.08^{d}	14.13±0.18^{bc}
5	10.60±0.34^{a}	10.06±0.29^{a}	8.83±0.44^{a}
6	14.13±0.18^{d}	14.16±0.16^{d}	14.33±0.16^{c}

The total haemoglobin of diabetic untreated rats (group 5) were significantly (p< 0.05) lower than the total haemoglobin of other rats in other group on days 56 and 84. The rats in groups 2 and 4 have their total haemoglobin values statistically comparable (p> 0.05) to those of the normal control rats (group 6) on days 56 and 84 post treatment

Table 3. Effects of chronic administration of *Cussonia arborea* root bark extract on the packed cell volume (%) of alloxan-induced diabetic rat

Group	Day 28	Day 56	Day 84
1	34.33±0.33^{b}	34.33±0.33^{c}	36.08±0.56^{c}
2	37.33±0.33^{c}	38.66±0.33^{d}	40.67±0.31^{e}
3	33.60±0.57^{b}	32.00±0.00^{b}	34.33±0.32^{b}
4	36.33±0.88^{c}	28.53±0.33^{d}	39.32±0.53^{d}
5	28.33±0.89^{a}	27.67±0.33^{a}	24.31±0.33^{a}
6	41.00±0.57^{d}	41.33±6.89^{e}	41.00±0.58^{e}

The packed cell volume of the rats in group 6 was significantly (p< 0.05) higher than the negative control (group 6) and other groups throughout the period of the experiment except on day 84 when the values weres statistically comparable (p> 0.05) to the packed cell volume value of the rats in group 2

responsible for uptaking and conjugating bilirubin [21]. Treatment of the rats with extract (125 mg/kg) resulted in significant (p<0.05) reduction of the total and unconjugated bilirubin and elevation of conjugated bilirubin compared to the negative control group (Tables 13-15). This indicates that the extract may have hepatocurative properties. Enhancement of bilirubin conjugation is a cardinal indication of the improvement in hepatic function and in general the overall health of the liver.

Assessment of the blood parameters revealed a positive effect in the erythrocytic parameters while increases in the bilirubin conjugation observed in the treated groups indicate improvement in hepatic function compared to the diabetic untreated group. In conclusion therefore, the results of the experiments showed that the plant extract especially at the tested dose of 125 mg/kg, posseses anti-anaemic properties and improves hepatocellular function in diabetic hepatopathy.

Table 4. Effects of chronic administration of *Cussonia arborea* root bark extract on mean corpuscular volume (fL) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	58.67±0.88^{b}	67.33±6.96^{b}	51.93±6.67^{a}
2	56.00±1.00^{ab}	55.33±0.67^{a}	54.53±0.57^{ab}
3	67.00±1.73^{c}	61.33±3.28^{ab}	53.46±1.46^{ab}
4	54.00±1.15^{a}	55.00±0.58^{a}	52.56±0.29^{ab}
5	58.33±1.85^{ab}	71.33±0.88^{b}	67.40±0.65^{c}
6	54.66±0.8^{ab}	53.33±0.67^{a}	54.90±1.02^{b}

The mean corpuscular volume of groups 2 and 4 rats were statistically comparable (p> 0.05) to that of the negative and normal control groups on day 28. On days 56 and 84, the MCV values of groups 2, 3, 4 and 6 were statistically comparable to one another but were significantly lower than that of the negative control group

Table 5. Effects of chronic administration of *Cussonia arborea* root bark extract on the mean corpuscular haemoglobin concentration (g/dL) in alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	33.86±0.12^{a}	34.36±0.49^{a}	37.00±2.00^{a}
2	34.90±0.41^{a}	36.03±0.29^{a}	34.33±0.66^{a}
3	33.46±1.53^{a}	34.03±1.54^{a}	38.00±1.00^{a}
4	34.66±0.29^{a}	35.20±0.20^{a}	35.33±0.33^{a}
5	34.26±3.69^{a}	36.40±1.44^{a}	36.00±1.52^{a}
6	34.50±0.94^{a}	34.43±0.93^{a}	35.00±0.57^{a}

No significant difference were observed among all the groups throughout the duration of the experiment

Table 6. Effects of chronic administration of *Cussonia arborea* root bark extract on the mean corpuscular haemoglobin (g/dL) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	19.96±0.31^{a}	23.46±2.68^{ab}	19.36±072^{a}
2	19.73±0.49^{a}	20.03±0.13^{ab}	18.70±0.26^{a}
3	22.30±0.26^{b}	20.73±1.08^{ab}	20.73±0.97^{a}
4	18.73±0.26^{a}	19.36±0.14^{a}	18.90±0.21^{a}
5	21.93±0.89^{b}	25.83±0.60^{c}	24.00±1.00^{b}
6	18.63±0.37^{a}	18.20±0.25^{a}	18.76±0.33^{a}

The mean corpuscular haemoglobin of all the rats in group 1, 2, 3 and 4 were significantly (p< 0.05) lower than that of the group 5 (negative control rats) but statistically comparable (p> 0.05) to that of group 6 rats on days 56 and 84 post treatment

Table 7. Effects of chronic administration of *Cussonia arborea* root bark extract on total White blood cell count (X10^{3} cells/µL) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	7.13±0.03^{b}	7.16±0.03^{bc}	7.20±0.06^{bc}
2	7.10±0.05^{b}	7.13±0.03^{bc}	7.27±0.08^{c}
3	7.03±0.03^{ab}	7.13±0.03^{bc}	9.97±0.12^{ab}
4	6.93±0.63^{a}	7.10±0.05^{b}	6.96±0.06^{ab}
5	7.00±0.06^{ab}	6.90±0.05^{a}	6.90±0.05^{a}
6	7.10±0.05^{b}	7.26±0.06^{c}	7.27±0.09^{c}

The total white blood cell counts of rats in groups 1 and 2 were significantly (p< 0.05) higher than that of the negative control group but were statistically comparable (p> 0.05) to that of the normal control rats on days 56 and 84 post treatment

Table 8. Effects of chronic administration of *Cussonia arborea* root bark extract on lymphocyte count (%) of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	57.66±0.66^{b}	59.00±0.57^{ab}	58.33±0.67^{ab}
2	58.33±0.33^{b}	59.33±0.66^{ab}	59.33±0.57^{b}
3	59.00±0.57^{b}	60.00±0.56^{ab}	57.33±0.33^{a}
4	58.33±0.32^{b}	59.00±0.58^{ab}	57.66±0.33^{ab}
5	56.00±0.58^{a}	61.33±1.20^{b}	57.06±0.56^{a}
6	58.00±0.56^{b}	58.00±0.58^{a}	58.33±0.33^{ab}

On day 28, the lymphocytes of groups 1, 2, 3 and 4 rats were significantly ($p<0.05$) greater than that of group 5 rats. On days 56 and 84 however, they appeared statistically comparable

Table 9. Effects of chronic administration of *Cussonia arborea* root bark extract on Neutrophil (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	40.66±0.33^{a}	39.33±0.33^{ab}	40.00±0.56^{a}
2	41.33±0.67^{a}	39.66±0.33^{ab}	40.66±0.66^{a}
3	40.60±0.58^{a}	39.00±0.57^{ab}	41.33±0.23^{a}
4	40.66±0.58^{b}	39.66±0.33^{ab}	41.00±0.58^{a}
5	43.00±0.58^{b}	37.33±1.45^{a}	41.67±0.33^{a}
6	41.00±0.058^{a}	40.66±0.66^{b}	40.33±0.33^{a}

No significant changes were observed in the neutrophil counts of all the rats in the treatment groups throughout the duration of the experiment. The neutrophil count of the rats in group 5 were significantly ($p< 0.05$) higher than that of the normal control rats

Table 10. Effects of chronic administration of *Cussonia arborea* root bark extract on Basophil (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.03±0.03^{a}	0.00±0.00^{a}	0.00±0.00^{a}
2	0.07±0.03^{a}	0.03±0.03^{a}	0.03±0.03^{a}
3	0.03±0.03^{a}	0.03±0.03^{a}	0.03±0.03^{a}
4	0.03±0.03^{a}	0.03±0.03^{a}	0.03±0.03^{a}
5	0.07±0.06^{a}	0.06±0.03^{a}	0.03±0.03^{a}
6	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

No significant changes were observed throughout the duration of the experiment

Table 11. Effects of chronic administration of *Cussonia arborea* root bark extract on Eosinophil (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.73±0.03^{a}	0.70±0.05^{a}	0.73±0.03^{abc}
2	0.73±0.06^{a}	0.97±0.22^{a}	0.67±0.06^{abc}
3	0.70±0.06^{a}	0.70±0.06^{a}	0.60±0.05^{ab}
4	0.73±0.03^{a}	0.90±0.15^{a}	1.03±0.21^{c}
5	0.80±0.05^{a}	0.87±0.06^{a}	0.56±0.03^{a}
6	0.77±0.03^{a}	1.00±0.25^{a}	1.00±0.20^{bc}

No significant changes were observed except on the day 84, where the eosinophil counts of group 6 rats were significantly ($p< 0.05$) greater than that of the negative control rats (group 5)

Table 12. Effects of chronic administration of *Cussonia arborea* root bark extract on Monocyte (%) count of alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.23±0.03^{ab}	0.30±0.05^{a}	0.27±0.03^{a}
2	0.20±0.05^{ab}	0.33±0.13^{a}	0.30±0.06^{a}
3	0.27±0.03^{b}	0.27±0.03^{a}	0.37±0.03^{ab}
4	0.23±0.03^{ab}	0.40±0.20^{a}	0.60±0.10^{b}
5	0.13±0.03^{a}	0.40±0.25^{a}	0.36±0.3^{ab}
6	0.23±0.03^{ab}	0.33±0.08+a	0.37±0.12^{ab}

No significant changes were observed in the monocyte counts of the rats in all the groups across the treatment period except for rats in group 3 which had a significantly ($p< 0.05$) higher monocyte count on day 28 when compared with the negative control group.

Table 13. Effects of chronic administration of *Cussonia arborea* root bark extract on serum total bilirubin (mg/dl) in alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.67±0.09^{ab}	0.99±0.01^{c}	0.91±0.02^{b}
2	0.78±0.01^{bc}	0.87±0.01^{bc}	0.81±0.1^{b}
3	0.97±0.03^{c}	1.15±0.08^{d}	1.063± 0.07^{c}
4	0.63± 0.09^{ab}	0.80±0.01^{b}	0.82±0.02^{b}
5	1.60±0.11^{d}	1.71±0.09^{e}	1.92±0.04^{d}
6	0.50±0.04^{a}	0.54±0.02^{a}	0.56±0.00^{a}

The total bilirubin levels of the rats in group 5 were significantly ($p< 0.05$) elevated compared to the normal control rats and other groups throughout the experimental period. Groups 2 and 4 rats have comparable ($p> 0.05$) total bilirubin values as seen on day 56. The total bilirubin levels of groups 1, 2 and 4 rats compare very well among one another on day 84 but were significantly ($p< 0.05$) higher than that of the normal control

Table 14. Effects of chronic administration of *Cussonia arborea* root bark extract on serum conjugated bilirubin (mg/dl) in alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.17±0.03^{a}	0.36±0.00^{a}	0.35±0.000^{ab}
2	0.17±0.000^{a}	0.45±0.03^{b}	0.47±0.00^{c}
3	0.21±0.05^{a}	0.34±0.00^{a}	0.35±0.00^{ab}
4	0.15±0.02^{a}	0.39±0.03^{ab}	0.37±0.01^{b}
5	0.37±0.06^{b}	0.35±0.02^{a}	0.33±0.01^{a}
6	0.23±0.02^{a}	0.44±0.00^{b}	0.34±0.01^{ab}

The conjugated bilirubin levels of rats in group 5 were significantly ($p<0.05$) different from those of the other groups on day 28 but compared very well with 1, 3, 4 and 5 on days 56 and 84 post treatment. On day 56, the conjugated bilirubin levels of group 2 rats were significantly ($p<0.05$) higher than that of the groups 1,3 and 5 but statistically comparable to those of the normal control rats

Table 15. Effects of chronic administration of *Cussonia arborea* root bark extract on serum unconjugated bilirubin (mg/dl) in alloxan-induced diabetic rats

Group	Day 28	Day 56	Day 84
1	0.61±0.00^{bc}	0.41±0.01^{b}	0.34±0.02^{b}
2	0.50±0.05^{abc}	0.63±0.00^{c}	0.56±0.01^{c}
3	0.76±0.08^{c}	0.81±0.07^{d}	0.71±0.06^{d}
4	0.48±0.08^{ab}	0.41±0.05^{b}	0.44±0.01^{b}
5	1.23±0.14^{d}	1.38±0.07^{e}	1.59±0.03^{e}
6	0.27±0.02^{a}	0.09±0.02^{a}	0.22±0.01^{a}

Unconjugated bilirubin levels of the normal control rats compared very well with those of the rats in groups 2 and 4 on day 28 but differed significantly ($p< 0.05$) from those of the other groups. On days 56 and 84, the unconjugated bilirubin levels of the normal control rats were significantly ($p< 0.05$) lower than that of other group

4. CONCLUSION

Treatment of diabetic rats with methanol root bark extract of *C. arborea* ameliorated diabetic anaemia and improved hepatic function especially at the dose of 125 mg/kg.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "principles of laboratory animal care" (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experimemts have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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