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# Effects of Aqueous Extract of *Triplochiton scleroxylon* on Red Blood Cells and Associated Parameters in Alloxan - Induced Diabetic Rabbits

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**Abstract:** The effect of aqueous extract of *Triplochiton scleroxylon* on the body weight, plasma glucose concentration and some hematological parameters in alloxan-induced diabetic rabbits were investigated. Rabbits of the same strain (New Zealand) weighing between 1.20 and 1.68kg were used. At least 100ml of the aqueous extract of the plant was administered to a set of test rabbits for a period of 28 days. Blood was collected for analyses intravenously from the larger vein at the back of the ear of the rabbits. Plasma glucose was analyzed by spectrophotometeric method. Hematological swelab auto - counter $920^{E+}$ (UK) was used for red blood cell counts, packed cell volume, hemoglobin and mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume and red cell distribution width. The aqueous extract of *Triplochiton scleroxylon* does not have any significant effect ($P>0.05$) on the body weight and some hematological parameters investigated. However, plasma glucose concentration decreased significantly ($P < 0.05$) on the $18^{th}$, $24^{th}$ and $28^{th}$ days of administration of herbal extract to alloxan - induced diabetic rabbits. The crude extract of this herb has an hypoglycemic properties and its use as an anti-diabetic agent may not interfere with the body weight and some hematological properties of rabbits and by extrapolation humans.

**Key words:** Aqueous extract, rabbits, plasma glucose

## Introduction
The exploitation of plants by man for the treatment of diseases has been in practice for a very long time (Sofowora, 1984; Gill, 1992). Over the years, a variety of medicinal plants have been very popular for the cure of a number of both human and animal diseases (Sofowora, 1984; Gill, 1992; Lamla, 1981).
Diabetes mellitus affects millions of people all over the world. No cure has proven effective without severe complication, resulting to plant exploitation for this purpose. Well over 400 medicinal plants are available globally for the medication of diabetes mellitus, with a few having been subjected to scientific authentication to ascertain their effectiveness as anti-diabetic agents (Onoagbe and Esekheigbe, 1999). *Triplochiton scleroxylon* is one of the over 30 herbs used locally in Nigeria to treat diabetes mellitus. These plants are widely cultivated in Nigeria because of their medicinal values. They are, therefore, available, cheap and massively used by rural and impoverished urban dwellers since insulin therapy is not only expensive but the route of its administration very painful (Onoagbe *et al.*, 1999). Unfortunately most of these indigenous herbs have not been scientifically investigated for proper classification as anti-diabetic herbs devoid of side effects.
This study was then designed to investigate the possible effects of the aqueous extract of the bark of this herb, *Triplochiton scleroxylon* on some hematological parameters in rabbits. This is with the view of proper

classification of this herb as an anti-diabetic plant safe for use as such and devoid of some life threatening complications.

## Materials and Methods
**Animals:** Rabbits of the same strain (New Zealand) weighing between 1.20 and 1.68kg were used. They were maintained under standard animal house conditions and allowed free access to food (growers mash) and water for 2 weeks of acclimatization.

**Chemical:** Glucose oxidase kit was obtained from Randox laboratories United Kingdom. Sodium fluoride from BDH chemicals Ltd (Poole, Dorset, U.K.) and alloxan was supplied by Sigma. All other chemicals were of analar grade. Lahor Public Health and Research Center, Benin City, Edo State Nigeria was used in the analyses of the hematological parameters.

**Medicinal plants:** The barks of Obeche (*Triplochiton scleroxylon*) were purchased from medicinal plant dealers at Oyingbo market, Lagos, Lagos State, Nigeria and identified by experts in Botany department, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

**Preparation and administration of plant extracts:** Aqueous extract of this herb was prepared and administered to test rabbits according to the procedure reported by Prohp *et al.*, 2006.

**Administration of alloxan:** Alloxan was dissolved in 0.9% Nacl solution (saline). Rabbits were then injected intramuscularly with portions of this solution at a dose of 150-mg./kg body weights.

**Collection of blood:** Blood was obtained intravenously through the larger vein at the back of the rabbit's ear with the aid of sterilized needle and syringe and transferred into appropriate sample tubes containing anticoagulant (lithium heparinized and fluoride oxalate sample tubes for enzyme and glucose assays respectively). Centrifugation was performed at 800g for 5 minutes to obtain clear plasma for enzyme and glucose assays (Onoagbe *et al.*, 1999).

## Results

The results of the effects of the aqueous extract of *Triplochiton scleroxylon* on body weight, plasma glucose and some hematological parameters have been presented in Tables 1-9, respectively. Analyses of results showed that the effects on body weights and hematological parameters were non significant ($P > 0.05$) whilst significant decreases ($P < 0.05$) were recorded for plasma glucose concentration on the 18th, 24th and 28th days of administration of aqueous extract of herb to alloxan-induced diabetic rabbits.

Table 1: Mean Body Weight of Rabbits (kg) of alloxan – induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 1.36±0.11 | 1.46±0.10 | 1.58±0.02 |
| 1 | 1.54±0.24 | 1.45±0.10 | 1.54±0.02 |
| 6 | 1.50±0.09 | 1.43±0.11 | 1.53±0.02 |
| 12 | 1.52±0.14 | 1.43±0.15 | 1.51±0.04 |
| 18 | 1.49±0.16 | 1.44±0.18 | 1.54±0.07 |
| 24 | 1.57±0.11 | 1.41±0.23 | 1.52±0.11 |
| 28 | 1.58±0.20 | 1.41±0.09 | 1.49±0.16 |

Values are mean body weight ± S.E.M. of three separate readings from nine rabbits. Values not significantly different ($P > 0.05$) from diabetic control

Table 2: Mean Plasma Glucose Concentration (mg/100ml) of alloxan-induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 90.00±2.04 | 172.67±7.32 | 164.32±4.62 |
| 1 | 84.45±3.26 | 176.52±2.57 | 168.91±4.58 |
| 6 | 71.25±5.60 | 177.33±7.23 | 163.67±3.72 |
| 12 | 92.18±4.12 | 190.25±2.53 | 161.35±3.50 |
| 18 | 74.36±6.32 | 180.00±5.30 | *97.33±2.36 |
| 24 | 53.81±4.51 | 194.06±4.59 | *99.25±3.26 |
| 28 | 72.12±5.35 | 179.58±2.35 | *84.06±6.05 |

Values are mean plasma glucose concentration ± S.E.M. of three separate readings from nine rabbits.
*Values significantly different ($P < 0.05$) from diabetic control.

Table 3: Mean Red Blood Cell Count (x $10^{12}$ cell/L) of alloxan - induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 5.41±0.28 | 5.00±0.16 | 4.60±0.27 |
| 1 | 5.04±0.68 | 5.14±0.21 | 4.61±0.71 |
| 6 | 5.03±0.05 | 5.06±0.49 | 4.33±0.39 |
| 12 | 5.27±0.027 | 4.88±0.37 | 5.35±0.09 |
| 18 | 5.22±0.36 | 4.68±0.71 | 5.21±0.10 |
| 24 | 5.24±0.36 | 5.35±0.49 | 4.89±0.28 |
| 28 | 5.30±0.41 | 5.25±0.43 | 5.37±0.03 |

Values are mean red blood cell counts ± S.E.M. of three separate readings from nine rabbits. Values not significantly different ($P > 0.05$) from diabetic control.

Table 4: Mean Packed Cell Volume (%) of alloxan - induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 34.33±2. 34 | 35.17±1.56 | 33.87±1.34 |
| 1 | 35.30±2.51 | 35.87±1.26 | 36.57±0.95 |
| 6 | 38.40±1.63 | 35.83±5.13 | 30.80±1.71 |
| 12 | 39. 53±3.33 | 33.30±1.69 | 38.73±2.35 |
| 18 | 38.83±2.68 | 33.85±4.95 | 37.57±3.18 |
| 24 | 38.90±3.67 | 38.85±3.35 | 34.83±3.35 |
| 28 | 41.10±4.12 | 38.20±3.00 | 38.20±2.60 |

Values are mean packed cell volume ± S.E.M. of three separate readings from nine rabbits. Values not significantly different ($P > 0.05$) from diabetic control

Table 5: Mean Hemoglobin Concentration (HB) (g/L) of alloxan-induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 101.67±5.13 | 96.00±1.16 | 87.33±6.69 |
| 1 | 91.00±6.21 | 96.00±5.13 | 85.33±10.37 |
| 6 | 107.00±5.16 | 96.33±15.41 | 81.00±2.52 |
| 12 | 105.67±8.11 | 88.67±7.36 | 98.00±5.59 |
| 18 | 110.67±5.21 | 94.50±9.39 | 97.00±7.81 |
| 24 | 105.00±2.58 | 109.00±9.00 | 84.00±11.34 |
| 28 | 103.67±4.12 | 98.50±11.50 | 99.00±8.00 |

Values are mean hemoglobin concentration ± S.E.M of three separate readings from nine rabbits. Values not significantly different ($P > 0.05$) from diabetic control.

Table 6: Mean Corpuscular Hemoglobin Concentration (MCHC) (g/L) of alloxan-induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 263.33±4.98 | 273.67±6.84 | 2.51.67±4.45 |
| 1 | 282.33±5.16 | 268.33±7.62 | 2.56.33±3.45 |
| 6 | 280.00±6.17 | 267.33±10.40 | 266.00±7.10 |
| 12 | 272.00±6.19 | 278.00±7.55 | 268.00±6.51 |
| 18 | 272.33±8.71 | 273.00±2.00 | 268.33±6.44 |
| 24 | 273.00±7.14 | 283.00±3.00 | 262.33±4.37 |
| 28 | 277.33±6.15 | 284.00±3.00 | 274.00±6.00 |

Values are mean corpuscular hemoglobin concentration ± S.E.M. of three separate readings from nine rabbits. Values not significantly different ($P > 0.05$) from diabetic control.

Table 7: Mean Cell Hemoglobin (MCH) (pg) of alloxan–induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 18.87±0.95 | 19.47±0.41 | 18.43±1.21 |
| 1 | 21.77±0.78 | 18.83±1.55 | 18.97±1.04 |
| 6 | 20.83±0.98 | 18.87±1.93 | 19.00±1.17 |
| 12 | 20.50±1.23 | 20.93±0.35 | 20.83±0.58 |
| 18 | 20.90±1.13 | 20.30±0.50 | 18.60±1.21 |
| 24 | 20.13±0.85 | 20.45±0.15 | 17.87±1.07 |
| 28 | 20.40±0.79 | 20.00±0.80 | 20.65±0.65 |

Values are mean cell hemoglobin ± S.E.M. of three separate readings from nine rabbits. Values not significantly different (P>0.05) from diabetic control.

Table 8: Mean Corpuscular Volume (fl) of alloxan–induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 72.70±1.45 | 70.37±3.00 | 69.73±3.20 |
| 1 | 79.27±1.56 | 70.00±3.87 | 78.37±2.58 |
| 6 | 73.03±1.27 | 70.30±4.71 | 71.37±2.58 |
| 12 | 75.60±3.15 | 75.37±3.15 | 70.13±3.19 |
| 18 | 78.00±2.67 | 72.40±0.50 | 70.20±3.93 |
| 24 | 78.17±2.19 | 75.00±1.90 | 68.10±3.03 |
| 28 | 78.50±1.98 | 73.30±2.10 | 72.15±0.15 |

Values are mean corpuscular volume ± S.E.M. of three separate readings from nine rabbits. Values not significantly different (P>0.05) from diabetic control.

Table 9: Mean Red Cell Distribution Width (RDW) (%) of alloxan–induced diabetic rabbits administered aqueous extract of obeche (*Triplochiton scleroxylon*)

| Time (days) | Non-diabetic control | Diabetic control | Triplochiton scleroxylon |
|---|---|---|---|
| 0 | 10.30±0.42 | 10.80±0.56 | 9.87±0.38 |
| 1 | 10.77±0.28 | 9.60±0.36 | 11.27±1.67 |
| 6 | 10.87±0.64 | 8.80±1.15 | 10.40±0.10 |
| 12 | 11.03±0.64 | 10.70±0.96 | 10.00±0.25 |
| 18 | 10.47±1.26 | 9.70±0.10 | 9.43±0.37 |
| 24 | 11.27±1.21 | 10.00±0.20 | 9.57±4.06 |
| 28 | 11.73±0.96 | 10.25±0.35 | 9.80±0.02 |

Values are mean cell distribution width ± S.E.M. of three separate readings from nine rabbits. Values not significantly different (P > 0.05) from diabetic control.

## Discussion

The use of herbs is increasingly gaining acceptance among Africans and the world over as alternatives to orthodox medicine for the treatment of some diseases (Gill, 1992; Lamla, 1981). Regardless of the highly advanced orthodox medicine, substantial amount of medicinal plants are used for the treatment of ailments in some developed countries. In United States of America, for example, medicinal plants constitute about 25% of all new refined prescriptions dispensed from community pharmacies (Trease and Evans, 1989). Studies have equally shown that the uneven distribution

of health personnel between rural and urban areas has markedly increased the use of medicinal herbs in the rural areas than in the cities of Africa (Onoagbe *et al.,* 1999). In most developing nations researches on medicinal plants are very popular in the chemical and biological sciences because of the availability of these medicinal plants most of which have not been identified and fully exploited for proper classification (Watts *et al.,* 1997). World Health Organization (WHO) has so far recorded the use of about 20,000 medicinal plants worldwide. In Britain alone, an estimated 6,000 – 7000 tonnes of herbs are used annually as ingredients in some 5500 different herbal products (Alberti and Zimmet, 1998). In Nigeria and other African countries herbs are used in unpardonable proportion occasioned by increasing rise in orthodox health care bills. According to Dalziel, (1987), the medicinal value of drugs is due to the presence of substances known as alkaloids, glycosides, resin, volatile oils, gum and tannins usually found in large concentrations in storage organs like roots, seeds, bark and leaves.

In this study, the aqueous extract of *Triplochiton scleroxylon* did not impose any acute fluid loss, proteolysis and lipolysis on the rabbits and as a result has no significant effect (P > 0.05) on their body weights. This agrees with the reports of Alberti and Zimmet, (1998) that in diabetes mellitus, acute fluid loss, proteolysis and lipolysis are responsible for loss of weight. On the 18th, 24th and 28th days of administration of extract to the rabbits, plasma glucose decreased significantly (P<0.05). This is indicative of the hypoglycemic properties of the aqueous extract of *Triplochiton scleroxylon*. Substances with hypoglycemic properties would be effective in the management of diabetes mellitus (Young and Maciejewski, 1997). Studies on the red blood cell and associated parameters such as red blood cell count, packed cell volume, hemoglobin concentration, mean corpuscular hemoglobin concentration, mean cell hemoglobin, mean cell volume and red cell distribution width showed that aqueous extract of this herb has no effect (P>0.05) on these parameters when compared with the control in rabbits. Values of red blood cell and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycytemia (American Diabetes Association, 2000). The aqueous extract of this herb may not have adverse effect on the bone marrow, kidney and hemoglobin metabolism. Only substances, which significantly affect the values of red blood cell and associated parameters, would have effects on the bone marrow, kidney and hemoglobin metabolism (Young and Maciejewski, 1997).

In conclusion aqueous extract of obeche (*Triplochiton scleroxylon*) has an hypoglycemic property for use as an

anti-diabetic herb. It is unlikely that its use can advance any adverse effects on red blood cell and associated parameters. The active principles of the aqueous extract of this herb when identified, isolated and administered in a purer form may have a better anti - diabetic properties. The same study is on going but streptozotocin rather than alloxan is used for selective and specific delineation of the β-cells of the pancreas for the purpose of inducing experimental diabetes mellitus in rabbits.

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