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Full Length Research Paper

Effects of aqueous extract of *Triplochiton scleroxylon* on some haematological parameters and blood glucose concentrations in non-diabetic rabbits

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Investigations on possible effects of aqueous extract of *Triplochiton scleroxylon* on some haematological parameters and glucose concentrations in non-diabetic rabbits were carried out. At least 100 ml of extract was administered daily for 15 days to test rabbits (New Zealand strain) through clean water troughs. Blood was drawn with the aid of sterile syringes from the large vein at the back of the ears of rabbits. Swelab auto-counter 920^{E+} (UK) was used in haematological analysis while blood glucose concentrations were determined by glucose oxidase procedures involving spectrophotometry. Aqueous extract of *T. scleroxylon* did not have significant effects ($P > 0.05$) on the haematological parameters (mean hemoglobin concentration, packed cell volume, white blood cell, lymphocyte and neutrophil counts) examined in non-diabetic rabbits when compared to normal control. However, it caused significant decrease ($P < 0.05$) in plasma glucose concentrations obtained on the 13th through 15th day of administration to the test rabbits. Aqueous extract of *T. scleroxylon* had hypoglycemic properties but does not contain lethal concentrations of chemical substances capable of adverse interference on the haematological parameters to cause any form of blood disorder.

Key words: *Triplochiton scleroxylon*, haematological parameters, non-diabetic rabbits.

INTRODUCTION

Plants are increasingly being explored and used as possible panacea for diabetes and other human diseases. *Triplochiton scleroxylon* is one of the medicinal plants commonly used in the rural and some urban areas in the western and southern parts of Nigeria to treat diabetes mellitus (Prohp et al., 2008; Prohp and Onoagbe, 2009a). This tree belongs to the family of African tropical medicinal plants whose active ingredients are believed to be present at the bark of the stem. It is well documented that *T. scleroxylon* is in the kingdom: Plantae, division: Magnoliophyta, class: Magnoliopsida, order: Malvales, family: Sterculiaceae (APG: Malvaceae), genus: Triplochiton and species: *scleroxylon* (Ritcher and Dallwitz, 2000). This tree is widely distributed in tropical West Africa along waterways and farms between humid evergreen and semi deciduous forests (Prohp et al., 2008). Several preliminary studies have proved valid the claims of its hypoglycaemia and anti-diabetic properties in normal, alloxan and streptozotocin-induced diabetic rabbits (Prohp et al., 2008; Prohp and Onoagbe, 2009a, b). However, there is inadequate information in literature about the possible side effects that could arise from the use of this plant in the treatment of diabetes mellitus.

This study was, therefore, to ascertain any possible side effects that may be associated with the use of this plant extract on some other haematological parameters in non-diabetic (normal) rabbits.

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MATERIALS AND METHODS

All the experimental protocols were in compliance with our Institu-

tional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (NIH publication, no. 92 - 3415, 1992).

Experimental animals

Adult male rabbits (New Zealand strain) with an average weight of 1.36 kg were used in this study. They were obtained from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The animals were maintained under laboratory conditions of room temperature, humidity and light. They were also allowed free access to standard laboratory diet supplied by Ewu feeds Ltd Ewu, Edo State, Nigeria and distilled water *ad libitum* for a period of two weeks to acclimatize to the new environment.

Chemicals

All chemicals used were of analytical grade.

Medicinal plant

The barks of *T. scleroxylon* were obtained from the forest of Uokha, Owan East local government area, Edo State, Nigeria. They were then identified by experts in the Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria, where a voucher specimen (UIH - 22329) had been deposited.

Preparation and administration of aqueous plant extract

The fresh barks of *T. scleroxylon* were washed with water, dried and cut into tiny strands. They were then ground into powder and 250 g of bark powder was boiled in 2500 ml of distilled water for 3, 2 and 1 h on the first, second and third day, respectively. After cooling to room temperature, it was filtered with sintered glass funnel under suction to eliminate particles. The filtered extract was transferred to clean jerry cans and stored at -21°C until used. The test animals were given not more than 100 ml of liquid extract daily for a total of 15 days as they were allowed to drink *ad libitum* from clean water troughs in accordance with the procedure reported by Prohp and Onoagbe (2009a, b).

Experimental procedure

Adult male rabbits (New Zealand strain) were allowed to acclimatize to the laboratory conditions for a period of two weeks, thereafter subjected to fasting overnight. They were randomly divided into two groups of three rabbits each and treated as follows:
Group 1: Served as normal control and received distilled water.
Group 2: Served as the test rabbits and given 100 ml of aqueous extract of *T. scleroxylon* daily.

Blood collection

Blood was drawn intravenously through the large vein at the back of the ears of the rabbits in accordance with the procedure reported by Prohp et al. (2006) into ethylene diamine tetra-acetic acid (EDTA) and fluoride oxalate sample tubes for haematological and glucose analyses, respectively.

Blood glucose assay

Glucose was determined by the glucose oxidase method according to procedure described by Randox Laboratories Ltd., United Kingdom.

Haematological analysis

Haematological Swelab auto counter 920^{E+} (UK) was used in the analysis of haematological parameters.

Statistical analysis

Results were expressed as mean ± S.E.M of three separate determinations. The significance of the difference between the means of the test and control animals was established using the student's t-test. Values lower than 0.05 probability level were considered significant.

RESULTS AND DISCUSSION

The results of the effects of aqueous extract of *T. scleroxylon* on hemoglobin concentration, parked cell volume, white blood cell, lymphocyte, neutrophil counts and plasma glucose concentration are presented in Tables 1 to 6. The aqueous extract of *T. scleroxylon* did not have significant effects ($P > 0.05$) on mean hemoglobin concentration (Table 1), parked cell volume (Table 2), white blood cell (Table 3), lymphocyte (Table 4) and neutrophil counts (Table 5) in non-diabetic rabbits when compared to control. However, plasma glucose concentrations observed following the administration of the extract to the test rabbits decreased significantly ($P < 0.05$) on the 13th and 15th days (Table 6).

Diverse complications arising from the use of some orthodox drugs have grossly stimulated the exploitation of plants as possible panacea in disease management and/or cure (Bodeker, 1994; Prohp et al., 2006). This has resulted in the current increases in orthodox health care bills in most African countries including Nigeria. It is, therefore, important to promote the knowledge and understanding of the already identified herbs/plants and their specific uses in the management of various diseases (Prohp et al., 2008). The use of medicinal plants stands at advantages over orthodox medicine because these plants grow everywhere and are therefore available, accessible and affordable by all classes of people (poor and rich, rural and urban dwellers). However, a well documented adverse effects of these medicinal plants need to be presented.

Findings from this study showed that aqueous extract of *T. scleroxylon* did not have significant effects ($P > 0.05$) on hemoglobin concentration, packed cell volume, white blood cell, lymphocyte and neutrophil counts (Tables 1 to 5), in control (non-diabetic) rabbits. These findings agreed with the results obtained for red blood cells, white blood cells and associated parameters in

Table 1. Mean hemoglobin concentration (g/dl) of non-diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*

S/N	Day	Control (non-diabetic)	Test
1	0	11.07 ± 0.23[a]	11.60 ± 1.38[a]
2	1	11.40 ± 0.20[a]	11.53 ± 0.29[a]
3	3	10.53 ± 0.53[a]	11.63 ± 0.49[a]
4	5	10.73 ± 0.20[a]	11.27 ± 0.33[a]
5	7	10.43 ± 0.13[a]	11.30 ± 0.17[a]
6	9	10.43 ± 0.59[a]	10.87 ± 0.13[a]
7	11	10.43 ± 0.43[a]	10.83 ± 0.62[a]
8	13	10.10 ± 0.49[a]	10.50 ± 0.59[a]
9	15	10.34 ± 0.43[a]	10.87 ± 0.13[a]

Values are mean hemoglobin concentration ± S.E.M of three separate determinations from six rabbits. Values of test rabbits with same alphabet (letter) are non-significant (P> 0.05) from control.

Table 2. Mean packed cell volume (%) of non-diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon.*

S/N	Day	Control (non-diabetic)	Test
1	0	33.30 ± 0.67[a]	35.00 ± 0.00[a]
2	1	34.30 ± 0.67[a]	34.70 ± 0.80[a]
3	3	31.70 ± 0.67[a]	23.00 ± 1.53[a]
4	5	33.00 ± 0.58[a]	34.00 ± 1.00[a]
5	7	32.30 ± 0.33[a]	34.00 ± 0.58[a]
6	9	30.70 ± 1.77[a]	32.70 ± 0.33[a]
7	11	31.30 ± 1.34[a]	32.70 ± 1.86[a]
8	13	30.30 ± 1.46[a]	31.70 ± 1.77[a]
9	15	31.30 ± 1.34[a]	32.70 ± 0.33[a]

Values are mean packed cell volume ± S. E. M of three separate determinations from six rabbits. Values of test rabbits with same alphabet (letter) are non-significant (P > 0.05) from control.

alloxan-induced diabetic rabbits (Prohp et al., 2006, 2008).Values of hemoglobin concentration and packed cell volume lower than normal, 80 to 130g/L and 33 to 50% in rabbits, respectively, were indicative of anemia while higher values suggested polycytemia (Mitruka and Rawnley, 1977). Substances that demonstrate significant effects on the above experimental parameters would have effects on bone marrow, kidney and also haemoglobin metabolism (Young and Maciejewski, 1997). It is well documented that reduction in white blood cell, neutrophil and lymphocyte counts is positively correlated with susceptibility to infection, leukaemia, arthritis and possible compromise of cellular and humoral mediated immunity (Bochner et al., 1991, 1994).

Since there was no significant difference in the hemoglobin concentration, packed cell volume, white blood cell, neutrophil and lymphocyte counts of the tested rabbits when compared to control (Tables 1 to 5), it was evident that the administration of bark extract of *T. scleroxylon* would not precipitate side effects in the blood.

Aqueous extract of *T. scleroxylon* caused significant ($P < 0.05$) decrease in the plasma glucose in non-diabetic rabbits on the 13th and 15th day of administration when compared to the control rabbits (Table 6). Decrease in plasma glucose concentration obtained on the 5th, 7th, 9th and 11th day was not significant when compared to control (Table 6). It is possible that this aqueous bark extract of *T. scleroxylon* induces hypoglycemia by triggering insulin release from the β-cell of the islet of Langerhan or by hepatic glucose reduction, with the amount of insulin released directly related to the volume of extract administered. Hypoglycemic properties of *Urena lobata*, *Sphenostylis sternocarpa*, *Irvingia grandifolia, Spondias mombin, Morinda lucida, Tetracera alnifolia, Strophanthus hispidus* and *Triumfetta rhomboide*a and *Uvaria chamae,* were reported when administered to rabbits and rats (Onoagbe and Esekheigbe, 1999; Onoagbe et al., 1999a,b).

Table 3. Mean white blood cell count ($10^2 \times mm^3$) of non-diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon.*

S/N	Day	Control (non-diabetic)	Test
1	0	62.33 ± 3.34[a]	55.67 ± 3.29[a]
2	1	69.00 ± 2.52[a]	57.33 ± 10.81[a]
3	3	96.33 ± 23.88[a]	82.00 ± 2.51[a]
4	5	84.67 ± 28.04[a]	74.33 ± 2.67[a]
5	7	76.33 ± 3.57[a]	74.33 ± 0.67[a]
6	9	69.66 ± 3.85[a]	71.00 ± 4.36[a]
7	11	68.33 ± 4.10[a]	69.67 ± 4.10[a]
8	13	88.00 ± 21.03[a]	91.00 ± 3.61[a]
9	15	92.00 ± 21.03[a]	95.00 ± 21.60[a]

Values are mean white blood cell count ± S. E. M of three separate determinations from six rabbits. Values of test rabbits with same alphabet (letter) are non-significant (P > 0.05) from control.

Table 4. Mean lymphocyte count (mm^3) of non-diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon.*

S/N	Day	Control (non-diabetic)	Test
1	0	44.00 ± 4.05[a]	40.33 ± 8.42[a]
2	1	58.00 ± 3.06[a]	51.33 ± 3.85[a]
3	3	60.67 ± 1.34[a]	77.33 ± 3.53[a]
4	5	51.00 ± 4.94[a]	39.00 ± 8.03[a]
5	7	57.00 ± 4.36[a]	70.67 ± 5.12[a]
6	9	60.33 ± 2.91[a]	69.33 ± 0.88[a]
7	11	54.67 ± 3.53[a]	62.00 ± 5.78[a]
8	13	57.00 ± 2.52[a]	55.33 ± 3.18[a]
9	15	56.67 ± 1.77[a]	55.67 ± 6.34[a]

Values are mean lymphocyte count ± S. E. M of three separate determinations from six rabbits. Values of test rabbits with same alphabet (letter) are non-significant (P > 0.05) from control.

Table 5. Mean neutrophil count (mm^3) of non-diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon.*

S/N	Day	Control (non-diabetic)	Test
1	0	55.67 ± 4.34[a]	58.67 ± 7.52[a]
2	1	55.67 ± 4.34[a]	48.67 ± 3.85[a]
3	3	31.67 ± 1.67[a]	32.00 ± 1.53[a]
4	5	33.00 ± 0.58[a]	34.00 ± 1.00[a]
5	7	32.33 ± 0.33[a]	34.00 ± 0.58[a]
6	9	30.67 ± 1.77[a]	32.67 ± 0.33[a]
7	11	43.67 ± 3.18[a]	36.33 ± 6.65[a]
8	13	43.00 ± 2.52[a]	44.67 ± 3.18[a]
9	15	42.67 ± 1.34[a]	44.00 ± 6.12[a]

Values are mean neutrophil count ± S. E. M of three separate determinations from six rabbits. Values of test rabbits with same alphabet (letter) are non-significant (P >0.05) from control.

Table 6. Mean plasma glucose concentration (mg/dl) of non-diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon.*

S/N	Day	Control (non-diabetic)	Test
1	0	56.60 ± 9.80[a]	62.00 ± 8.50[a]
2	1	62.67 ± 10.20[a]	64.11±8.20[a]
3	3	68.30 ± 1.31[a]	68.60 ± 7.83[a]
4	5	85.01 ± 4.00[a]	69.66 ± 7.19[a]
5	7	64.20 ± 2.04[a]	62.62 ± 2.40[a]
6	9	70.30 ± 6.55[a]	60.60 ± 6.54[a]
7	11	66.30 ± 9.72[a]	45.70 ± 6.06[a]
8	13	76.16 ± 2.13[a]	42.01 ± 1.17[b]
9	15	71.29 ± 7.47[a]	43.12 ± 1.79[b]

Values are mean plasma glucose ± S. E. M of three separate determinations from six rabbits. Values of test rabbits with different alphabet (letter) are significant ($P < 0.05$) from control.

Conclusion

Aqueous bark extract of *T. scleroxylon* does not contain deleterious chemical substances likely to alter homeostatic haematological values to cause blood dysfunctions in rabbits. This justifies the use of the aqueous bark extract of *T. scleroxylon* in some parts of Nigeria in the treatment of diabetes mellitus.

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