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Full Length Research Paper

Anti–diabetic studies of aqueous extract of *Triplochiton scleroxylon* on platelets and associated parameters in alloxan-induced diabetic rabbits

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The effects of aqueous extract of *Triplochiton scleroxylon* (TS) on platelet and associated parameters, namely, platelet counts, volume and distribution width in alloxan-induced diabetic rabbits (New Zealand strain) were carried out. Plasma glucose concentration was also monitored as the test rabbits were treated with at least 100 ml of the aqueous extract daily through clean drinking troughs for a period of 28 days. Haematological swelab auto counter 920E$^+$ (UK) was used in blood analysis. Analysis of results showed that extract of TS did not have any significant effects ($P>0.05$) on the platelet counts, volume and distribution width. However, plasma glucose concentration decreased ($P<0.05$) on the 18, 24 and 28th days following treatment with aqueous extract of *Triplochiton scleroxylon.* The use of this extract in the treatment of diabetes mellitus in some part of Nigeria is not likely to accentuate any of the abnormalities or diseases/disorders of the platelets.

Key words: Anti–diabetic, *Triplochiton scleroxylon*, platelets, alloxan–induced diabetic rabbits.

INTRODUCTION

Diabetes mellitus is a disease that is increasingly affecting millions of people all over the world. And this disease has no known cure in spite of its age long existence. Currently there are over 150 millions diabetics worldwide and this is likely to increase to 300 million or more by 2025 (Tanko et al., 2007). The need to unravel or unearth a cure for this disease is never a misplaced priority. Insulin, sulphonylureas, biguanides and other orthodox drugs used in the treatment of diabetes mellitus had resulted to severe life - threatening complications in patients for example hypoglycemia, hepatitis, risks of lactic acidosis and liver damage (Lebovitz, 2004). Over the years the attempt has been to explore plants as a possible panacea. The use of several other medicinal plants for the treatment of other human and animal diseases has also been reported (Sofowora, 1984; Gill, 1992).

Triplochiton scleroxylon (epo arere, obeche; Nigeria, wawa; Ghana, ayous; Cameroon, samba; Ivory Coast) is one of the herbs commonly used by some Nigerian diabetics to treat their conditions (Prohp et al., 2006). This plant belongs to the family Sterculiaceae and is widely distributed in tropical West Africa along waterways and farms between humid evergreen and semi deciduous forests (Prohp et al., 2008). Studies have shown that the aqueous extract of the bark of this plant has hypoglycemic and anti-diabetic properties but do not have adverse effects on red blood cells, white blood cells and their associated parameters in alloxan - induced diabetic rabbits (Prohp et al., 2006; Prohp et al., 2008).

The objective of this continuing research is to examine the possible effects of this herbal extract on platelet and associated parameters in rabbits. This is with the view of understanding whether its use would aggravate any disorders of platelets that may have negative impact on blood clot formation.

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METHODOLOGY

All experimental protocols were in compliance with our ethical

committee guidelines as well as internationally accepted practices for laboratory animal use and care as contained in US guidelines (NIH publication, revised in 1985).

Materials

Animals

Rabbits (male) of the same strain (New Zealand) weighing between 1.56 and 1.68 kg were used. They were maintained under standard animal house conditions and allowed free access to food (growers mash) and water *ad libitum* for 2 weeks to acclimatize to the new environment.

Chemicals

Alloxan was supplied by sigma. All other chemicals were of analytical grade. Facilities in Lahor Public Health and Research Centre, Benin City, Edo State, Nigeria were used in the analysis of haematological parameters.

Medicinal plants

The barks of *Triplochiton 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.

Methods

Preparation and administration of aqueous plant extract

The barks of *Triplochiton scleroxylon* were washed with water, dried and cut into tiny bits. They were then ground and 250 g of ground bark of the plant boiled in 2.5 L of distilled water for 3, 2 and 1 h (s) on the first, second and third days respectively. After cooling to room temperature, it was filtered with sintered glass funnel under suction to eliminate debris. The clear extract was transferred to clean jerry cans and stored at -21°C until used. The test animals were given not more than 100 mls (equivalent to 121 mg) of aqueous extract daily for 28 days (Onoagbe et al., 1999a).

Experimental procedure

Adult male rabbits (New Zealand strain) after acclimatization for a period of two weeks were fasted overnight and randomly divided into three groups of three rabbits each and treated as follows:

Group 1: Served as normal (non-diabetic) control and received distilled water through clean drinking troughs for 28 days.
Group 2: Served as diabetic control and received distilled water through clean drinking troughs for 28 days.
Group 3: Served as the test diabetic rabbits given 100 mls of aqueous extract of *Triplochiton scleroxylon* daily through clean drinking troughs for a period of 28 days.

Blood collection

Blood was drawn intravenously through the large vein at the back of the ears of the rabbits into EDTA sample tubes for haematological analysis. Flouride oxalate sample tubes were used to collect blood for glucose assay.

Administration of alloxan

Alloxan was dissolved in 0.9% NaCl solution (saline). Test rabbits were then injected intramuscularly with portions of this solution at a dose of 150 mg/kg body weight.

Blood glucose assay

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

Haematological analysis

Haematological parameters were analysed with the aid of the haematological Swelab autocounter 920E^{+} (UK) system. The reference method used was hemacytometer counting of platelets in whole blood diluted with 0.85% NaCl solution (Maxie, 1977; Malok et al., 2007).

Statistical analysis

Results have been expressed as mean ± standard error mean of three determinations. Data were analyzed using the student's t – test for significance of the difference between the means of the test and control animals. Values lower than 0.05 were considered to be significant.

RESULTS

The results have been presented in Tables 1 to 4. The aqueous extract has no significant effects ($P > 0.05$) on the platelet counts, platelet volume and platelet distribution width (Tables 1, 2 and 3). However, mean plasma glucose concentration decreased significantly ($P<0.05$) on the 18, 24 and 28th days of the experiment (Table 4).

DISCUSSION

In Africa and other parts of the world, plants are used traditionally in large proportion in the management and/or control of diabetes mellitus and other diseases. Despite the highly advanced orthodox medicine, many find respite from using medicinal plants to manage their diabetes condition. That might have arisen from uncontrolled increases in orthodox health care bills and diversified complications associated with the use of most hypoglycemic drugs. The use of medicinal plants to manage diseases is not just restricted to Nigeria alone as some other nations of the world and from all social strata also use herbs. As a matter of fact, many rural communities in Africa still have areas where traditional herbal medicine is the major and in some cases the only source of health care available (Aregbeyen, 1983;

Table 1. Mean platelet counts (× 10^9 cell/L) of alloxan-induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/No.	Day(s)	Non-diabetic control (normal)	Diabetic control	Diabetic group +100 ml extract daily
1	0	589.00±25.94	674.00 ± 100.60	565.33 ± 115.66
2	1	717.67 ± 60.36	602.67 ± 135.73	662.67 ± 67.76
3	6	611.33 ± 119.83	528.00 ± 94.69	565.33 ± 90.35
4	12	562.33 ± 151.64	584.67 ± 119.87	658.67. ± 134.75
5	18	502.62 ± 47.69	583.00 ± 120.00	598.00 ± 201.55
6	24	549.33 ± 49.98	595.67 ± 19.89	688.33 ± 208.95
7	28	531.33 ± 42.65	578.33 ± 20.00	663.00 ± 137.23

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

Table 2. Mean platelet volume (FL) of alloxan–induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/No.	Day (s)	Non-diabetic control (normal)	Diabetic control	Diabetic group +100 ml extract daily
1	0	7.93 ± 0.25	7.50 ± 0.46	7.53 ± 0.51
2	1	8.17 ± 0.52	7.73 ± 0.07	7.20 ± 0.06
3	6	8.43 ± 0.66	8.23 ± 0.13	8.87 ± 0.72
4	12	6.60 ± 0.43	8.50 ± 0.91	7.17 ± 0.24
5	18	6.77 ± 0.61	7.95 ± 0.15	7.40 ± 0.12
6	24	7.23 ± 0.22	6.70 ± 0.10	7.30 ± 0.16
7	28	7.87 ± 0.68	7.10 ± 0.60	7.00 ± 0.10

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

Table 3. Mean platelet distribution width (FL) of alloxan–induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/No.	Day(s)	Non-diabetic control (normal)	Diabetic control	Diabetic group +100 ml extract daily
1	0	11.13 ± 0.22	10.00 ± 0.86	11.00 ± 0.30
2	1	11.20 ± 0.36	10.53 ± 0.27	10.23 ± 0.07
3	6	10.82 ± 0.16	11.90 ± 1.29	10.13 ± 0.35
4	12	8.83 ± 0. 62	11.25 ± 0.55	10.37 ± 0.09
5	18	9.64 ± 0.33	12.07 ± 0.48	12.77 ± 1.07
6	24	10.17 ± 0.26	10.10 ± 0.70	9.90 ± 0.20
7	28	10.70 ± 0.52	10.20 ± 0.40	10.95 ± 0.25

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

Trease and Evans, 1989; Bodeker, 1994; Prohp et al., 2006).

Triplochiton scleroxylon (TS) is one of the over 30 indigenous medicinal plants commonly used by some Nigerian diabetics to treat their diabetes conditions (Onoagbe et al., 1999a). However, the side effects of this plant and its safety margins have not been fully documented. Earlier studies have shown that the use of the aqueous bark extract of TS may not predispose to compromise of cellular and humoral immunity and may also not stimulate or trigger any of the abnormalities associated with the reduction of white blood cell differentials (Prohp et al., 2008). It has also been reported that aqueous bark extract of TS may not have adverse effects on the bone marrow, kidney and hemoglobin metabolism (Prohp et al., 2006).

Table 4. Mean plasma glucose concentration (mg/dl) of controls and treated streptozotocin–induced diabetic rabbits.

S/No.	Day(s)	Non-diabetic control (normal)	Diabetic control	Diabetic group +100 ml extract daily
1	0	90.00±2.04	172.67±7.32	164.32±4.62
2	1	84.54±3.26	176.52±2.57	168.91±4.58
3	6	71.25±5.60	177.33±7.23	163.67±3.72
4	12	92.18±4.12	190.25±2.53	161.35±3.50
5	18	74.36±6.32	180.00±5.30	145.33*±2.36
6	24	53.81±4.51	194.06±4.59	142.25*±3.26
7	28	72.12±5.35	179.58±2.35	125.06*±6.05

Values are mean ± S.E.M. of three separate determinations. *Values are significantly different (P< 0.05) when compared with diabetic control.

Findings from this study showed that the extract of TS did not have significant effect (P > 0.05) on platelet counts, volume and distribution width in alloxan induced diabetic rabbits. This may be due to absence of chemical substances in the aqueous extract capable of interfering with active release of platelets. Variation in platelet size can be a sign of platelet release while elevated level is indicative of increased megakaryocyte shedding of platelets. A decreased level is however, common in thrombocytopenia. Low and too high number of platelets would lead to excessive bleeding and blood clot formation respectively. The latter may obstruct or occlude blood vessels supplying the brain and/or heart leading to stroke and/or heart attack respectively (http://medicaldictionary.thefreedictionary.com/); (http://en.wikipedia.org/wiki/Platelet). Platelet count obtained in this study fall within the range reported for normal platelet counts in feline (http://wwwpeteducation.com=2011). Significant decreases in plasma glucose following treatment with the aqueous extract on the 18, 24 and 28th days (Table 4) agree with documented evidences of the hypoglycemic and anti - diabetic properties of this plant (Prohp et al., 2006, 2008; Prohp and Onoagbe, 2009ab). It is possible that this extract reduced blood glucose by stimulating insulin release from the β-cells of the pancreas that may have survived alloxan destruction (Onoagbe et al., 1999b). Effect of phytochemicals (flavonoids) on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by Mahesh and Menon (2004) and Sri-Balasubashini et al., (2004) as the mechanism by which medicinal plants used in the treatment of diabetes mellitus reduce hyperglycaemia in streptozotocin-induced diabetic rats. It is very possible that aqueous extract of *Triplochiton scleroxylon* reduce hyperglycaemia (high blood glucose) in alloxan-induced diabetic rats by similar mechanism.

Aqueous bark extract of TS, used in various parts of Nigeria to treat diabetes mellitus may not predispose to any disorder or diseases of the platelets (thrombocytopathy) and do not contain deleterious chemical substances capable of interfering with the biological function and active release of platelets.

Conclusion

On the basis of this research, aqueous bark extract of *Triplochiton scleroxylon* is safe and has demonstrated anti-diabetic potentialities in alloxan-induced diabetic rabbits. However, further research is on-going to determine the safety limits and possible side effects of this extract for proper classification of the plant as a safe hypoglycemic and anti-diabetic herb.

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