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EVALUATION OF THE BODY WEIGHT AND SOME PLASMA COMPONENTS IN ALLOXAN–INDUCED DIABETIC RABBITS TREATED WITH AQUEOUS EXTRACT OF *TRIPLOCHITON SCLEROXYLON.*

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ABSTRACT: Aqueous bark extract of *Triplochiton scleroxylon* used as an anti - diabetic herb in some parts of Nigeria, was examined for possible effects on body weight and some plasma components, viz., urea, creatinine and calcium concentrations in alloxan – induced diabetic rabbits. Test rabbits (New Zealand strain) of average weight, 1.60 kg, were on daily oral administration of about 100 ml of extract through clean water troughs, for 28 days. Sterile syringes were used to collect blood intravenously for analysis, from the large veins at the back of the ears of the rabbits. Standard procedures involving spectrophotometry were used to determine glucose, urea, creatinine and calcium concentrations in the plasma of experimental rabbits. The weights of the rabbits were obtained with the aid of a laboratory weighing balance. Significant decreases ($P < 0.05$) in plasma glucose concentration were observed on the 12^{th}, 18^{th} and 28^{th} days of experiment when compared to diabetic control. However, no significant difference ($P > 0.05$) was observed on the body weight, urea and creatinine concentration with respect to diabetic controls. Although plasma calcium concentration decreased on the 12^{th} day in the test rabbits, the decrease was not significant ($P > 0.05$) when compared to diabetic control. Findings so far, have shown that aqueous extract of *T. scleroxylon* has anti -diabetic properties without toxic side effects and this could justify the use of this plant in the treatment of diabetes mellitus in some parts of Nigeria.

Key words: *Triplochiton scleroxylon*, Body weight, Urea, Creatinine, Calcium, Alloxan – induced diabetic rabbits.

INTRODUCTION

Diabetes mellitus is a major metabolic disease and the leading cause of irreversible blindness and chronic renal failure. It is characterised by hyperglycemia, altered metabolism of lipids, carbohydrates, proteins and an increased risk of complications from vascular diseases and mortality (Garcia et al., 1974; Kannel and McGee, 1979). Chronic hyperglycemia causes glycation of body proteins and this has been associated with a wide scale of diabetic complications such as diabetic cataract, retinopathy, nephropathy and cardiovascular diseases (Bucala and Cerami, 1992; Sharma, 1993; West, 2000; Bayraktutan, 2002). All these impose a huge burden on healthcare services (WDD, 2002). And in its age long existence this disease has no known and permanent cure without adverse complications. Plants are increasingly being explored globally and used as possible panacea for diabetes and other human diseases. However, the literature is porous of the safety margins of some of these herbs and/or possible side effects that could arise from their uses in the treatment of diabetes and other human diseases (Onoagbe et al., 1999a; Prohp et al., 2006, 2008). The bark of *Triplochiton scleroxylon*, is used by many Nigerian diabetics especially in the southern part of the country, to treat their diabetic state (Prohp et al., 2006, 2008).

Triplochiton scleroxylon (Sterculiaceae (Malvaceae)) is a large, deciduous African forest tree usually distributed in humid ever green semi-deciduous forest along water ways in the tropical West Africa (Russel et al., 1997; Ritchter and Dallwitz, 2000). The tree is associated with altitudes below 500 m, mean annual rainfall from 1100 to 1800 mm, a seasonal and two-peaked rainfall distribution, average temperatures 20 - 35 °C and well drained ferruginous soils (Hall and Bada, 1979). In the dry forest zone, this tree occurs in clusters, either within the dry forests or at its boundaries with the moist forest. Trees found in the dry forest zone have smaller leafs, narrower crowns and shorter bole lengths (Jones, 1975). Within the natural range, three sections are generally recognized, namely: Sierra Leone to Togo, Benin to Nigeria and Cameroon to Congo (Hall and Bada, 1979). In Côte d'Ivoire and Benin, the leaves of *T. scleroxylon* are prepared as a cooked vegetable or sauce in traditional cuisine. The bark is used to cover the roof and walls of huts and also applied in traditional medicine to treat oedemas and as an anodyne. *Triplochiton scleroxylon* is a food plant of the silkworm *Anaphe venata*, the larvae of which are a good source of protein and commonly eaten. Some common vernacular names of this plant are: African whitewood, African maple, ayous, obeche, wawa and samba. The Nigerian name obeche and the Ghana name wawa have been adopted as alternative British Standard names for the timber of this species; obeche is the usual trade name in Britain. The names ayous and samba refer to timber originating in Cameroon and the Ivory Coast, respectively (Hall and Bada 1979; Onilude et al., 2002; Dick et al., 2004; Attah et al., 2005).

Findings from preliminary studies have shown that aqueous bark extract of this plant could actually be safe for the treatment of diabetes mellitus (Prohp et al., 2006, 2008; Prohp and Onoagbe, 2009a, b), hence the need for proper scrutiny of this plant. It is the objective of this study, therefore, to ascertain any possible consequences of the use of this extract on body weight and some plasma components in alloxan – induced diabetic rabbits; with the view of building up data for proper classification of the bark aqueous extract of *T. scleroxylon* as safe for the treatment of diabetes mellitus.

MATERIALS AND METHODS

All the experimental protocols were in compliance with our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National Institute of Health, 1992).

Experimental Animals

Adult male rabbits (New Zealand strain) with an average weight of 1.60 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 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

Alloxan was supplied by Sigma. All other chemicals 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.

METHODS

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 ground bark powder of the plant 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 ^{o}C until used. The test animals were given not more than 100mls of liquid extract daily for 28days 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 three groups of three rabbits each and treated as follows:

Group 1: Served as normal control and received distilled water.

Group 2: Served as diabetic control and received distilled water.

Group 3: Served as the test diabetic rabbits and treated with 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 lithium – heparin and fluoride oxalate sample tubes. The plasma samples were then analyzed for some plasma components and glucose concentrations respectively using standard procedures.

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 150mg/kg body weight.

Blood glucose assay

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

Blood Urea, Creatinine and Calcium assays

Assays of these blood components were carried out according to procedures described in the Kits provided by Randox Laboratories Ltd., United Kingdom.

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 he student's t-test. Values lower than 0.05 probability level were considered significant.

RESULTS

On the 12th, 18th and 28th days of daily administration of about 100ml of aqueous bark extract of *T. scleroxylon* to the alloxan – induced diabetic rabbits, mean plasma glucose concentration decreased significantly ($P<0.05$). The decrease observed on the 24th day was not significant ($P>0.05$) when compared to diabetic control (Table 1). The values of the mean body weight, plasma urea, creatinine and calcium of alloxan – induced diabetic rabbits obtained after 28 days of administration of aqueous extract of TS to alloxan – induced diabetic rabbits were not significant ($P>0.05$) when compared to diabetic control (Tables 2 -5).

Table 1: Mean plasma glucose concentration (mg/dl) of alloxan – induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/N	Day	Normal control	Diabetic control	Test
1	0	66.67 ± 8.72^a	172.67 ± 7.32^b	164.00 ± 4.59^b
2	1	80.00 ± 6.42^a	175.33 ± 8.52^b	167.00 ± 7.03^b
3	6	82.33 ± 5.60^a	171.67 ± 7.23^b	165.67 ± 4.26^b
4	12	76.00 ± 6.10^a	177.33 ± 8.42^b	117.00 ± 3.72^c
5	18	82.00 ± 2.61^a	180.00 ± 5.30^b	110.48 ± 2.01^c
6	24	74.00 ± 2.88^a	179.00 ± 0.05^b	131.24 ± 2.00^b
7	28	81.30 ± 4.61^a	179.50 ± 11.53^b	112.12 ± 1.40^c

Values are mean ± S. E.M of 3 separate determinations from nine rabbits. Mean in the same row with different superscript letters are significantly different ($P<0.05$) when compared to diabetic control.

Table 2: Mean body weight of alloxan – induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/N	Day	Normal control	Diabetic control	Test
1	0	1.60 ± 0.08^a	1.45 ± 0.10^a	1.58 ± 0.09^a
2	1	1.48 ± 0.13^a	1.45 ± 0.10^a	1.54 ± 0.02^a
3	6	1.60 ± 0.09^a	1.48 ± 0.11^a	1.53 ± 0.02^a
4	12	1.63 ± 0.07^a	1.47 ± 0.15^a	1.51 ± 0.04^a
5	18	1.65 ± 0.12^a	1.44 ± 0.18^a	1.54 ± 0.07^a
6	24	1.68 ± 0.10^a	1.41 ± 0.23^a	1.52 ± 0.11^a
7	28	1.68 ± 0.08^a	1.61 ± 0.09^a	1.49 ± 0.16^a

Values are mean ± S. E.M of 3 separate determinations from nine rabbits. Mean in the same row with different superscript letters are significantly different (P<0.05) when compared to diabetic control.

Table 3: Mean plasma urea concentration (mg/dl) of alloxan – induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/N	Day	Normal control	Diabetic control	Test
1	0	54.67 ± 12.62[a]	51.33 ± 15.51[a]	48.67 ± 15.78[a]
2	1	44.00 ± 0.81[a]	32.33 ± 2.85[a]	33.33 ± 4.38[a]
3	6	23.00 ± 11.01[a]	31.67 ± 5.49[a]	47.33 ± 4.81[a]
4	12	33.33 ± 4.61[a]	37.33 ± 3.53[a]	46.67 ± 3.72[a]
5	18	54.67 ± 6.08[a]	48.00 ± 12.14[a]	38.00 ± 11.39[a]
6	24	43.33 ± 4.62[a]	56.00 ± 0.01[a]	54.33 ± 6.75[a]
7	28	43.33 ± 0.80[a]	54.00 ± 2.01[a]	51.33 ± 1.77[a]

Values are mean ± S. E.M of 3 separate determinations from nine rabbits. Mean in the same row with different superscript letters are significantly different (P<0.05) when compared to diabetic control.

Table 4: Mean plasma creatinine concentration (mg/dl) of alloxan – induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/N	Day	Normal control	Diabetic control	Test
1	0	0.43 ± 0.18[a]	0.67 ± 0.04[a]	1.10 ± 0.02[a]
2	1	0.77 ± 0.08[a]	0.43 ± 0.09[a]	0.63 ± 0.09[a]
3	6	0.33 ± 0.05[a]	0.47 ± 0.07[a]	0.73 ± 0.08[a]
4	12	0.40 ± 0.01[a]	0.37 ± 0.03[a]	0.50 ± 0.05[a]
5	18	0.40 ± 0.02[a]	0.27 ± 0.01[a]	0.33 ± 0.07[a]
6	24	0.43 ± 0.01[a]	0.35 ± 0.02[a]	0.40 ± 0.06[a]
7	28	0.50 ± 0.00[a]	0.35 ± 0.04[a]	0.47 ± 0.07[a]

Values are mean ± S. E.M of 3 separate determinations from nine rabbits. Mean in the same row with different superscript letters are significantly different (P<0.05) when compared to diabetic control.

Table 5: Mean plasma calcium concentration (mg/dl) of alloxan – induced diabetic rabbits administered aqueous extract of *Triplochiton scleroxylon*.

S/N	Day	Normal control	Diabetic control	Test
1	0	14.67 ± 0.32[a]	13.77 ± 0.50[a]	14.57 ± 0.20[a]
2	1	15.27 ± 0.08[a]	14.73 ± 0.20[a]	14.77 ± 1.02[a]
3	6	15.47 ± 0.72[a]	15.20 ± 0.53[a]	13.43 ± 1.33[a]
4	12	16.47 ± 0.11[a]	15.60 ± 0.12[a]	9.33 ± 0.17[a]
5	18	14.40 ± 0.87[a]	15.77 ± 1.67[a]	13.10 ± 0.90[a]
6	24	14.20 ± 1.04[a]	15.95 ± 1.05[a]	15.27 ± 1.79[a]
7	28	15.27 ± 0.45[a]	14.85 ± 0.35[a]	14.90 ± 0.55[a]

Values are mean ± S. E.M of 3 separate determinations from nine rabbits. Mean in the same row with different superscript letters are significantly different ($P<0.05$) when compared to diabetic control.

DISCUSSION

Plant research is now a global phenomenon, stemming from the failure of some orthodox medications to provide a cure to some human and animal diseases without complications. Arising from the exploitation of plants, over 400 plant species have been reported to have activities that are significant for the treatment of diabetes mellitus (Oliver – Bever, 1986; Rai, 1995) whilst many others have found usefulness in the management of other diseases (Mohammed et al., 2006). However, the uses of some of these plants have not been linked to side effects since toxicity studies in most of them have not been fully documented. It is necessary to carry out toxicity studies on medicinal plants even though they have been used for decades, to determine acceptable from non – acceptable toxicity levels (Jasper et al., 2003). The toxicological evaluation of plant extract generally seeks to determine its possible collateral effects to ensure the safety of use (Idu et al., 2008).

T. scleroxylon is one of the over 30 medicinal plants, indigenous to Nigeria and used in the

treatment of diabetes mellitus especially in some areas in the southern part of Nigeria. So far some toxicity studies on this plant have shown that its use in the treatment of diabetes mellitus is safe (Prohp et al., 2006, 2008; Prohp and Onoagbe, 2009a, b). However, more work is still being carried out for proper classification of *T. scleroxylon* as an anti – diabetic plant with tolerable toxicity limits.

Significant decreases in plasma glucose concentration observed on the 12th, 18th and 28th days of administration of plant extract to alloxan – induced diabetic rabbits were indicative of the anti – diabetic potential of this plant (Table 1). This finding agrees with the documented reports on this plant (Prohp et al., 2006, 2008; Prohp and Onoagbe, 2009a, b). 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).

However, no significant effect was observed on the body weights of the rabbits following exposure to this extract through clean water troughs for 28 days (Table 2). It could be that the extract was well tolerated physiologically and did not cause any form of enlargement of internal organs to affect the weights of experimental animals (Oyewole et al., 2007).

The normal values for plasma urea, creatinine and calcium in rabbits in the ranges of 20.0 – 55.5, 0.5 – 2.6 and 12.0 – 20.0 mg/dl respectively have been reported (Jones, 1975 and http://www.medirabbit.com). The results obtained in this study fall within the normal values for all the plasma components examined (Tables 3 – 5). The use of aqueous extract of *T. scleroxylon* will not lead to renal impairment (Tables 3 and 4) and kidney dysfunction or any of the health risks associated with hypocalcemia (Table 5). One of the most important means of assessing the degree of renal failure is to measure the concentration of urea and creatinine (Davis and Berndt, 1994). A significantly elevated plasma urea concentration above 15mmol/L (blood urea nitrogen, 42mg/dl), usually indicates impaired glomerular function. Measurement of plasma creatinine may occasionally help to resolve any doubt (Carter and Thompson, 1949). Extremely high levels of blood calcium may result in shock, kidney failure and death. Prolonged and moderate hypercalcemia results in the deposit of calcium phosphate crystals in the kidney and eye (Heller, 1999). Severe hypocalcemia is marked by irregular heartbeat, muscle spasm and burning or prickling feelings of the hands, feet, lips and tongue (Curhan et al., 1997).

Conclusion

Aqueous bark extract of *T. scleroxylon* is safe for the treatment of diabetes mellitus as findings so far have not shown that its use would predispose to serious side effects.

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