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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Anti - Diabetic Properties and Toxicological Studies of *Triplochiton scleroxylon* on the Heart Enzymes in Normal and Streptozotocin - induced Diabetic Rabbits

T.P. Prohp[1] and I.O. Onoagbe[2]
[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences,
College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
[2]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria

Abstract: The effects of orally administered aqueous bark extract of *Triplochiton scleroxylon* on the activities of some heart enzymes were examined. Some of the enzymes investigated in the plasma of normal and streptozotocin - induced diabetic rabbits were glutamate oxaloacetate transaminase, creatine kinase and lactate dehydrogenase. Rabbits of New Zealand strains, weighing between 1.45 to 1.95kg were used. Experimental diabetes was induced in the test rabbits by intra - peritoneal injection of streptozotocin at the dose of 70mg/kg body weight. Blood was collected for analyses, intravenously from the large veins at the back of the ears of the rabbits. Glucose concentration decreased significantly ($P < 0.05$) on the 13th day in normal rabbits but in streptozotocin – induced diabetic rabbits significant decreases were observed on the 12th, 24th and 28th days of administration of the extracts. Some of the heart enzymes investigated in normal and streptozotocin - induced diabetic rabbits were not affected significantly ($P > 0.05$) after 13 and 28 days of administration of the extract respectively. On the basis of this research, extract of *Triplochiton scleroxylon* may be useful in the treatment of diabetes mellitus with the added advantage that it may not contain destructive chemical substances capable of damaging the heart.

Key words: Heart enzymes, diabetic rabbits, *Triplochiton scleroxylon*

INTRODUCTION

Diabetes mellitus is one of the oldest diseases of man that affects millions of people all over the world. In its age long existence, it has no known cure (Onoagbe and Esekheigbe, 1999). The use of insulin and other orthodox drugs as panacea had lead to diverse complications some of which are life - threatening. Consequently, plants are being explored as a possible remedy in nearly all countries of the world (Sofowora, 1984; Gill, 1992; Marrif *et al*., 1995; Liaguat *et al*., 1994; Kako *et al*., 1996). *Triplochiton scleroxylon* is one of the over 30 medicinal plants used by Nigerian diabetics to treat their conditions, especially amongst the rural and impoverished urban dwellers (Onoagbe *et al*., 1999). It is of the family, sterculiaceae and is identified by the following common names: Epo arere, obeche (Nigeria), samba (Ivory Coast), ayous (Cameroon), wawa (Ghana) and abachi (Germany, Holland). This plant is widely distributed in tropical West Africa from Guinea to Cameroon along waterways and on abandoned farms in the transition zone between the humid evergreen and semi deciduous forests (Richter and Dallwitz, 2000).

Earlier investigations had shown that the aqueous bark extract of *Triplochiton scleroxylon* did not have significant effects on the red blood cell and associated parameters and white blood cell differentials in alloxan - induced diabetic rabbits (Prohp *et al*., 2006b; Prohp and Onoagbe, 2008). Studies further showed that aqueous extract of this plant did not have significant effects on the liver specific enzymes in normal and streptozotocin - induced diabetic rabbits (Prohp and Onoagbe, 2008).

This work therefore ascertains possible effects of the aqueous bark extract of this plant on the heart of rabbits. This is with the view of understanding some of the side effects that may be associated with the use of this herb as an anti - diabetic antidote.

MATERIALS AND METHODS

Animals: Male and female rabbits of the New Zealand strain, weighing between 1.45 and 1.95kg were used. They were maintained under standard animal house conditions and allowed free access to food (growers mash) and water for a period of 2 weeks to acclimatize to the new environment.

Chemicals: Enzyme kits used were obtained from Randox Laboratories Ltd, United Kingdom. Chloroform was procured from BDH Chemicals Ltd (Poole, Dorset, UK) while streptozotocin was purchased from Sigma Chemicals Company Ltd (St Louis, USA). All other chemicals were of Analar grade and were purchased from standard suppliers.

Medicinal plants: The barks of *Triplochiton scleroxylon* were obtained from medicinal herb dealers at Oyingbo market, Lagos. They were identified by experts in Botany

department of the Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Preparation and administration of aqueous plant extracts: The barks of *Triplochiton scleroxylon* were washed with water, dried and cut into tiny pieces or bits. They were then ground and boiled in distilled water (1gm/10ml) for 3 days. Boiling was for 3, 2 and 1 hour(s) for the first, second and third day respectively. After cooling to room temperature, it was filtered with sintered glass funnel to eliminate debris. The clear extract was transferred to clean jerry cans and stored at - 21°C until used. The test animals were allowed free access to the extracts for the desired period of the experiment. The drinking troughs were replenished immediately they were depleted of extracts (Onoagbe *et al*., 1999).

Administration of streptozotocin: Streptozotocin was dissolved in saline solution. The rabbits (diabetic control and test rabbits) were injected intra - peritoneally with portions (0.5ml) of this solution at a dose of 70mg per kg body weight after about 2 weeks of acclimatization. The use of appropriate doses of streptozotocin allows acute or mild diabetes to be established in experimental animals (Junod *et al*., 1969). Diabetes was confirmed by identifying glucose in the urine of rabbits besides the observed blood glucose level two to three times higher (3 days after) following streptozotocin injection.

Blood collection: Blood was drawn intravenously from the large vein at the back of the ears of rabbits into sample tubes containing heparin and sodium fluoride (final concentration, 5mM), EDTA (for GGT assay) and lithium heparin (for all the other enzyme assays) as described by Randox Laboratories Ltd., United Kingdom. Centrifugation was performed at 800g for 5 minutes to obtain clear plasma for glucose and enzyme assays respectively (Onoagbe *et al*., 1999).

Biochemical analyses: The glucose and enzyme assays were performed in accordance with the procedures described by Randox Laboratories Ltd, U.K.

Experiment A (Hypoglycemic studies): A total of six rabbits (3 controls and 3 tests) were used in this study. Glucose assay was carried out on days 0, 1, 3, 5, 7, 9, 11 and 13.

Experiment B (Toxicological studies)

Enzyme assays: Enzyme (glutamate oxaloacetate transaminase, creatine kinase and lactate dehydrogenase) assays were carried out on days 0, 1, 3, 5, 7, 9, 11 and 13 in normal rabbits. However, in streptozotocin - induced diabetic rabbits, enzyme assays were conducted at 0, 1hr, 3hr, 6hr, 1, 6, 12, 18, 24 and 28 day (s) of experiment.

Experiment C (Anti - diabetic studies): A total of nine rabbits (3 non - diabetic controls, 3 diabetic controls and 3 treated diabetic rabbits) were used in this study. Glucose assay was conducted at 0, 1hr, 3hr, 6hr, 1, 6, 12, 18, 24 and 28 day (s) of experiment.

Blood glucose assay: Glucose was determined by the glucose oxidase method according to procedure described by Randox Laboratory Ltd, United Kingdom.

Statistical analysis: Results were expressed as mean±S.E.M. Data were analyzed with t - test for comparison between the two groups. The significance level was set at $P<0.05$.

RESULTS

Results have been presented in Tables 1-8. Plasma glucose concentration reduced significantly ($P < 0.05$) on the 13th day of administration of the extract to normal rabbits (Table. 4). In treated streptozotocin induced diabetic rabbits, significant decreases ($P<0.05$) in glucose concentration were obtained on the 12th, 24th and 28th days of administration of the medicinal extract (Table 5). However, effects on the activities of some heart enzymes in the plasma viz: Glutamate oxaloacetate transaminase, creatine kinase and lactate dehydrogenase studied were not significant ($P>0.05$) in normal (Tables 1-3) and streptozotocin - induced diabetic rabbits (Tables 6 - 8) when compared with the normal and diabetic controls respectively.

DISCUSSION

In most developing nations studies on medicinal plants are very popular in the chemical and biological sciences because of the availability of these plants most of which have not been identified and fully explored for proper classification (Watts *et al*., 1997).

In contemporary rural Africa, there is no doubt about the efficacy of herbal medicine as many Africans, both in rural and in urban places rely on the use of herbal medicine when they are ill. 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. China harnesses the legacy of traditional medicine to provide adequate and sustainable health care coverage for her vast urban and rural population (Aregbeyen, 1983; Bodeker, 1994). In United States of America, for example, medicinal plants constitute about 25% of all newly refined prescriptions dispensed from community pharmacies (Trease and Evans, 1989). It is clear from studies that the uneven distribution of health personnel between

Table 1: Mean plasma glutamate oxaloacetate transaminase activities (U/l) of non - diabetic rabbits administered aqueous bark extract of Triplochiton scleroxylon

Days	Control	*Triplochiton scleroxylon*
0	67.02±4.48	59.08±4.10
1	70.10±8.10	58.40±9.30
3	60.00±6.20	63.70±2.90
5	75.00±5.00	79.20±5.00
7	95.70±6.70	63.10±3.40
9	85.05±5.10	70.20±4.10
11	90.00±0.01	70.00±4.10
13	85.30±5.10	95.30±5.00

Values are mean±S. E. M of 3 separate determinations from 6 rabbits. Values not significantly different from control (P > 0.05).

Table 2: Mean plasma creatine kinase activities (U/l) of non - diabetic rabbits administered aqueous bark extract of Triplochiton scleroxylon

Days	Control	*Triplochiton scleroxylon*
0	91.60±9.10	153.60±27.10
1	91.30±11.50	95.60±11.18
3	127.20±19.90	183.00±26.30
5	172.00±20.70	194.30±18.90
7	170.00±15.80	194.30±18.90
9	64.00±8.00	89.00±13.90
11	62.00±18.00	97.00±15.30
13	113.00±12.10	98.60±19.80

Values are mean±S.E.M of 3 separate determinations from 6 rabbits. Values not significantly different from control (P > 0.05)

Table 3: Mean plasma lactate dehydrogenase activities (U/l) of non – diabetic rabbits administered aqueous bark extract of Triplochiton scleroxylon

Days	Control	*Triplochiton scleroxylon*
0	144.00±14.00	172.00±13.80
1	195.00±15.30	172.00±11.20
3	110.00±14.00	124.00±12.00
5	162.00±12.00	128.00±16.90
7	93.00±14.01	101.30±19.70
9	163.00±10.10	132.00±14.70
11	148.00±15.70	159.00±10.20
13	101.43±13.80	155.30±18.60

Values are mean±S. E. M of 3 separate determinations from 6 rabbits. Values not significantly different from control (P > 0.05).

Table 4: Mean plasma glucose concentration (mg/dl) of non – diabetic rabbits administered aqueous bark extract of Triplochiton scleroxylon

Days	Control	*Triplochiton scleroxylon*
0	56.60±9.80	42.00±8.50
1	62.60±10.20	72.00±18.20
3	68.30±1.30	84.60±7.80
5	85.00±14.00	93.60±17.00
7	64.00±2.40	76.60±2.40
9	70.30±6.50	60.60±6.50
11	56.30±9.70	45.00±16.00
13	91.60±8.20	42.00±9.80*

Values are mean±S.E.M of 3 separate determinations from 6 rabbits. *Significantly different from control (P < 0.05).

Table 5: Mean plasma glucose concentration (mg/100ml) of Triplochiton scleroxylon treated streptozotocin - induced diabetic rabbits

Days	Non - diabetic control	Diabetic control	Triplochiton scleroxylon
0	73.03±9.76	353.58±10.71	360.00±15.00
1hr	79.38±18.80	353.58±10.71	353.58±10.78
3hr	79.40±4.21	361.27±10.00	370.41±9.50
6hr	77.76±2.23	360.00±15.00	323.07±3.57
1	79.38±18.80	345.00±52.74	296.43±3.57
6	83.33±24.60	371.40±0.00	219.09±29.29
12	98.55±10.10	392.85±37.68	206.67±5.91*
18	94.20±5.42	470.00±113.10	179.10±27.60
24	88.87±13.90	512.49±24.92	168.81±12.60*
28	83.33±24.60	561.12±9.69	158.37±16.65*

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

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).

Over 400 medicinal plants have so far been investigated globally and about 30 of them are indigenous to Nigeria and commonly used by Nigerian diabetics to treat their ailments (Watt and Breyer - Branwijk, 1962; Satyavati *et al*., 1987; Bailey and Day, 1989; Onoagbe *et al*., 1999). *Triplochiton scleroxylon* belongs to the family of tropical plants (Russel *et al*., 1997) and is commonly used by some Nigerian diabetics as panacea for their conditions.

In this study, blood glucose concentration ranged between 56 - 98 mg/100ml in normal rabbits (Table 4). This range agrees with values reported by Onoagbe *et al*. (1999) Okpala *et al*. (2005) and Prohp *et al*. (2006a,b). However, Bispang (1963) and Mitruka and Rawnley (1977) reported 102 - 149mg/100ml and 77 - 140mg/100ml respectively. Studies also show that rabbits with acute or mild diabetes have blood glucose concentrations in the range of 350 to 500mg/dl and values above 200mg/dl (Jennard, 2000). Experimental values (Table 5) were indicative of the diabetic status of streptozotocin - induced diabetic rabbits.

Glucose concentration decreased significantly (P<0.05) on the 13th day of administration of the aqueous extract of this herb to the normal rabbits (Table 4). However, in streptozotocin - induced diabetic rabbits, significant decreases (P<0.05) in glucose concentrations were recorded on the 12th, 24th and 28th days of experiment (Table 5). The hypoglycemic and anti - diabetic effects of this extract could be attributable to the presence of some phytochemicals viz. alkaloids, flavonoids, triterpenoids, glycosides and saponins which are common in plants with known hypoglycemic effect (Okpala *et al*., 2005).

The activities of some of the heart specific enzymes studied here viz: Glutamate oxaloacetate transaminase, creatine kinase and lactate dehydrogenase were not significantly increased (P>0.05) following the

Table 6: Mean plasma glutamate oxaloacetate transaminase activities (U/l) of Triplochiton scleroxylon treated streptozotocin - induced diabetic rabbits

Days	Non - diabetic control	Diabetic control	*Triplochiton scleroxylon*
0	37.67±5.91	46.33±4.34	26.67±7.64
1hr	37.67±5.91	17.83±9.20	26.00±4.86
3hr	36.91±10.22	15.75±7.40	21.20±3.90
6hr	21.67±10.22	30.83±1.45	19.50±6.50
1	31.83±1.20	25.00±1.00	30.33±9.21
6	22.33±5.25	22.00±7.50	31.33±5.34
12	18.50±2.93	14.33±5.17	28.00±8.51
18	13.67±0.67	14.33±5.17	19.17±9.67
24	28.33±1.17	28.33±1.17	34.00±2.76
28	13.00±0.00	13.00±0.00	10.00±1.73

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

Table 7: Mean plasma creatine kinase activities (U/l) of Triplochiton scleroxylon treated streptozotocin – induced diabetic rabbits

Days	Non - diabetic control	Diabetic control	*Triplochiton scleroxylon*
0	246.60±10.81	263.33±19.84	297.13±14.30
1hr	102.54±10.99	234.11±15.20	248.25±16.15
3hr	189.21±14.02	271.24±10.79	231.48±4.99
6hr	172.86±16.49	288.56±14.50	250.95±10.10
1	159.20±14.65	181.43±16.49	237.46±23.88
6	56.67±9.19	113.33±24.74	64.76±12.59
12	62.06±9.91	107.93±21.99	107.94±20.98
18	64.76±13.81	35.08±8.75	37.78±8.98
24	48.57±12.6	59.37±12.24	89.05±11.22
28	126.82±22.66	105.25±19.48	94.44±16.36

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

Table 8: Mean plasma lactate dehydrogenase activities (U/l) of Triplochiton scleroxylon treated streptozotocin - induced diabetic rabbits

Days	Non - diabetic control	Diabetic control	*Triplochiton scleroxylon*
0	153.81±25.63	156.18±27.25	131.58±25.93
1hr	246.98±21.83	156.50±15.40	161.58±24.27
3hr	192.48±21.20	140.67±17.20	162.40±20.51
6hr	151.92±17.99	137.62±24.32	156.34±16.32
1	143.01±15.04	133.33±18.63	167.30±22.59
6	107.93±15.04	161.90±18.05	180.63±12.54
12	91.74±10.61	94.44±9.75	70.16±10.99
18	64.76±4.76	24.29±8.00	16.19±5.00
24	21.59±9.62	45.87±8.75	88.25±10.24
28	53.97±6.72	33.49±2.75	62.06±7.28

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

administration of the aqueous bark extract of *Triplochiton scleroxylon* to normal (Tables 1 - 3) and streptozotocin - induced diabetic (Tables 6 - 8) rabbits for 13 and 28 days respectively. That could be due to the fact that the aqueous extract of this herb may not contain harmful or lethal chemical agents capable of causing adverse effects on the hearts of rabbits leading to the leakage of some of these heart enzyme markers to the plasma.

Elevated levels of diagnostic enzymes in plasma are reflections of their reduced clearance or increased proliferation of cells, increased rate of cell turnover, increased cell damage or increased rate of enzyme synthesis (induction of microsomal enzymes by certain drugs) (Raju and Mandala, 2005). Activities of creatine kinase and lactate dehydrogenase obtained in this study compared favourably with normal values as reported by Raju and Mandala (2005) in humans. However, the activities of glutamate oxaloacetate transaminase obtained was higher than the range of between 8 - 20 U/l reported by Raju and Mandala (2005). Values earlier reported by Prohp *et al*. (2006a) in rabbits agreed with the experimental results.

Aqueous bark extracts of *Triplochiton scleroxylon* have proven hypoglycemic and anti - diabetic properties and may not contain chemical substances likely to cause any damages to the heart.

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