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

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Abstract: This study investigates the activities of liver function enzymes viz. Alkaline phosphatase (ALP), glutamate pyruvate transaminase (GPT), gamma glutamyl transferase (GGT) and glutamate oxaloacetate transaminase (GOT), following the administration of *Triplochiton scleroxylon* to normal and streptozotocin - induced diabetic rabbits. Rabbits (New Zealand strains) used weighed between 1.45 to 1.95kg. Experimental diabetes was induced in rabbits by intra - peritoneal injection of streptozotocin at the dose of 70mg/kg body weight. Blood for analyses was collected intravenously from the large veins at the back of the ears of the rabbits. Analysis of results showed that glucose concentration decreased significantly ($P < 0.05$) on the 13th day in normal rabbits whilst in streptozotocin - induced diabetic rabbits significant decreases were observed on the 12th, 24th and 28th days following the administration of the extracts. However, the aqueous bark extract of this herb did not have significant effects ($P > 0.05$) on the activities of liver function enzymes investigated in normal and streptozotocin - induced diabetic rabbits. The aqueous bark extract of *Triplochiton scleroxylon* may therefore be safe for and useful in the treatment of diabetes mellitus and may not contain chemicals capable of damaging the liver and interfering adversely with its central roles in metabolism.

Key words: Anti-diabetic, toxicology, triplochiton, streptozotocin and liver enzymes

INTRODUCTION

Diabetes mellitus is one of the oldest diseases of man. It is the most common metabolic disorder that affects millions of people all over the world (Onoagbe *et al.*, 1999; Onoagbe and Esekheigbe, 1999). In its age long existence, diabetes mellitus has no known cure (Onoagbe and Esekheigbe, 1999). Plants have been exploited as panacea for diabetes mellitus for a very long time due to life - threatening complications and other problems associated with the use of insulin and other orthodox drugs (Sofowora, 1984; Gill, 1992; Marrif *et al.*, 1995; Liaquat *et al.*, 1995 and 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). *Triplochiton scleroxylon* belongs to 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). It is widely distributed in tropical West Africa from Guinea to Cameroon mainly along waterways and on abandoned farms in the transition zone between the humid evergreen and semi deciduous forests (Richter and Dallwitz, 2000). This plant belongs to the family of tropical medicinal plants (Russel *et al.*, 1997).

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 (red blood cell counts, packed cell volume, haemoglobin concentration, mean cell haemoglobin, mean cell volume and red cell distribution width) and white blood cell differentials (neutrophils, lymphocytes, basophils and eosinophils) in alloxan - induced diabetic rabbits (Prohp *et al.*, 2006b; Prohp *et al.*, 2008).

The objective of this work is to ascertain any possible effects of the aqueous bark extract of this plant on the livers of rabbits considering the altruism of the liver in cellular metabolism. 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: Rabbits (male and female) of the same strain (New Zealand), 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: All the enzyme kits used were obtained from Randox Laboratories Ltd, United Kingdom. Chloroform

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

Days	Control	*Triplochiton scleroxylon*
0	13.80±0.01	13.80±0.03
1	13.80±0.02	13.80±0.01
3	20.70±4.80	13.80±0.01
5	20.70±4.88	18.40±3.76
7	13.80±0.02	13.80±0.01
9	13.80±0.04	13.80±0.01
11	20.70±4.88	13.80±0.01
13	13.80±0.02	18.40±3.76

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 glutamate pyruvate transaminase activities (U/l) of non - diabetic rabbits administered aqueous bark extract of Triplochiton scleroxylon

Days	Control	*Triplochiton scleroxylon*
0	60.12±10.10	68.14±15.24
1	55.00±5.00	46.70±13.20
3	61.03±5.00	56.70±11.90
5	75.00±5.00	54.70±10.10
7	69.02±6.20	68.70±3.70
9	64.40±5.20	50.00±10.00
11	71.01±3.10	58.00±15.00
13	55.00±5.10	50.00±8.10

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

was obtained 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: Aqueous bark extract of *Triplochiton scleroxylon* was prepared and administered to experimental animals (rabbits) according to the procedure of Onoagbe *et al*. (1999), as reported by Prohp *et al*. (2006 b; 2008).

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 (Jennard, 2000).

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).

Hypoglycemic studies: A total of six normal 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 in accordance with the procedures described by Randox Laboratories Ltd, United Kingdom.

Toxicological studies

Enzyme assays: Plasma enzyme (alkaline phosphatase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and gamma glutamyltransferase) assays were carried out on days 0, 1, 3, 5, 7, 9, 11 and 13 in normal rabbits. However, in streptozotocin - induced diabetic rabbits, plasma enzyme assays were conducted at 0, 1hr, 3hr, 6hr, 1, 6, 12, 18, 24 and 28 day (s) of experiment. The procedure used was as described by Randox Laboratories Ltd., United Kingdom.

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. Analytical procedure was as described by Randox Laboratories 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 Figs. 1 - 10 and Tables 1 - 10. Plasma glucose concentration reduced significantly ($P < 0.05$) on the 13th day of administration of the extract to normal rabbits (Fig. 5). 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 (Fig. 10). Aqueous bark extract of this plant has hypoglycemic and anti - diabetic effects. However, effects on the activities

Table 3: Mean plasma λ - glutamyltransferase activities (U/l) of non - diabetic rabbits administered aqueous bark extract of Triplochiton scleroxylon

Days	Control	*Triplochiton scleroxylon*
0	8.04±0.88	8.98±0.23
1	7.78±1.11	9.26±1.33
3	7.80±1.10	8.53±0.98
5	7.70±1.00	7.33±0.63
7	8.07±0.88	8.53±0.37
9	8.13±1.43	8.87±0.66
11	9.63±0.37	8.13±0.77
13	9.90±1.82	9.60±0.94

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 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 5: 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).

of some liver enzymes in the plasma viz: Alkaline phosphatase, glutamate pyruvate transaminase, gamma glutamyl transferase and glutamate oxaloacetate transaminase studied were not significant (P > 0.05) in normal and streptozotocin - induced diabetic rabbits when compared with the normal and diabetic controls respectively (Figs. 1 - 4, 6 - 9).

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

Days	Non-diabetic control	Diabetic control	*Triplochiton scleroxylon*
0	15.54±2.78	11.96±2.61	10.73±2.38
1hr	7.36±1.36	5.52±0.52	14.96±2.87
3hr	7.17±1.21	12.48±1.80	7.32±1.78
6hr	8.89±1.02	15.64±0.92	8.52±2.52
1	17.48±0.92	10.00±0.02	7.97±0.50
6	13.80±2.76	31.52±5.38	20.24±5.97
12	10.12±1.13	14.90±2.46	13.49±1.07
18	10.92±0.02	11.84±0.92	14.11±1.69
24	11.97±1.50	16.44±2.13	13.49±1.07
28	14.41±2.39	21.74±5.04	11.04±2.50

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 glutamate pyruvate transaminase activities (U/l) of Triplochiton scleroxylon treated streptozotocin - induced diabetic rabbits

Days	Non-diabetic control	Diabetic control	*Triplochiton scleroxylon*
0	33.33±2.33	35.33±1.80	44.83±6.86
1hr	22.67±5.46	34.0±3.47	38.00±4.73
3hr	14.75±7.29	29.27±7.21	31.24±8.71
6hr	19.5±4.29	23.33±5.16	27.70±4.23
1	27.67±3.45	23.32±2.33	22.83±9.87
6	24.67±4.34	28.17±2.80	25.17±5.35
12	25.5±4.73	25.5±4.00	37.33±7.36
18	24.17±4.21	27.67±3.18	43.83±3.94
24	10.33±3.72	17.17±1.83	23.17±1.49
28	16.33±0.66	15.00±0.00	13.50±0.00

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 λ - glutamyltransferase activities (U/l) of Triplochiton scleroxylon treated streptozotocin - induced diabetic rabbits

Days	Non-diabetic control	Diabetic control	*Triplochiton scleroxylon*
0	16.95±2.67	11.10±4.49	15.84±4.81
1hr	9.25±4.19	6.95±1.53	9.56±1.14
3hr	5.71±0.07	8.80±1.11	9.29±2.17
6hr	5.55±1.98	9.28±2.08	12.91±4.78
1	18.49±6.23	16.95±7.10	14.44±1.12
6	15.16±1.15	19.98±3.12	14.90±7.41
12	7.37±3.30	9.37±4.33	8.15±0.68
18	8.53±0.12	12.12±3.70	11.96±2.20
24	13.33±6.71	10.60±3.70	11.60±3.70
28	12.94±9.61	14.82±7.07	9.22±1.36

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

DISCUSSION

Medicinal plants are now increasingly being explored in nearly all the countries of the world as the panacea for different diseases of humanity. 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 newly refined prescriptions dispensed from community pharmacies (Trease and Evans, 1989). It is

Table 9: 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 10: 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.

clear from studies 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 plants most of which have not been identified and fully explored for proper classification (Watts *et al.*, 1997).

Diabetes mellitus is one of those diseases without credible cure and whose existence could be dated to antiquity (Dalziel, 1956; Sofowora, 1984; Gill, 1992). The uses of oral hypoglycemic drugs and insulin injection over the years have led to both chronic and acute complications with life - threatening side effects.

Out of over 400 medicinal plants so far investigated globally about 30 are indigenous to Nigeria and commonly used by Nigerian diabetics to treat their ailments (Watt and Breyer - Brandwijk, 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 Nigerians as panacea for their conditions.

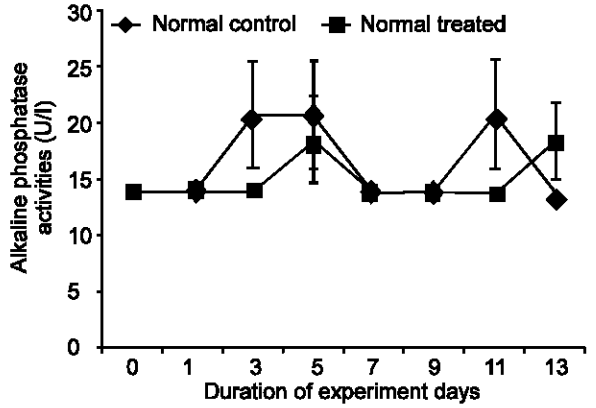

Fig. 1: Mean plasma alkaline phosphatase activities (U/l) of non - diabetic rabbits administered aqueous bark extract of *Triplochiton scleroxylon*

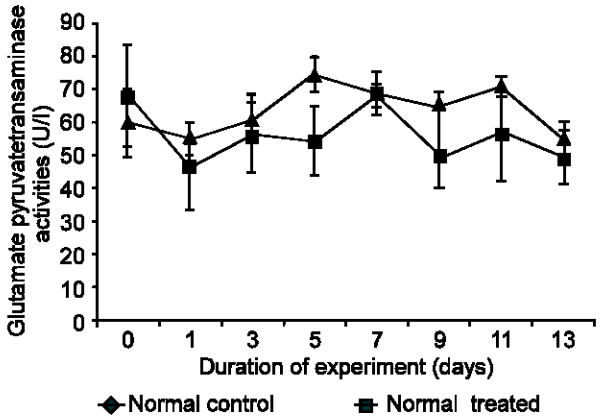

Fig. 2: Mean plasma glutamate pyruvate transaminase activities (U/l) of non - diabetic rabbits administered aqueous bark extract of *Triplochiton scleroxylon*

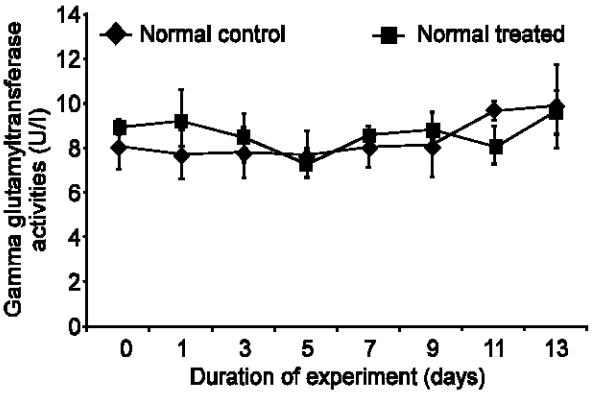

Fig. 3: Mean plasma gamma glutamyltransferase activities (U/l) of non - diabetic rabbits administered aqueous bark extract *Triplochiton scleroxylon*

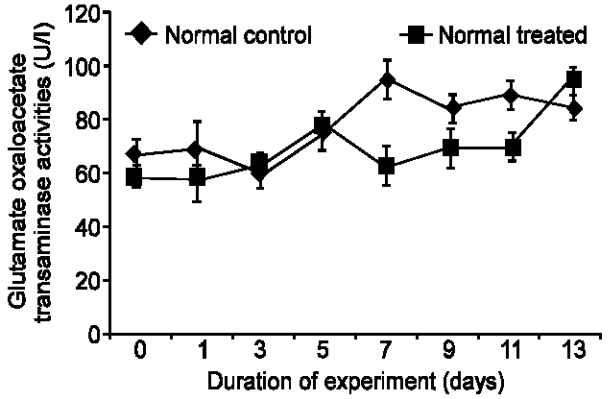

Fig. 4: Mean plasma glutamate oxaloacetate transaminase activities (U/l) of non - diabetic rabbits administered aqueous bark extract of *Triplochiton sceroxylon*

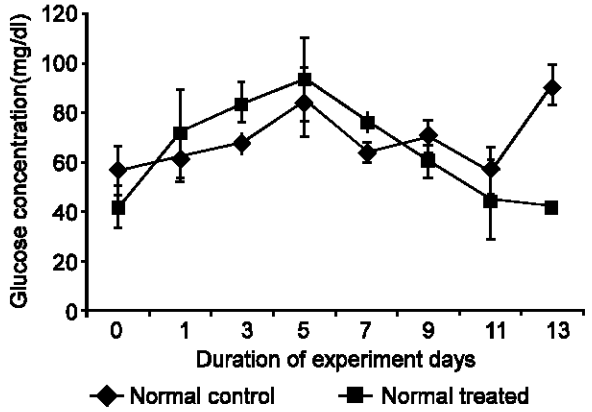

Fig. 5: Mean plasma glucose concentration (mg/dl) of non - diabetic rabbits administered aqueous bark extract of *Triplochiton scleroxylon*

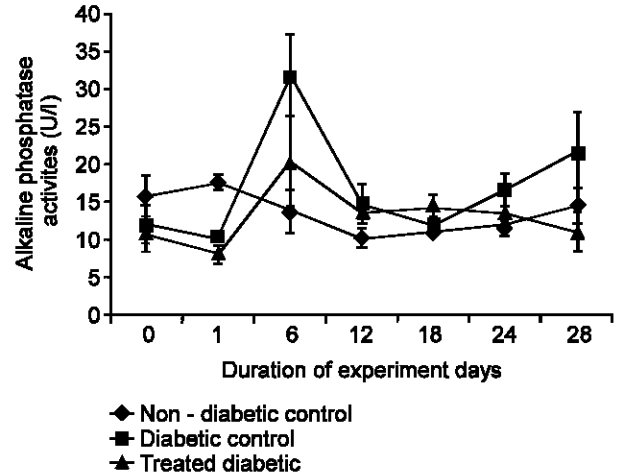

Fig. 6: Mean plasma alkaline phosphatase activities (U/l) of *Triplochiton scleroxylon* treated streptozotocin - induced diabetic rabbits.

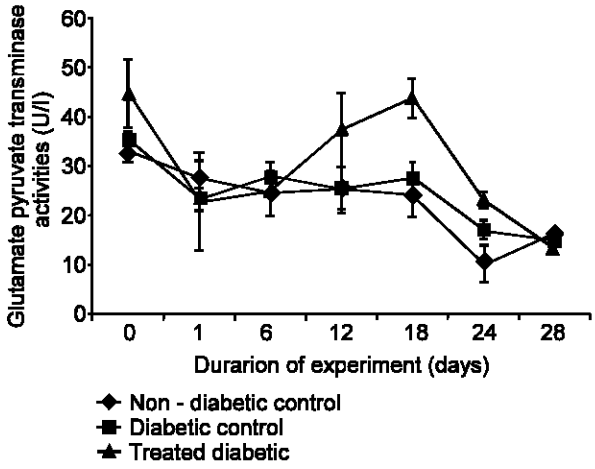

Fig. 7: Mean plasma glutamate pyruvate transaminase activities (U/l) of *Triplochiton scleroxylon* treated streptozotocin - induced diabetic rabbits

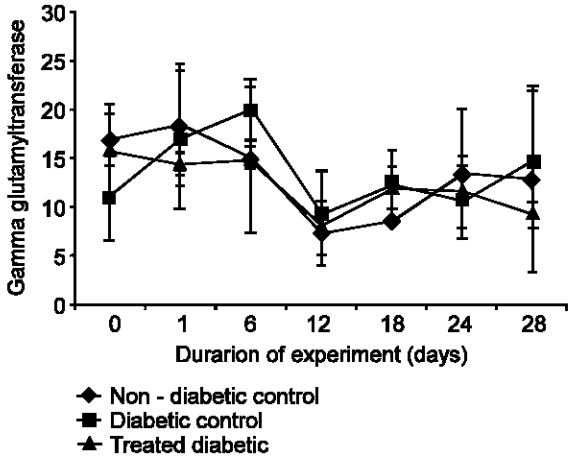

Fig. 8: Mean plasma gamma glutamyltransferase activities (U/l) of *Triplochiton scleroxylon* treated streptozotocin - induced diabetic rabbits

In this study, blood glucose concentration in normal rabbits ranged from 56 - 98 mg/100ml (Fig. 5). 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. 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 (Fig. 10) 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 (Fig. 5). However, in

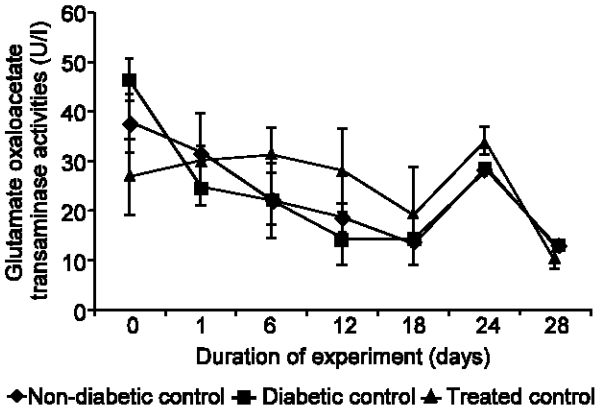

Fig. 9: Mean plasma glutamate oxaloacetate transaminase activities (U/l) of *Triplochiton scleroxylon* treated streptozotocin - induced diabetic rabbits

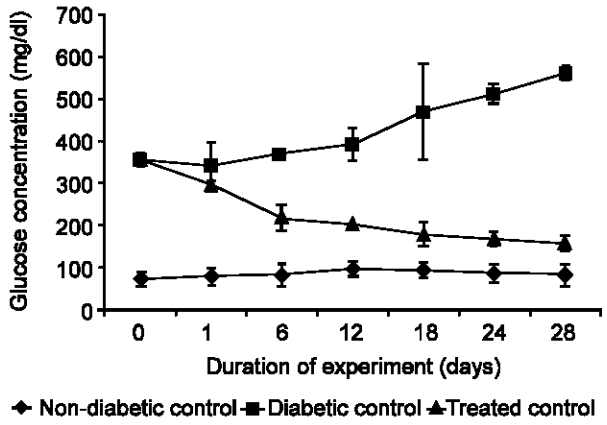

Fig. 10: Mean plasma glucose concentration (mg/dl) of *Triplochiton scleroxylon* treated streptozotocin - induced diabetic rabbits

streptozotocin - induced diabetic rabbits, significant decreases ($P < 0.05$) in glucose concentrations were recorded on the 12th, 24th and 28th days of experiment (Fig. 10). 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).

This study also showed that the aqueous extract of this plant did not have significant effects ($P > 0.05$) on the activities of some liver specific enzymes in the plasma viz. alkaline phosphatase, glutamate pyruvate transaminase, glutamate oxaloacetate transaminase and γ - glutamyltransferase investigated. This could be due to the fact that the aqueous bark extract of *Triplochiton scleroxylon* may not contain toxic chemical substances capable of causing severe lesions to the livers of experimental rabbits. Increased levels of alkaline phosphatase, glutamate pyruvate transaminase and gamma glutamyltransferase in the plasma are however, mainly associated with liver diseases. Also invitro hemolysis or delayed separation of plasma from whole blood could cause elevated plasma glutamate pyruvate transaminase activities (Kaplan *et al.*, 1997; Nelson and Cox, 2001).

Biochemical, histological and histochemical studies are currently going on, for proper classification of *Triplochiton scleroxylon* as an anti - diabetic herb. Aqueous extract of this herb may therefore not contain deleterious chemicals capable of any form of liver damage.

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