EVALUATION OF THE ANTI-DIABETIC EFFECT OF AQUEOUS LEAF EXTRACT OF *TAPINANTHUS BUTUNGII* IN MALE SPRAGUE-DAWLEY RATS

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SUMMARY: The prevalence of diabetes mellitus is between 2-7% of the population, increasing with age to between 10-14% of the patients aged above 40 years. Despite significant achievements in treatment modalities and preventive measures, the prevalence of diabetes has risen exponentially in the last decade. There is therefore a continued need for new and more effective therapies. Our aim was to evaluate the hypoglycemic, anti-hyperglycemic and anti-diabetic effects of aqueous extract of fresh green leaves of Tapinanthus butungii in rats.

Young adult, male Sprague-Dawley rats weighing 180-200 g were used. Diabetes mellitus was induced in the group of diabetic test rats by intraperitoneal injections of alloxan (150 mg/kg). Hyperglycemic state was induced by administration of subcutaneous injections of 50% Dextrose in water (5 g/kg). Single doses of aqueous leaf extract of Tapinanthus butungii (200, 300 or 400 mg/kg p.o.) were administered to normoglycemic, hyperglycemic and diabetic rats. The hypoglycemic, anti-hyperglycemic and anti-diabetic effects of these single doses were compared with those of glibenclamide (10 mg/kg), chlorpropamide (250 mg/kg), insulin lente (0.1 I.U./kg) and distilled water (2 ml/kg). Blood glucose levels were estimated before treatment, 0h, 1h, 2h, 4h, 8h, 10h and 12h after administration of extract.

Aqueous leaf extract of Tapinanthus butungii produced significant dose-dependent reductions ($p < 0.05$-0.001) in blood glucose concentrations of normoglycemic, hyperglycemic and diabetic rats comparable to glibenclamide, chlorpropamide and insulin.

Our results suggest that the leaves of Tapinanthus butungii have strong and remarkable anti-diabetic properties. Further studies are, however, required on the characterization of the constituents of the leaf extract of Tapinanthus butungii.

Keywords: Tapinanthus butungii, blood glucose, diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia, due to absolute or relative deficiency or diminished effectiveness of circulating insulin. It is the most common of the serious metabolic diseases. *Diabetes mellitus* has been recognized as a clinical syndrome since ancient times, and remains a crippling global health problem today. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The urban population in developing countries is projected to double between 2000 and 2030 (1).

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Despite major inroads into understanding the pathophysiology and treatment of this insidious disease, it has continued to be a major health problem worldwide. The possibility of its management by the oral administration of hypoglycemic agents has stimulated great interest in recent years. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of *diabetes mellitus*, there is increasing demand by patients to use the herbal preparations with anti-diabetic activity. Current therapies seem to be insufficient to prevent diabetic complications, with a two- to four-fold likelihood for developing cardiovascular events (2). *Diabetes mellitus* is a major cause of morbidity (such as blindness, kidney failure, lower-extremity amputation, and cardiovascular disease) and premature mortality (3). Type 2 *diabetes mellitus*, is a serious, costly disease affecting approximately 8 percent of adults in the United States (4). Present modality of treatment only prevents some of its devastating complications (5, 6) and does not eliminate all the adverse consequences. There is also a worldwide increase in the incidence of Type I diabetes and it will be 40% higher in 2010 than in 1998 (7). Despite the large armamentarium presently available, the progressive deterioration of diabetes control is such that treatment is still insufficient, with the majority of type 2 diabetes patients eventually requiring insulin therapy to achieve targeted glycemic levels (8), and an estimated 75% dying of diabetes-related complications from cardiovascular disease (9). Because of these limitations, there is a continuous need for development of novel health promotion strategies and therapeutic modalities. The current shift to the use of herbal preparations may therefore be due to presumed effectiveness, relatively low cost, presumed less side effects and low toxicity even though the biologically active constituents are unknown most often. While physicians advocate aggressive use of drugs to tighten glucose control and attenuate cardiovascular disease risk factors, many patients are more inclined toward use of alternative therapies that include diet, food supplements and herbal medicine. The use of herbs has more than tripled over the last 10 years (10), and a whole new industry referred to as 'nutraceuticals' has evolved. The use of dietary supplements is widespread among people motivated by general health concerns. Estimates of use range from 40 to 68 percent for the U.S. population (11). Little scientific evidence exists to support the numerous herbs used to improve diabetes-related metabolic disorders (12). For a long time diabetics have been treated orally with several medicinal plants or their extracts based on folk medicine (13). Based on the World Health Organization recommendations on *diabetes mellitus* (14), investigations of hypoglycemic agents of plant origin used in traditional medicine are important. The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth and for many years, the search for antidiabetic products will continue to focus on plants and other natural resources. Investigators have consistently found that several plant products showed unique hypoglycemic activities in diabetic animal models (15). West Africa has several thousands of such plants (16). *Tapinanthus butungii* is a plant parasitizing on *Albizia glaberrima.* It belongs to the family Loranthaceae commonly referred to as mistletoe. A wide range of biological activity, including causing hypoglycemia, has been ascribed to this group of plants especially in folkloric medicine.

In this present study, the effects of aqueous leaf extract of *Tapinanthus butungii* on blood glucose levels were evaluated in normoglycemic, hyperglycemic and alloxan-induced diabetic male Sprague-Dawley rats and compared with those of glibenclamide, chlorpropamide and human insulin lente with a view to providing a pharmacological rationale for the folkloric use of fresh green leaves of *Tapinanthus butungii* by the people of Western Nigeria for the treatment of *diabetes mellitus*.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Tapinanthus butungii* were obtained from the botanical garden of the University of Lagos. Identification and authentication of the leaves were carried out at the Forestry Research Institute of Nigeria, Ibadan where voucher specimen was deposited (FHI 10636). Fresh leaves of *Tapinanthus butungii* were air-dried and reduced to coarse powder. The powdered plant material obtained (520 g), was subjected to aqueous extraction in the Pharmacognosy Department of the Faculty of Pharmacy, University of Lagos. Briefly, the powdery material was placed in distilled water and allowed to boil, simmering for one hour. The extract was evaporated under reduced pressure at 40°C until all the solvent had been removed, to give an extract sample with a yield of 18.2%. The powder obtained (94.62 g, 18.20% yield) was stored at 4°C before use and was prepared in distilled water for pharmacological studies. The animals treated with *Tapinanthus butungii* were either given 200, 300 or 400 mg/kg body weight of the leaf extract.

Animal material

One hundred and ninety-two adult male Sprague-Dawley rats weighing 180-220g were used for the experiments. They were procured from the Animal House of the College of Medicine, University of Lagos. They were allowed to acclimatise and maintained under standard photoperiodic condition in the Rat Room of the Department of Anatomy for two weeks. They were allowed unrestricted access to rat chow purchased from Pfizer Mill and pipe-born water in the Anatomy Department. The rats were weighed, and randomly divided into three main groups (G1-3) of 64 rats each. The animals in the 3 main groups were subdivided into 8 subgroups (G1a, G1b, G1c, G1d, G1e, G1f, G1g, G1h, G2a…G3h), of 8 rats each. G1 were normoglycemic, G2, hyperglycemic and G3, diabetic rats. Subgroups a - h were: (a) control rats treated with intraperitoneal injections of distilled water; (b) control rats treated with distilled water via gastric intubation; (c) rats treated with 200 mg/kg body weight of aqueous leaf extract of *Tapinanthus butungii*; (d) rats treated with 300 mg/kg body weight of aqueous leaf extract of *Tapinanthus butungii*; (e) rats treated with 400 mg/kg body weight of aqueous leaf extract of *Tapinanthus*; (f) rats administered 10 mg/kg of glibenclamide; (g) rats given 250 mg/kg of chlorpropamide; and (h) those given 0.1 I.U./kg body weight of human insulin lente. The extract, glibenclamide and chlorpropamide were administered by gastric intubation, while insulin was given subcutaneously. All animals were observed for clinical signs of drug toxicity (such as tremors, weakness, lethargy, refusal of feeds, weight loss, hair-loss, coma and death) throughout the duration of the experiment and four weeks thereafter. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (17) and were approved by the Departmental Committee on the Use and Care of Animals.

Induction of experimental diabetes

Diabetes was induced (in the group of diabetic rats) with alloxan. 2 g of crystalline powdered alloxan purchased from NAFCO Nigeria Limited was taken and dissolved in 50 mls of distilled water to yield a concentration of 40 mg/ml. 150 mg/kg body weight of alloxan was administered intraperitoneally (18) to 64 of the animals, after an overnight fast (access to only water) of 12 hours to make them more susceptible to developing diabetes (19, 20). Only rats with serum glucose levels greater than 400 mg/dl were used in experiments.

Induction of hyperglycemic state

Following a pilot study, we designed the following protocol. Rats were injected 5 g/kg of 50% dextrose in water subcutaneously. The hyperglycemic state was assessed in dextrose-treated rats by measuring non-fasting blood concentration of glucose 30 minutes post-dextrose injection. Only rats with serum glucose levels greater than 110 mg/dl were used in experiments.

Experimental procedure

All animals were maintained under the same laboratory conditions of temperature (ambient temperature maintained between 26-28°C), humidity and light (L:D; 12:12) and were allowed free access to food (rat chow from Pfizer, Nigeria) and water. The alloxan-treated rats, also allowed unrestricted access to water and food, were left undisturbed for 48 hours, during which time diabetes developed and reached a steady state in the animals. All the rats used in this study were fasted for a period of 12 hours (but still allowed free access to drinking water) before they were treated with either distilled water, the plant extract, glibenclamide, chlorpropamide or human insulin lente. Immediately before and 0, 1, 2, 4, 6, 8, 10 and 12 hours after acute administrations of the test compounds, blood samples were taken from the tail vein of each animal for blood glucose analysis in the diabetic rats. Blood glucose level was assayed by allowing a single drop of fresh whole blood from the vein to drop on the strip provided with the glucose monitor. The blood glucose monitor used was One Touch Basic made by Lifescan (Johnson and Johnson Company) and the results were read off on the meter 45 seconds after application of samples to the strips. The technical performance of the glucometer used was evaluated by comparison with standard laboratory method of blood glucose measurement (spectrophotometer) at the beginning, midway and at the end of the experiment as previously described by Ajala *et al*., (21).

In order to minimize the effects of circadian rhythm on our results, the experiment was structured in such a way that the serial blood glucose estimation of half of the rats were commenced at 8 a.m. and subsequently at 9 a.m., 11 a.m., 1 p.m., 3 p.m., 5 p.m. and 7 p.m., while those of the other half were commenced at 8 p.m. and subsequently at 9 p.m., 11 p.m., 1 a.m., 3 a.m. and 5 a.m.. The serial blood glucose levels taken were the means of these two divisions.

Data analysis

Results were expressed as means ± standard deviations (SD) and subjected to statistical analysis using one-way analysis of variance (ANOVA) and the Scheffe's post-hoc test. The significance level considered was $p < 0.05$.

RESULTS

Activity in normoglycemic rats

The hypoglycemic effect of the extract was observed within 4 hours after oral administration (Table 1). The percentage of maximum reduction in blood glucose concentrations caused by the aqueous leaf extract of *Tapinanthus butungii* at 200, 300 and 400 mg/kg body weight were 11.46, 13.42 and 17.75%, respectively and these values are statistically ($p < 0.01 - 0.001$) different from those of the controls.

Table 1: Effects of aqueous leaf extract of *Tapinanthus butungii* (200, 300 or 400 mg/kg *per oral*), glibenclamide (10 mg/kg *per oral*), chlorpropamide (250 mg/kg *per oral*) and human insulin lente (0.1 I.U./kg *subcut*) on blood glucose concentrations of normoglycemic rats.

Treatment Group	Before Treatment	AFTER TREATMENT							Maximum Reduction	% Maximum Reduction
	0h	1h	2h	4h	6h	8h	10h	12h		
COIP	71.75±5.38	73.34±5.23	71.45±5.15	72.01±6.54	73.15±5.14	73.13±5.45	73.28±6.31	74.05±5.13	0.30'	0,42
COGI	72.45±5.02	72.44±4.43	72.78±5.45	73.02±6.99	73.41±5.37	72.07±4.22	73.01±4.47	72.23±4.34	0,38	0,53
TB2	72.95±5.10	74.24±6.15	72.04±5.12	69.25±4.71	67.18±4.51	65.79±3.44	64.59±3.55*	67.85±4.61	8.36*	11.46**
TB3	73.45±5.50	74.24±6.22	71.04±5.09	65.45±4.08	64.43±4.52	63.79±3.44*	63.59±3.55	64.90±4.01	9.86*	13.42**
TB4	73.41±5.18	74.08±6.87	69.12±5.01	64.39±4.02*	64.15±3.45*	62.11±3.01*	60.38±3.67*	62.11±3.91*	13.03***	17.75***
GC	72.24±5.33	63.02±3.26*	59.65±3.22*	55.45±2.87**	55.31±2.56**	50.22±2.39**	56.90±2.78**	58.93±3.02**	22.02***	30.48***
CP	71.64±4.05	62.45±3.45	55.45±2.70	49.55±2.33**	50.77±2.21**	56.33±2.71**	60.01±2.61*	61.45±3.79*	22.09***	30.83***
IL	73.11±5.36	44.34±2.02***	39.56±2.22***	40.35±2.11***	55.52±2.22**	65.14±3.75	67.24±4.22	70.32±4.0	33.55***	45.89***

Values given represent the mean±SD of 8 observations. *p< 0.05; **p<0.01; ***p<0.001 (student's t-test)

COIP : Control group of rats that had 2 mg/kg of distilled water intraperitoneally

COGI : Control group of rats that had 2 mg/kg of distilled water through gatric intubation

TB2 : Group of rats that had aqueous leaf extract of *Tapinanthus butungii* at the dose of 200 mg/kg

TB3 : Group of rats that had aqueous leaf extract of *Tapinanthus butungii* at the dose of 300 mg/kg

TB4 : Group of rats that had aqueous leaf extract of *Tapinanthus butungii* at the dose of 400 mg/kg

GC : Group of rats treated with 10 mg/kg of glibenclamide

CP : Group of rats treated with 250 mg/kg of chlorpropamide

IL : Group of rats treated with 0.1 I.U./kg of human insulin lente

Activity in hyperglycemic rats

Aqueous leaf extract of *Tapinanthus butungii* caused dose-dependent reductions of blood glucose concentrations in rats pretreated with 50% dextrose in water. The 400 mg/kg body weight of the extract caused reductions in glucose levels comparable to glibenclamide and chlorpropamide (Table 2).

Activity in alloxan-induced diabetic rats

In diabetic rats, the extract exhibited significant antiglycemic effect within the first 2 hours of administration (Table 3). Of great significance is the fact that the extract caused considerable decline in blood glucose levels in the diabetic rats far in excess of those caused by glibenclamide and chlorpropamide.

Our results also showed that there were no significant differences in blood glucose levels of rats that had distilled water intraperitoneally and those that were similarly treated by gastric intubation (Tables 1-3).

There were no obvious signs of toxicity (such as tremors, weakness, lethargy, refusal of feeds, weight loss, hair-loss, coma and death) observed in any of the animals throughout the duration of our observation.

DISCUSSION

Although *Tapinanthus butungii* has been advocated as a traditional plant treatment in folkloric medicine in Southern Nigeria, scientific studies to evaluate its efficacy are lacking. The present study reports for the first time the scientific basis for its use. *Diabetes mellitus* is a metabolic disorder characterized by insufficient insulin secretion and/or insensitive target tissues to metabolic actions of insulin. Though, insulin is presently one of the most important therapeutic agents known to medicine, efforts have continued to seek for insulin substitutes from synthetic or plant sources for treatment of diabetes (22). It is clear from the results of this experimental animal study that the tested aqueous leaf extract of *Tapinanthus butungii* induced sig-

Table 2: Effects of aqueous leaf extract of *Tapinanthus butungii* (200, 300 or 400 mg/kg *per oral*), glibenclamide (10 mg/kg *per oral*), chlorpropamide (250 mg/kg *per oral*) and human insulin lente (0.1 I.U./kg *subcut*) on blood glucose concentrations of hyperglycemic rats.

Treatment Group	Before Treatment	AFTER TREATMENT							Maximum Reduction	% Maximum Reduction
	0h	1h	2h	4h	6h	8h	10h	12h		
COIP	114.45±7.23	79.45±6.11	75.67±5.85	74.39±4.01	73.98±4.96	73.11±4.89	73.34±5.05	69.96±4.86	44,49	38,87
COGI	116.77±8.53	79.99±6.58	76.653±5.89	74.66±5.14	74.87±5.82	73.55±5.25	73.47±5.11	73.11±5.70	43,66	37,39
TB2	115.71±8.01	81.45±7.99	75.02±6.32	70.34±5.92	68.45±5.32	65.67±5.91	63.78±6.17*	64.94±5.47	51.93*	44.88*
TB3	116.91±8.13	80.76±7.95	71.02±5.59	69.23±4.92	67.09±5.21	62.18±5.15*	61.36±6.04*	60.92±5.25*	55.99*	47.89*
TB4	117.89±8.69	77.11±7.03	70.98±4.22	68.54±4.43	66.49±3.64	60.65±4.21*	59.85±4.21*	57.34±4.17	60.55**	51.36**
GC	116.40±8.37	65.64±5.26	56.45±4.32**	52.45±3.61**	51.27±3.56**	49.41±3.20***	51.45±3.77**	55.49±4.01**	66.99**	57.55**
CP	117.23±8.97	64.87±5.54	48.65±3.28***	50.54±3.37**	52.46±3.67**	55.39±3.77**	54.91±3.88*	59.56±4.12*	68.58**	58.50**
IL	117.93±8.99	54.26±3.42**	46.33±3.11***	47.84±3.21***	48.64±3.51***	51.77±4.25**	57.45±4.41**	61.65±5.01*	71.60***	60.71***

Values given represent the mean±SD of 8 observations. *p< 0.05; **p<0.01; ***p<0.001 (student's t-test)

COIP : Control group of rats that had 2 mg/kg of distilled water intraperitoneally

COGI : Control group of rats that had 2 mg/kg of distilled water through gatric intubation

TB2 : Group of rats that had aqueous leaf extract of *Tapinanthus butungii* at the dose of 200 mg/kg

TB3 : Group of rats that had aqueous leaf extract of *Tapinanthus butungii* at the dose of 300 mg/kg

TB4 : Group of rats that had aqueous leaf extract of *Tapinanthus butungii* at the dose of 400 mg/kg

GC : Group of rats treated with 10 mg/kg of glibenclamide

CP : Group of rats treated with 250 mg/kg of chlorpropamide

IL : Group of rats treated with 0.1 I.U./kg of human insulin lente

nificant dose-dependent reductions ($p < 0.05$-0.001) in blood glucose concentrations of normoglycemic, hyperglycemic and alloxan-induced diabetic rats. The fact that this extract caused significant reductions in blood glucose levels in alloxan-induced diabetic rats suggests that *Tapinanthus butungii* may act in yet undetermined ways apart from stimulating insulin production from the pancreatic islets since these would have been severely damaged by alloxan. However, stimulation of the undamaged or residual pancreatic islets to produce insulin cannot be ruled out since both glibenclamide and chlorpropamide caused slight but insignificant reductions in blood glucose levels in alloxan-induced diabetic rats. In addition, significant reductions in blood glucose levels in hyperglycemic rats by the extract may suggest that *Tapinanthus butungii* could, at least in part, stimulate insulin production and glucose utilization, like glibenclamide and chlorpropamide, to bring its hypoglycemic effect in the mammalian experimental model used. Although the present findings confirm the antiglycemic potential in the fresh leaf of *Tapinanthus butungii*, the precise mechanism of its hypoglycemic action requires further studies for appropriate elucidation. It is, however, interesting to note that the plant extract is more effective in reducing the blood glucose concentrations of diabetic and hyperglycemic rats than in reducing the blood glucose concentrations of normoglycemic rats. The reason for this is not completely clear. One attractive speculation is that once normoglycemia is achieved, the extract triggers on a mechanism that tends to prevent further drop in blood glucose level. A second possibility is the 'dampening' effect caused by the carbohydrate-rich fiber content of the extract that is more pronounced in the normoglycemic rats. This may also explain the observance of an initial rise in the blood glucose levels of normoglycemic rats treated with the extract. These possibilities are to be addressed in the next phase of our study. Also included in the next phase of our study are the effects of this extract on serum insulin levels and lipid profile.

Table 3: Effects of aqueous leaf extract of *Tapinanthus butungii* (200, 300 or 400 mg/kg *per oral*), glibenclamide (10 mg/kg *per oral*), chlorpropamide (250 mg/kg *per oral*) and human insulin lente (0.1 I.U./kg *subcut*) on blood glucose concentrations of diabetic rats.

Treatment Group	Before Treatment	AFTER TREATMENT							Maximum Reduction	% Maximum Reduction
	0h	1h	2h	4h	6h	8h	10h	12h		
COIP	512.51±14.02	522.33±14.69	527.15±15.45	510.98±14.86	531.14±15.53	534.49±15.89	557.23±15.73	566.18±15.96	1,53	0.30'
COGI	520.01±14.32	523.61±14.12	518.18±14.23	533.24±15.76	535.59±15.67	536.57±15.90	551.38±15.77	562.59±15.98	1,83	0,35
TB2	502.01±15.39	498.65±14.98	442.76±14.03	388.24±13.87*	320.54±13.87*	289.39±13.96*	281.56±14.68*	285.23±14.12*	220.45***	43.91***
TB3	501.89±15.45	488.34±14.88	402.54±14.12	358.02±13.01*	298.34±12.93*	254.22±12.64*	243.45±13.01*	199.43±11.92*	302.46***	60.26***
TB4	539.54±15.87	475.06±14.87	396.84±14.98*	270.98±10.98**	230.65±12.32**	192.09±9.87***	192.63±9.46***	165.03±8.65***	374.51***	69.41***
GC	520.12±15.43	534.26±15.58	516.14±15.59	511.67±15.47	490.74±14.36	475.35±14.24	460.45±14.55	453.43±13.49	80,83	15,13
CP	499.98±14.96	498.72±15.21	495.12±15.10	486.14±9.35	433.23±14.02	405.14±14.26	405.02±13.25	424.13±14.49*	94,84	18,97
IL	510.83±15.13	170.24±8.87***	138.25±7.56***	349.12±13.02*	344.76±13.67*	511.55±15.11	513.34±15.18	515.35±15.25	372.58***	72.94***

Values given represent the mean±SD of 8 observations. *p< 0.05; **p<0.01; ***p<0.001 (student's t-test)

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TB4 : Group of rats that had aqueous leaf extract of *Tapinanthus butungii* at the dose of 400 mg/kg

GC : Group of rats treated with 10 mg/kg of glibenclamide

CP : Group of rats treated with 250 mg/kg of chlorpropamide

IL : Group of rats treated with 0.1 I.U./kg of human insulin lente

CONCLUSIONS

The results of this experimental animal study clearly demonstrates that aqueous leaf extract of *Tapinanthus butungii* has a pronounced and remarkable blood-glucose-lowering potential in hyperglycemic and alloxan-induced diabetic male Sprague-Dawley rats comparable to that produced by glibenclamide and chlorpropamide. *Tapinanthus butungii*, therefore, represents an effective antihyperglycemic dietary adjunct for the treatment of diabetes and a potential source for discovery of new orally active agent(s) for future diabetes therapy. Further comprehensive chemical and pharmacological investigations are, however, required for characterization of its constituents and appropriate elucidation of its mechanism of action.

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