Physiology and Endocrinology

HYPOGLYCAEMIC EFFECTS OF ALLIUM CEPA, ALLIUM SATIVUM AND ZINGIBER OFFICINALE AQUEOUS EXTRACTS ON ALLOXAN-INDUCED DIABETIC RATTUS NOVERGICUS

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SUMMARY: *This study compared the hypoglycaemic effects of the increasing dosages of A. cepa, A. sativum and Z. officinale aqueous extract on alloxan –induced diabetic Rattus novergicus for possible use in the management of diabetes mellitus. Diabetes mellitus was induced in 108 out of a total of 117 adult Rattus novergicus using 150mg/kg b wt of alloxan monohydrate. Increasing dosages (200, 250 and 300mg/kg bw ip) of A. cepa, A. sativum and Z. officinale aqueous extracts were given to the diabetic rats for six weeks while the control rats got either normal saline (1ml) or increasing dosage of glibenclamide (2.5, 3.8 and 5.0mg/kg b wt ip) during the same period. Blood glucose level from each treatment was evaluated using o – toluidine method. F-LSD was employed to test significant differences (P < 0.05) among treatment means. Increasing dosages of plants aqueous extracts produced a dose-dependent significant (P < 0.05) reductions in the blood glucose levels of diabetic rats after 6 weeks of treatment when compared with that of the control rats. Among the extracts studied, A. sativum at 300mg/kg bw ip gave the best hypoglycaemic effect (79.7% from 314.1±40.4 to 63.9±2.9), A. cepa followed with 75.4% (297.8±37.5 to 73.4±3.0) and Z. Officinale with 56.7% (303.3±35.8 to 147.2±2.3). From the experimental findings, it is possible to conclude that the three plant extracts studied exhibited promising hypoglycaemic activity in alloxan-diabetic rats. The hypoglycaemic effects could represent a protective 2 mechanism against the development of hyperglycaemia common in diabetes mellitus.*
Key words: allium cepa, allium sativum, zingiber officinale, hypoglycemia, diabetes, rat

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia (high blood sugar) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (1). The effect of diabetes mellitus includes long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present characteristic symptoms such as thirst, polynuria, blurring of vision and weight loss (1). In its most severe forms, ketoacidosis or non-ketotic hyperosmolar state may develop and lead to stupor, coma and in absence of effective treatment death (1). Diabetes mellitus is characterized by recurrent or persistent hyperglycaemia and other signs, as distinct from a single illness or condition. Diabetes mellitus can be diagnosed by demonstrating any one of the following:

i. fasting plasma glucose level at or above 126mg/dl (7.0mmol/l).

ii. plasma glucose at or above 200mg/dL or 11.1mmol/l two hours after a 75g oral

glucose load as in a glucose tolerance test.

iii. random plasma glucose at or above 200mg/dl or 11.1mmmol/l (1).

In 2006, according to the World Health Organization, at least 171 million people world wide may suffer from diabetes (2). The incidence is increasing rapidly and it is estimated that by the year 2030, this number will double (2).

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Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries (3). The greatest increase in prevalence is however expected to occur in Asia and Africa, where more patients will likely be found by 2030. In 2005, there were about 20.8 million people with diabetes in the United States alone. ADA (2) reported that there were about 6.2 million people undiagnosed and about 41 million people that would be considered prediabetic in the United States. The national diabetes information clearing house estimates 3 that diabetes costs $132 billion in the United States alone every year. ADA (2) pointed out that 1 in 3 Americans born after year 2000 may develop diabetes in their lifetime. Statistical projections from India suggested that the number of diabetes will rise from 15 million in 1995 to 57 million in the year 2025, thus making India the country with the highest number of diabetics in the world (4-5). Although there is a paucity of data on the prevalence of diabetes in Nigeria and other African countries, available data suggested that diabetes was emerging as a major health problem in Africa (6). The prevalence of diabetes in Nigeria was estimated to be between 1.4 to 2.7% of the population (7-9) and over 90% of these are non–insulin dependent diabetes mellitus (10). Diabetes mellitus has been reported to be the major cause of blindness, kidney failure, lower-extremity amputation, cardiovascular diseases and premature mortality (11).

Diabetes has increasing cases in rural and poor populations throughout the world, despite major investigation into understanding the pathophysiology and treatment of diabetes mellitus, it has continued to be a major health problem worldwide (12). The possibility of its management by the oral administration of hypoglycaemic agents has stimulated great research interest in over the years. Though different types of oral hypoglycaemic agents are available along with insulin for the management of diabetes mellitus, there is increased demand by patients for the use of herbal preparations with hypoglycaemic activity (12). The use of herbal medicine is wide spread (13). The use of herbs has more than tripled over the last 10 years (14). Estimates of use of herbal preparation range from 40 to 60 % among the United States population (15). The growing public interest and awareness of herbal medicine have led the pharmaceutical industry and biomedical researchers to pay more attention to medicinal plants (16). The annual sale of medicinal herbs and related commodities in the United States now exceeds two billion dollars (17). 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 may be often unknown (12). Recently, there has been a resurgent interest in the herbal treatments of diabetes. For a long time diabetes have been treated orally with several medicinal plants or their extracts based on herbal medicine (18). Little scientific evidence exists to support the numerous herbs used to improve diabetes related metabolic disorder (19). 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 anti-diabetic products will continue to focus on plants and other natural resources (12). The cost of administrating modern antidiabetic drugs is beyond the reach of most people in the low income group and those living in the rural areas, hence the use of plants for the treatment of common diseases such as diabetes are very common. Herbal medicine therefore can solve the economic problem of the poor. Investigators have consistently found that several plant products showed unique hypoglycaemic activities in diabetic animal model (20). West Africa has several thousand of such plants (21). Nigeria is blessed with medicinal plants which are used for the treatment of various diseases.

In line with the WHO (22) expert committee on diabetes which recommends that traditional methods of treatment of diabetes should be further investigated. Also considering the economic resource constraints and cheapness of these herbal products, the present study was designed to determine the effect of increasing dosage of plant extracts on the blood glucose level of alloxan - induced diabetic *R. novergicus* for possible use of the most effective hypoglycaemic dosage in the control of hyperglycaemia characteristic of diabetes mellitus.

These will thus provide a pharmacological basis for the use of the most hypoglycaemic dosage of the plant extracts in adult onset Type 2 diabetes mellitus in some parts of Nigeria, Africa and the World at large. This is more so as many modern pharmaceuticals used in conventional medicine today also have natural plant origin. Example Metformin was derived from the flowering plant *Galya officinalis* (Goat's Rue or French lilac) which was a common traditional remedy for diabetes. There is therefore no doubt that antidiabetic medicinal plant might provide an important source of new oral hypoglycaemic compounds for development as pharmaceutical entities or as simple dietary adjuncts to existing therapies. Compounds that stimulate insulin biosynthesis and secretion or promote peripheral glucose uptake and utilization in herbal products have high potentials in diabetes management.

MATERIALS AND METHODS
Plant Material: The *A. sativum*, *Z. officinale* and *A. cepa* used for the experiment were bought from the Ogige market, Nsukka, Nigeria. The plants were identified (21) to species level at the Herbarium Unit, Department of Botany University of Nigeria, Nsukka where voucher specimen were kept.

Animal Model: A hundred and seventeen (117) adult white wistar strain albino rats (*R. norvegicus*) weighing 200 to 250g,

bred in the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. They were fed *ad labium* with 30% crude protein (Guinea feed) commercial feed. They were allowed to acclimatize under standard photoperiodic condition in a clean rat cage in the Postgraduate Research Laboratory, Department of Zoology, University of Nigeria, Nsukka. All animals were maintained under the standard laboratory condition for temperature (26± 20°C) and light (12 hours day length) and were allowed free access to food and water.

Extract Preparation: The methods of Akah *et al.* (23) and Habib *et al.* (13) were used. Fresh health plant each of *A. cepa*, *A. sativum* and *Z. officinale* (2000g) were washed, cut into small pieces and homogenized in a waring blender. The resulting mixture was soaked in 2L of distilled water. The mixture was allowed to stand for twenty four hours with intermittent shaking. Following filtration, the filtrate was heated to dryness in a water bath and the weight of the crude extract determined. The extract was kept in refrigerator (40°C) thereafter. The extract was later reconstituted in normal saline (0.85% NaCl) at a concentration of 1g/ml before administration.

Induction of Diabetes Mellitus: The methods of Osinubi *et al.* (12) and Battu *et al.* (24) were used to induce diabetes in the rats. Two grams of crystalline powdered alloxan monohydrate was dissolved in 50 mls of normal saline to yield a concentration of 40 mg/ml. 150 mg/kg body weight of alloxan per rat was administered intraperitoneally after overnight fast (access to only water) of twelve hours to make them more susceptible to developing diabetes. Only rats with serum glucose levels between (250 – 400 mg/dl) after two weeks were considered diabetic and used for the experiment.

Experimental Design: The study was carried out on alloxan-induced diabetic rats for six weeks (25). The animals were fasted for sixteen hours before each experiment and blood sample collected from the eye of the rats. Blood glucose levels were determined before the plant extract treatments of the animals (initials) and subsequently evaluated weekly for six weeks. The experimental design was the three by three Latin square design using 117 rats divided into two major groups:

Group I: nine non- diabetic rats (non diabetic control).

Group II: a hundred and eight alloxan -induced diabetic rats.

The group I rats were divided into 3 subgroups (Ia, Ib, Ic) of 3 rats each in different cages and receives 1.0ml of normal saline intraperitoneally daily.

The Group II rats (alloxan induced diabetic) were divided into 4 subgroups (IIa, IIb, IIc, IId). Subgroups IIa, IIb and IIc were divided into 3 replicates (IIa1, IIa2, IIa3, IIb1, IIb2, IIb3 and IIc1, IIc2, IIc3) respectively, each replicate (3 rats each) received either 200 mg/kg, 250 mg/kg or 300 mg/kg of *A. cepa*, *A. sativum* and *Z. officinale* aqueous extracts intraperitonially daily.

The subgroups IId was the diabetic control (27 rats) and were divided into 3 replicates (IId1, IId2 and II3) each replicate had three rats and were administered 2.5 mg/kg/, 3.8 mg/kg and 5.0 mg/kg of antidiabetic drug (glibenclamide) daily for six weeks.

Blood Glucose level determination: The glucose in a protein-free supernatant prepared from whole blood, serum or plasma

was heated with a solution of a primary aromatic amine, O- toluidine, in glacial acetic acid. A green colour is produced, probably a glycosylamine, the absorbance of which was read using a spectrophotometer (26).

Data analysis: The data collected were pooled and analyzed for their central tendencies using descriptive statistic, values were expressed as mean ± standard deviation of the observations. F-LSD was employed to test the significant differences (P < 0.05) among treatment means. All analyses were performed using Genstat (27) statistical software package for windows.

RESULTS

The increasing dosage (200, 250 and 300mg/kg bw ip) of *A. cepa*, *A. sativum* and *Z. officinale* aqueous extracts produced dose-dependent, significant (P< 0.05) reductions in the blood glucose levels of diabetic rats after 6 weeks of treatment when compared with that of the control rats. The comparative hypoglycaemic effects of the plant extracts studied compared with glibenclamide and normal saline in alloxan-induced diabetic and normal rats showed that *A. cepa* at 200mg/kg reduced fasting blood glucose levels by 62.9% (292.3±29.0 to 108.2±4.6) after six weeks of treatment. *A. cepa* at 250mg/kg reduced it by 69.7% (296.3±37.8 to 89.8±4.3). *A. cepa* at 300mg/kg lowered it by 75.4 %(297.8±37.5 to 73.4±3.0). *A. sativum* at 200mg/kg reduced fasting blood glucose level by 70.1% (293.0±35.0 to 87.6±6.3) (Table 1). *A sativum* at 250mg/kg reduced it by 76.6 % (311.1±29.6 to 72.8±3.2). *A. sativum* at 300mg/kg reduced it by 79.7 % (314.1±40.4 to 63.9±2.9). *Z. officinale* at 200mg/kg reduced fasting blood glucose levels by 51.4% (303.3±35.8 to 147.2±2.3) after six weeks of treatment *Z. officinale* at 250mg/kg reduced it by 56.9% (319.0±58.0 to 137.3±2.2). *Z. officinale* at 300mg/kg reduced it by 56.7% (297.2±32.8 to 128.6±2.1). Glibenclamide at 2.5mg/kg reduced fasting blood glucose levels by 76.4% (313.0±40.3 to 73.8±4.6) after six weeks of treatment (Table 1). Glibenclamide at 3.8mg/kg reduced it by 80.1 % (319.4±54.0 to 63.6±2.2). Glibenclamide at 5.0mg/kg reduced it by 81 % (310.7±35.0 to 59.0±1.6). The most effective percentage reduction in blood glucose level was observed at 300mg/kg bw ip for the three extracts studied. Normal saline at 1ml/kg bw ip had no effect on fasting blood glucose level by 0% (55.4±5.0 to 55.4±5.2). These values were statistically different when their F-LSD value (15.317) was used to test the significance differences between the means at P=0.05. Among the extracts studied, after 6 weeks of treatment, *A. sativum* at 300mg/kg bw ip was the most hypoglycaemic (79.7%), *A. cepa* followed with 75.4% and *Z. Officinale* with 56.7%. The comparative hypoglycaemic effects of the increasing dosage of the plant extracts studied at 200, 250 and

Table 1:The comparative effects of the increasing dosage of plant extract on blood glucose level of alloxan - diabetic Rattus novergicus.

Treatment	Dosage	Blood glucose level(mg/dl) per week							% reduction after 6 weeks
		WK0	WK1	WK2	WK3	WK4	WK5	WK6	
NS	1.0ml	55.4±5.0	55.4±5.0	55.4±5.0	55.4±5.2	55.4±5.2	55.4±5.2	55.4±5.2	0.0
AC	200mg	292.3±29.0	178.6±17.0	149.4±4.3	134.2±6.4	118.2±2.2	115.3±2.8	108.2±4.6	62.9
AC	250mg	296.3±37.8	167.0±21.4	137.6±5.2	123.8±4.1	110.9±4.5	100.6±2.1	89.8±4.3	69.7
AC	300mg	297.8±37.5	137.0±14.7	121.9±4.2	108.6±5.0	96.8±4.2	83.6±4.6	73.4±3.0	75.4
AS	200mg	293.0±35.0	146.7±16.1	123.9±4.6	111.8±4.5	100.2±2.4	97.1±1.6	87.6±6.3	70.1
AS	250mg	311.1±29.6	134.7±15.4	105.2±4.0	96.1±2.8	87.6±2.6	80.3±2.1	72.8±3.2	76.6
AS	300mg	314.6±40.4	117.9±15.0	99.9±3.0	90.1±2.6	84.1±2.4	75.1±3.7	63.9±2.9	79.7
ZO	200mg	303.3±35.8	239.0±26.4	193.8±7.9	172.6±4.9	160.8±3.4	152.8±2.0	147.2±2.3	51.4
ZO	250mg	319.0±58.0	237.1±39.0	188.2±3.7	177.0±4.9	157.9±2.6	150.0±4.8	137.3V2.2	56.9
ZO	300mg	297.2±32.8	204.9±21.4	179.3±5.2	164.0±5.1	151.0±4.6	139.0±2.4	128.6±2.1	56.7
GL	2.5mg	313.0±40.3	152.6±20.6	118.0±5.0	108.7±2.1	102.8±4.6	88.8±3.8	73.8±4.6	76.4
GL	3.8mg	319.4±54.0	126.1±22.4	94.1±4.8	82.7±2.9	75.2±3.5	68.0±2.3	63.6±2.2	80.1
GL	5.0mg	310.7±35.0	108.4±11.4	85.0±2.7	77.8±2.2	72.6 ±1.8	65.3±2.0	59.0±1.6	81.0

Values given represent the Mean SD of 9 observations. NS = Normal saline represents Non Diabetic Control, AC = Allium cepa, AS = Allium sativum, ZO = Zingiber officinale and GL = glibenclamide represents Diabetic control. P < 0.05, FLSD = 15.317

300mg/kg b wt compared with normal saline at 1ml/kg and Glibenclamides at 2.5mg, 3.8mg and 5.0mg indicated that *A. cepa* and *A. sativum* at 300mg/kg bw ip caused significant percentage reduction in glucose levels.

DISCUSSION

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial/heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies (28). Traditional plant remedies have been used for centuries in the treatment of diabetes (18), but only a few have been scientifically evaluated. Alloxan is known for its selective pancreatic islet β–cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals (29-30). Generalised increase in the level of blood glucose during diabetes have been consistently reported both in animal models (31-34) and humans especially those suffering from insulin- dependent diabetes mellitus (35). In our study, increase in blood glucose level was observed on induction of diabetes mellitus in the rat models. *A. sativum* ability to reduce fasting blood glucose level have been earlier reported in alloxan and streptozotocin - induced rats and mice (36). S – allyl cysteine sulfoxide (alliin) a sulphur containing amino acid in garlic (200mg/kg b wt) has potential to reduce diabetic condition in rat almost to the same extent as did glibenclamide and insulin (37). This report is in agreement with the findings of

this research on the hypoglycaemic effect of garlic, it reduced blood glucose level in a dose - dependent manner. Garlic can act as a hypoglycaemic agent by increasing either the pancreatic secretion of insulin from the beta cell or it release from bound insulin (38). The antioxidant effect of s- allyl cysteine sulfoxide contained in garlic contributed to its beneficial effects in diabetes (37). Another mechanism of action may be due to spare insulin from sulphydryl group, garlic (allicin) can effectively combine with compounds like cysteine and thus enhances serum insulin. S – allyl cysteine sulphoxide (SACS) is one of such compound present in garlic which has demonstrated antidiabetic effects in experimentally induced diabetic rats (39). Onions extract was shown to reduce hyperglycaemia in a dose - dependent manner following a glucose tolerance test, in a human study (40). Our result is in line with Sharma *et al.* (40) on onions extract reduction of blood glucose level in a dose-dependent manner with the highest percentage reduction occurring at 300mg/kg bw ip. An active ingredient (allyl propyl disulfide) in onions has been found to have antidiabetic properties (41), although other active sulphurous compounds may be present (42). A compound called SMCS, isolated from onions, produced similar effects as insulin in a group of diabetic rats (25). It is not unlikely that the plant extracts like glibenclamide induced hypoglycaemia by stimulating insulin release and action , thereby enhancing cellular uptake and utilization of glucose in animals. It remains unclear whether

the cellular glucose uptake was due to increased insulin secretion or decreased insulin degradation rate. An Indian homeopathic journal reported in the late 1970s that freshly squeezed ginger juice had hypoglycaemic effects in both diabetic and non diabetic rats (43). Kalejaiye *et al.* (44) reported that acute dose of aqueous extracts of *Z. Officinale* rhizome exhibit hypoglycaemic activity. Our findings on the dose- dependent hypoglycaemic effect of ginger, with the most effective percentage reduction at 300mg/kg b wt suggested that ginger might increase insulin levels (45). Since the mechanism of the hypoglycaemic effects of these plant extracts studied still remain speculative, further studies are required to unravel the pathway of hypoglycaemic action of the plant extracts and to shed more light on the hypoglycaemic constituents of the plants. Extracts may act in yet undetermined ways apart from stimulating insulin production from the pancreatic islets since these

would have been severely damaged by alloxan. Increased peripheral utilization and inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney, if any can also contribute to a glucose lowering effect (46). Conclusively, it is evident from this research that plant extracts studied contain hypoglycaemic agents capable of lowering blood glucose level in alloxan - diabetic rats, although the detailed mechanisms involved needs further investigations. Plant extracts might have corrected the hyperglycaemic induced imbalance in glucose metabolism by normalizing the blood glucose homeostasis. Glucose lowering capacity of plant extracts may be attributed to bioactive substances which might have enhanced the utilization and inhibition of glucose synthesis by increasing the $NADP+$ and $NADPH$ ratio in the body (47), the consequence of which is a drop in glucose level in the blood of hyperglycaemic rats.

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