# EVALUATION OF THE ACTIVITY OF *ZINGIBER OFFICINALE (GINGER)* AQUEOUS EXTRACTS ON ALLOXAN-INDUCED DIABETIC RATS.

## [1]OZOUGWU, J. C. and [2]EYO, J. E.

[1&2] Physiology and Biomedical Research Unit, Department Of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria.

## Summary

In view of the varied medicinal applications of *Z. officinale,* the effects of the increasing dosages of its aqueous extracts on alloxan - induced diabetic *Rattus novergicus* for possible use in the management of diabetes mellitus was evaluated. Diabetes mellitus was induced in 54 out of a total of 63 adult *Rattus novergicus* using 150mg/kg of alloxan monohydrate.  Increasing dosages (200, 250 and 300mg/kg) of  *Z. officinale* aqueous extracts were given to the diabetic rats for six weeks while the control rats  got  either normal saline (1ml) or increasing dosages of glibenclamide ( 2.5, 3.8 and 5.0mg/kg) during the same period. Blood glucose level, total serum lipids and total serum cholesterol were assessed with routine methods.  F-LSD was employed to test significant differences ($P < 0.05$) among treatment means. Increasing dosages of *Z. officinale* aqueous  extracts produced  a dose-dependent significant ($P < 0.05$)  reductions  in the blood glucose levels, total  serum lipid  and  total  serum  cholesterol when  compared with that  of  the control rats. The  best percentage reduction in blood glucose level, total serum lipids and cholesterol were observed at 300mg/kg. It is possible to conclude from the experimental findings that *Z. officinale* studied exhibited promising hypoglycaemic and hypolipidaemic activity in alloxan-induced diabetic rats. It's hypoglycaemic and hypolipidaemic effects could represent a protective mechanism against the development of hyperglycaemia and hyperlipidaemia characteristic of diabetes mellitus.

**\*Corresponding Author:** Ozougwu, Jevas C. Physiology and Biomedical Research Unit, Department Of Zoology, University of Nigeria, Nsukka, Enugu State, Nigeria. E-mail: jevaschubby@yahoo.com  Tel (Mobile): +2348034006816.

## Introduction

*Z. officinale* is one of the most widely used members of the family Zingiberaceare and is a common condiment for various foods and beverages. *Z. officinale* has a long history of medicinal use dating back to 2,500 years in China and India for conditions such as headaches, nausea, and rheumatism and cold (1). They are characterized in traditional Chinese medicine as spicy and hot. *Z. officinale* has been reported to warm the body and treat cold extremities, improve a weak and tarchy pulse, address a pale complexion and strengthen the body after blood loss (2).

.The major pungent, compounds in *Z. officinale,* from studies of the lipophilic rhizome extracts have yielded potentially active gingerols which can be converted to shogaols, zingerone and paradol (3). The compounds 6 - gingerol and 6 - shogaol have been shown to have a number of pharmacological activities, including antipyretic, analgesic, antitussive and hypotensive effects (4). *Z. officinale* extracts exhibit inhibition of platelet aggregation and thromboxane synthesis *in vitro* (5,6) which has led to concerns  *Z. officinale* extracts may prolong bleeding, however, several European studies using Z. officinale orally did not find any significant anti coagulant effects *in vivo* (7). There is scanty of research work on the hypoglycaemic and hypolipidaemic effects of *Z. officinale* and hence the need to embark on this study became necessary.

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 (8). In 2006, according to the World Health Organization, at least 171 million people world wide suffer from diabetes (9). The incidence is increasing rapidly and it is estimated that by the year 2030, this number will double (9). Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries (10). The greatest increase in prevalence is however expected to occur in Asia and Africa, where more patients will likely be found by 2030. The national diabetes information clearing house estimates that the management of diabetes mellitus costs $132 billion in the United States alone every year.  (9) 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 (11, 12*)*. 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 (13). 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. In line with the (14) expert committee on diabetes which recommends that traditional methods of management of diabetes should be further investigated. Also considering the economic resource constraints and cheapness of these herbal products, this present study was designed to determine the effects of increasing dosages of *Z. officinale* (Ginger) on alloxan induced diabetic *R. novergicus* and its possible mechanisms of action, for possible use in the control of hyperglycaemia and hyperlipidaemia characteristic of diabetes mellitus.

## Materials and Methods

**Plant Material**

The *Z. officinale* used for the experiment was bought from the Ogige Market, Nsukka, Nigeria. The plants were identified (15) to species level at the Herbarium unit, Department of Botany, University of Nigeria, Nsukka where voucher specimen were kept.

**Animal Model**

Sixty three (63) 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 Physiology Research Laboratory, Department of Zoology, University of Nigeria, Nsukka. All animals were maintained under the standard laboratory condition for temperature ($26 \pm 2^0$C) and light (12 hours day length) and were allowed free access to food and water.

**Preparation of Plant Extracts**

The methods of (16) and (17) were used. Fresh health plant of *Z. officinale* (2000 g) 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 filtrates were heated to dryness in a water bath and the weight of the crude extract determined. The extract was kept in refrigerator ($4^0$ 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*. (13) and (18) were used to induce diabetes in the rats. 150 mg of alloxan per kg body weight of 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. The animals were fasted for sixteen hours before each experiment and blood sample collected from the eye of the rats. All parameters assessed were determined before the 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 63 rats divided into two major groups:

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

Group II: fifty four alloxan induced diabetic rats.

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.

Group II (fifty - four alloxan induced diabetic rats) were divided into 2 subgroups (IIa, IIb). Subgroups IIa, (twenty seven rats) were divided into 3 replicates (IIa$_1$, IIa$_2$, IIa$_3$)

respectively, each replicate had three rats and received 200 mg/kg, 250 mg/kg or 300 mg/kg of Z. *officinale* aqueous extracts intraperitonially daily.

The subgroups IIb was the diabetic control (twenty- seven rats) and were divided into 3 replicates (IId1, IId2 and II3) each replicate had three rats and were administered 2.5mg/kg/, 3.8mg/kg and 5.0mg/kg of standard 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 produced, probably a glycosylamine, the absorbance of which was measured using a spectrophotometer at 630 nm wavelength. (19).

**Determinations of Total Serum Cholesterol**

The cholesterol of the serum was oxidised to a tetraene derivative by ferric ions derived from ferric perchlorate using four test tubes marked test, control, standard and blank. The absorbance was measured using spectrophotometer at 590nm wavelength and compared with that of a pure solution of cholesterol (19).

**Determination of Total Lipids in Serum**

Serum 0.05ml, was pipetted into a test tube (15ml), containing 2.00ml of concentration Sulphuric acid (d= 1.84). The tube was swirled carefully, closed with a glass ball and placed in a bath of boiling water for 10 minutes. After cooling in cold water, 0.1ml was transferred into another test tube (15ml), containing 2.5ml of phosphoric acid- vanillin reagent acid the solution was mixed carefully. The intensity of the pink colour that develops reaches its maximum after 30 minutes; it begins to fade after about 50minutes. The absorbance of the sample was measured at 546nm against the blank. The amount of lipid was read off an analytical care, which is obtained by analyzing four different amounts of total lipid of serum. Instead of total lipids, triolein was used as reference material. In this case, the values must be multiplied by a factor of 0.76. A standard solution of triolein (10g/L) was used (19).

**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 (20) for windows.

<div align="center">

**Results**

</div>

**Blood glucose levels**

The increasing dosages (200, 250 and 300mg/k) of 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 (Table 1). Z. *officinale* at 200mg/kg reduce fasting blood glucose levels by 51.4% (303.3±35.8 to 147.2±2.3), at 250mg/kg it reduced it by 56.9% (319.0±58.0 to 137.3±2.2). while at 300mg/kg it reduced it by 56.7 %( 297.2±32.8 to 128.6±2.1).

 Glibenclamide at 2.5mg/kg reduce fasting blood glucose levels by 76.4% (313.0±40.3 to 73.8±4.6), at  3.8mg/kg  it reduced it  by 80.1  %  (319.4±54.0  to  63.6±2.2)  while  at 5.0mg/kg it reduced it by 81 % (310.7±35.0 to59.0±1.6). The most effective percentage reduction in blood glucose level was observed at 300mg/kg.  Normal saline at 1ml/kg had no effect on fasting blood glucose level.

**Total Serum Lipids**

The  increasing  dosages  (200,  250  and  300mg/kg)  of  *Z. officinale*  aqueous  extracts produced a  dose- dependent, significant (P < 0.05) reductions  in the total serum  lipids of diabetic rats  after  6 weeks  of  treatment when  compared with that  of  the control rats (Table 2). *Z. officinale* at 200mg/kg reduce total serum lipids by 15.6% (183.3±8.1 to 154.9±6.4), at 250mg/kg it reduced it by  18.0%  (184.1±8.8 to  150.9±7.9) while at 300mg/kg it reduced it by 19.8 % (183.0±8.3 to 146.7±6.9)**.** Glibenclamide at 2.5mg/kg reduce total serum lipids by 22.9% (183.7±7.4 to 141.6±4.9), at 3.8mg/kg it reduced it by 27.1 % (183.3±7.7 to 133.7±3.7) while at 5.0mg/kg it reduced it by 33.1 % (182.9±8.3 to 122.4±4.4).  The   most   effective percentage reduction in   total   serum lipids    was observed at 300mg/kg   Normal saline at 1ml/kg had no effect on total serum lipids.

**Total Serum Cholesterol**.

The increasing dosage (200, 250 and 300mg/kg) of *A. sativum* aqueous extracts produced dose -dependent significant (P < 0.05)   reductions  in the total serum  cholesterol of diabetic rats  after  6 weeks  of treatment when  compared with that  of  the  control  rats (Table 3). *Z. officinale* at 200mg/kg reduce total serum cholesterol by 7.5% (129.1±6.0 to 119.4±2.9), at 250mg/kg it reduced it by 12.9% (129.4±5.6 to  112.7±3.9) while at 300mg/kg reduced it by 16.1 % (130.7±4.4 to 109.7±7.5).  Glibenclamide at 2.5mg/kg reduced total serum cholesterol by 22.9% (129.4±4.4 to 99.7±3.2), at 3.8mg/kg it reduced it by 29.5% (129.1±4.3 to 91.0±3.7) while at 5.0mg/kg it reduced it by 32.9 % (129.4±3.7 to 86.8±3.1) after 6 weeks of treatment. The   most   effective percentage reduction in total  serum cholesterol   was  observed at 300mg/kg   Normal  saline at 1ml/kg  had no effects on  total serum  cholesterol.

**Table 1:Effects of the increasing dosage of *Z. officinale* extracts on blood glucose level of alloxan diabetic *Rattus novergicus*.**

| | | Blood glucose level(mg/dl) per week | | | | | | | % reduction after 6 weeks |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | Dosage | WK0 | WK1 | WK2 | WK3 | WK4 | WK5 | WK6 | |
| NS | 1.0ml | 55.4±5.6 | 55.3±5.0 | 55.4±4.0 | 55.4±5.0 | 55.7±5.8 | 55.4±5.6 | 55.3±4.1 | 0.01 |
| 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 | 137.3±2.2 | 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, ZO = *Zingiber officinale* and GL = glibenclamide represents Diabetic control. P < 0.05, FLSD = 15.317**

**Table 2: Effects of the increasing dosage of *Z. officinale* extracts on total serum lipids of alloxan Diabetic *Rattus novergicus*.**

| Treatment | Dosage | WK0 | WK1 | WK2 | WK3 | WK4 | WK5 | WK6 | %reduction after 6 weeks |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Total serum lipids(mg/dl) per WEEKS | | | | | |
| NS | 1.0ml | 144.1±6.9 | 144.4±4.2 | 144.8±5.9 | 144.3±6.5 | 144.5±6.9 | 144.1±7.9 | 144.0±7.9 | 0.02 |
| ZO | 200mg | 183.4±8.1 | 176.3±7.0 | 176.1±4.7 | 170.6±4.3 | 165.8±3.0 | 163.8±2.0 | 154.9±6.4 | 15.6 |
| ZO | 250mg | 184.1±8.8 | 174.7±5.1 | 169.4±4.8 | 168.4±2.1 | 164.8±1.1 | 158.7±1.9 | 150.9±7.9 | 18.0 |
| ZO | 300mg | 183.0±8.3 | 173.0±4.0 | 166.9±1.5 | 163.7±1.4 | 160.3±1.2 | 156.1±0.8 | 146.7±6.9 | 19.8 |
| GL | 2.5mg | 183.7±7.4 | 166.1±2.1 | 161.3±2.7 | 151.8±2.2 | 144.9±2.9 | 135.8±2.6 | 141.6±4.9 | 22.9 |
| GL | 3.8mg | 183.3±7.7 | 164.8±2.3 | 158.7±2.6 | 154.6±2.7 | 141.0±1.4 | 137.8±3.7 | 133.7±3.7 | 27.1 |
| GL | 5.0mg | 182.9±8.3 | 163.6±1.8 | 155.0±3.1 | 146.1±2.9 | 136.0±3.8 | 126.8±3.9 | 122.4±4.4 | 33.1 |

**Values given represent the Mean±SD of 9 observations. NS = Normal saline represents Non Diabetic Control, ZO = *Zingiber officinale* and GL = glibenclamide represents Diabetic control. $P < 0.05$, FLSD =4.428**

**Table 3: Effect of the increasing dosage of *Z. officinale* extracts on serum cholesterol of alloxan diabetic *Rattus novergicus*.**

| Treatment | Dosage | WK0 | WK1 | WK2 | WK3 | WK4 | WK5 | WK6 | %reduction after 6 weeks |
|---|---|---|---|---|---|---|---|---|---|
| | | Total serum cholesterol(mg/dl) per weeks | | | | | | | |
| N.S | 1.0ml | 91.1±6.2 | 91.0±3.2 | 91.2±4.2 | 91.0±3.2 | 91.1±3.2 | 91.1±4.2 | 91.0±5.2 | 0.0 |
| ZO | 200mg | 129.1±6.0 | 132.2±5.6 | 130.4±0.4 | 125.7±3.5 | 122.3±2.9 | 120.2±4.7 | 119.4±2.9 | 7.5 |
| ZO | 250mg | 129.4±5.6 | 130.3±4.1 | 129.4±4.1 | 124.1±3.4 | 121.8±2.4 | 114.6±4.6 | 112.7±3.9 | 12.9 |
| ZO | 300mg | 130.7±4.4 | 128.2±6.0 | 125.2±5.1 | 123.2±4.5 | 123.0±5.2 | 113.0±9.5 | 109.7±7.5 | 16.1 |
| GL | 2.5mg | 129.4±4.4 | 121.8±3.5 | 112.9±2.6 | 111.9±3.4 | 105.7±2.0 | 104.7±6.7 | 99.7±3.2 | 22.9 |
| GL | 3.8mg | 129.1±4.3 | 119.1±2.9 | 110.8±2.4 | 105.8±2.1 | 101.6±3.5 | 97.3±2.5 | 91.0±3.7 | 29.5 |
| GL | 5.0mg | 129.4±3.7 | 115.8±2.8 | 109.3±2.0 | 102.9±2.3 | 96.0±3.4 | 97.6±5.3 | 86.8±3.1 | 32.9 |

**Values given represent the Mean±SD of 9 observations. NS = Normal saline represents Non Diabetic Control, ZO = *Zingiber officinale* and GL = glibenclamide represents Diabetic control. P < 0.05, FLSD =3.67**

## Discussion

### Hypoglycaemic effects

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 (21). Traditional plant remedies have been used for centuries in the treatment of diabetes (22), 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 (23, 24).Generalised increase in the level of blood glucose during diabetes have been consistently reported both in animal models (25,26,27,28) and humans especially those suffering from insulin dependent diabetes mellitus (29). Ginger extract reduced blood glucose levels in a dose dependent manner, producing its best effects at 300mg/kg which corroborates some earlier reports (30, 31, 32, 32). Ginger extracts may act by stimulating insulin production from the pancreatic islets or by. increasing peripheral utilization and inhibition of the proximal tubular reabsorption mechanism for glucose in the kidney which have a glucose lowering effect (33). It is not unlikely that ginger extracts might have corrected the hyperglycaemic induced imbalance in glucose metabolism by normalizing the blood glucose homeostasis through enhanced utilization and inhibition of glucose synthesis by increasing the NADP+ and NADPH ratio in the body, the consequence of which is a drop in glucose level in the blood of hyperglycaemic rats (34). The mechanism of the hypoglycaemic effects still remains speculative, further studies are required to unravel the mechanism of its hypoglycaemic action.

### Hypolipidaemic effects

Alteration in serum lipids profile are known in diabetes , which are likely to increase the risk of coronary heart disease (35, 36, 37). The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (38) Hypercholesterolemia has been reported to occur in alloxan diabetic rats (39, 40). Ginger extract reduced the elevated total serum cholesterol and lipids levels in diabetic rat models in a dose dependent manner, producing its best effects at 300mg/kg which is in line with some earlier reports (41, 42, 43). The mechanism of hypolipidaemic action may be due to decreased oxidative load, by directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds (44). or by increasing the synthesis of antioxidant molecules.

## Conclusions

In conclusion, the levels of total serum cholesterol , total serum lipids and blood glucose levels which were actually raised in alloxan induced diabetic rats can be lowered by ginger aqueous extracts. This hypoglycaemic and hypolipidaemic effects may be protective against the development of atherosclerosis, hyperlipidaemia and hyperglycaemia common in diabetes mellitus.

# References

1. Grant, K. L. and Lutz, R. B. (2001). Ginger. *American Journal of Health and Systemic Pharmacy*, 57: 945 – 947.

2. Chang, C. P., Chang J. Y., Wang, F. Y. and Chang, J. G. (1995). The effect of Chinese medicinal herb *Zingiberis rhizoma* extract on cytokine secretion by human peripheral blood mono nuclear Cells. *Journal of Ethnopharmacology*, 48: 13 – 19.

3. Govindarajan, V. S. (1982). Ginger – chemistry, technology and quality evaluation, part I critical review. *Food Science and Nutrition,* 17: 189 – 258.

4. Suekawa, M., Ishige, A. and Yuasa, K. (1984). Pharmacological studies on ginger I. pharmacological actions of pungent constituents, 6 – gingerol and 6 – shogaol. *Journal of pharmacobiodyn*, 7: 836 – 848.

5. Kiuchi, K., Shibuya, M. and Sankawa, U. (1982). Inhibitors of prostaglandin biosynthesis from ginger. *Chemical pharmacy Bulletin,* 30: 754 – 757.

6. Srivastava, K. C. (1989). Effects of onion and ginger consumption on platelet thromboxane production in humans. *Prostaglandins, Leukotriene and Essential Fatty Acids,* 35: 183 – 185.

7. Bordia, A., Verma, S. K. and Srivastava, K. C. (1997). Effect of ginger (*Zingiber officinale Rose)* and ferugreek (*Trigonella foerum graecum L*) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 56: 379 – 384.

8. WHO. (1999). Definition, diagnosis and classification of diabetes mellitus and its complications. World Health Organization Department of Nonncomunicable Disease Surveillance. (http://whglibdoc.who.int/hg/1999/WHO-NCD-NCS-99.2pdf). 60 pp. Retrieved on 7/6/2007.

9. ADA. (2005).Total prevalence of diabetes and pre-diabetes        15 pp. American DiabetesAssociation,(http://www.diabetes.org/diabetesstatistics/prevalence.jsp ). Retrieved on 07/06/2007.

10. ADA. (2005).Total prevalence of diabetes and pre-diabetes. 15 pp. American DiabetesAssociation,(http://www.diabetes.org/diabetesstatistics/prevalence.js). Retrieved on 07/06/2007.

11. King, H., Aubert, R. E. and Herman, W. H. (1998). Global burden of diabetes 1995 – 2005: Prevalence, numerical estimates and projections. *Diabetes Care*, 21: 1414 – 1431.

12. Boyle, J. P., Honeycutt, A. A., Narayam, K. M., Hoerger, T. J., Geiss, L. S., Chens, H. and Thompson, T. J. (2001). Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the United State. *Diabetes Care*, 24: 1936 – 1940.

13. Osinubi, A. A., Ajayi, O. G. and Adesiyun, A. E. (2006). Evaluation of the anti-diabetic effect of aqueous leaf extracts of *Tripinanthus butungil* in male spragne Dawley rats. *Medical Journal of Islamic World Academy of Science*, 16(1): 41 – 47.

14. WHO. (1980). The World Health organization Committee on diabetes mellitus: Second Report. Technical Report Series 646. World Health Organization, Geneva.

15. Gbile, Z. O. (1980). *Vernacular Names of Nigerian Plants* (Yoruba). Forestry Research Institute of Nigeria, Ibadan.

16. Akah, P., Njoku, O., Nwanguma, A. and Akunyili, D. (2004). Effects of aqueous leaf extracts of *Vernonia amygdolina* on blood glucose and triglyceride levels of alloxan − induced diabetic rats. *Animal Research International*, 1(2): 90 − 94.

17. Habib, M. Y., Islam, M. S, Awal, M. A. and Khan, M. A. (2005). Herbal products: A novel approach for diabetic patients. *Pakistan Journal of Nutrition,* 4(1): 17 − 21.

18. Battu, G. R., Mamidipalli, S. N., Parimi, R., Viriyala, R. K., Patchula, R. P. and Mood, L. R. (2007). Hypoglycemic and antihyperglycemic effect of alcoholic extract of *Benincasa hispida* in normal and in alloxan induced diabetic rats. *Pharmacognosy Magazine*, 3: 101 − 105.

19. Sood, R. (1999). *Medical Laboratory Technology Methods and Interpretations.* 5th edition, Jaypee Brothers Medical publishers Limited, New Delhi, India.

20. Genstat for Windows Discovery Edition 2 (2007). Lawes Agricultural Trust, Rothamsted, United Kingdom.

21. Ugochukwu, N. S., Babady, N. E., Cobourne, M. and Gasset, S. R. (2003). The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *Journal of Biosciences*, 28(1): 1- 5.

22. Akhtar, F. M. and Ali, M. R. (1984). Study of the antidiabetic effect of a compound medicinal plant prescription in normal and diabetic rabbit. *Journal of Pakistan Medical Association*, 34: 239 − 244.

23. Zarrow, M. X., Yochim, J. M. and McCarthy, J. L. (1964). *Experimental Endocrinology; A Source Book of Basic Techniques.* Academic Press. New York.

24. Nafisa, P. C., Chakradnar, V. L., Vandana, S. P. and Suresh, R. N. (2007). An experimental evaluation of the antidiabetic and antilipidaemic properties of a standardized *Momordica charantia* fruit extract. *BMC Complementary and Alternative Medicine*, 7: 29 − 55.

26. Tukuncu, N. B., Bcyraktar, M., and Varli, K. (1998). Reversal of defective nerve conductions with vitamin E supplementation in type 2 diabetes. *Diabetes Care*, 21: 1915 − 1918.

27. Sharpe, P. C., Yue, K. M., Catterwood, M. A., Mcmarter, D. and Tribble, E. R. (1998). The effects of glucose induced oxidative stress on growth and extracellular matrix gene expression of vascular smooth muscle cells. *Diabetologia*, 41: 1210 -1219.

28. Hamme, H. R., Martins, S., Federlin , K., Geisen, K. and Brownee, M. (1991). Aminoguanidine treatment inhibits the development of experimental diabetes retinopathy. *Proceedings of National Academy of Sciences*, 88: 11555 - 11558.

29. Bell, B. M., Hayes, J. R., and Stout, R.W. (1984). Lipoprotein, insulin and glycaemic control in diabetes. *Hormonal metabolic research,* 16: 252 − 260.

30. Sharma, M. and Shukla, S. (1977). Hypoglycaemic effect of ginger. *Journal of Research in Indian Medicine, Yoga Homeopathy*, 12: 127 - 130.

31. Kalejaiye, O. F., Iwalewa, E. O., Omobuwajo, O. R. and Oyedapo, O. O. (2002). Hypoglycaemic effect of Nigerian *Zingiber officinale* rhizome on experimental diabetic rats. *Nigerian Journal of Natural Product and Medicine,* 6: 33 − 35.

32. Klemens, J. A. (2006). Herbs that lower blood Sugar. *Bonsaius ferreus*, 5 pp. (www.naturaldatabse.com) Retrieved on 25/07/2007.

33. Subramanian, A., Pushpagandan, P., Ragesekharan, S., Evans, D. A., Latha, P.G., and Valsaraj. R. (1996). Effect of artemisia pallens wall on blood glucose levels in normal and alloxan – induced diabetic rats. *Journal of Ethnopharmacology*, 50:13- 17.

34. Black, S. (1962). Regulation of sulfoxide and disulfides page 992 - 996 In: Kolowick and Kaplan , N. (eds). *Methods in enzymology. Volume 5*, Academic Press, New York.

35. Laakso, M. (1996). Lipids and lipoproteins as risk factors for coronary heart disease in non insulin dependent diabetes mellitus. *Annals of Medicine*, 28: 341- 345.

36. Steiner, G. (1999). Risk factors for macrovascular disease in Type 2 diabetes. Classic lipid abnormalities. *Diabetes Care*, 22(3): 6 - 9.

37. Massing, M.W., Sueta, C. A., Chowdhury, M., Biggs, D. P. and Simpson, R.J. (2001). Lipid management among coronary artery disease patients in diabetes mellitus or advanced age. *American Journal of Cardiology*, 87: 646 -664.

38. Hardman, J. G. and Limberd, L. E. (2001). Insulin, oral hypoglycaemic agents and the pharmacology of the endocrine pancreas, Pages 1383 - 1399. *In*: Goodman and Gilman's. *The Pharmacological Basis of Therapeutics (10$^{th}$ edition).* McGraw Hill Company limited, U S A.

39. Sharma, S. R., Dwivedi, S. K. and Swarup, D. (1996). Hypoglycaemic and hypolipidaemic effects of *Cinnamon tomala* nees leaves. *Indian Journal of Experimental Biology*, 34: 372 - 374.

40. Pushparaj, P., Tan, C. H. and Tan, B. K. (2000). Effect OF *Averrhoa bilimli* leaf extract on blood glucose and lipids in streptozotocin diabetic rats. *Journal of Ethnopharmacology*, 72: 69 - 76.

41. Kemper, K. J. (1999). Ginger (*Zingiber officinale*) 18pp (http://www. Mcp.edu/herbal/default.htm). Retrieved on 18/10/2007

42. Bhandari, U., Sharma, J. N. and Zafari, R. (1998). The protective action of ethanolic ginger *(Zingiber officinale)* extract in cholesterol fed rabbits. *Journal of Ethnopharmacology*, 61: 167- 171.

43. Tanabe, M., Chen, Y. D., Saito, K. and Kano, Y. (1993). Cholesterol biosynthesis inhibitory components from *Zingiber officinale* Roscoe. *Chemical Pharmacy Bulletin*, 41: 710 -713.

44. Gupta, S. K., Prakash, J. and Srivastava, S. (2002). Validation of traditional claim of Tulsi, *Ocium sanctum linn* as a medicinal plant. *Indian Journal of Experimental Biology*, 40: 765 -773.