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Anti-diabetic activity of ethanolic extract of *Zingiber officinale roscoe*

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ABSTRACT

Diabetes mellitus is a most common endocrine disorder, affecting more than 300 million people worldwide. For these therapies developed along the principles of allopathic are often limited in efficacy, Carry the risk of adverse effects, and are often too costly, especially for the developing world. In order to identify complementary or alternative approaches to existing medications, we studied the anti-diabetic potential of leaves of *Zingiber officinale*. The acute oral toxicity studies of the extracts revealed no toxic effects up to the levels of 2000mg/kg. The aqueous and alcoholic extracts of 20 and 30mg/kg body weight of *Zingiber officinale* was screened for the presence of hypoglycemic and antidiabetic activity. In this study diabetes was induced by a single IP dose Alloxan monohydrate in 72hrs fasted rats. The FBGL was carried on 7th, 14th and 21st day and OGTT was measured on 8th, 15th and 22nd day. Glibeclamide was taken as the standard and the results are quite comparable with it. The studies were indicated that the rhizome of *Zingiber officinale* are effective in regeneration of insulin secreting β -cells and thus possess antidiabetic activity. The aqueous and alcoholic extracts showed significant effect in decreasing the Fasting blood Glucose level and oral glucose tolerance test of rats and it's also showed good hypoglycemic activity in normal glycemic rats. The preliminary phytochemical analysis of the extracts of *Zingiber officinale* revealed the presence of Alkaloids, Tannins, Anthraquinones, Flavonoids, Saponins, Triterpenes, Sterols, Coumarinas the possible biologically active principles.

Keywords: *Zingiber officinale*, Alloxan monohydrate, Glibenclamide, FBGL and OGTT.

INTRODUCTION

Diabetes Mellitus (DM)

Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This

may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be.

Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and in all age groups, the general public has a concern about its control and treatment¹.

Classification of DM

Diabetes is classified by underlying cause. The most common forms of diabetes are categorized as

Type 1, or Insulin-dependent diabetes mellitus (IDDM) - an autoimmune disease in which the body's own immune system attacks the pancreatic beta cells, rendering it unable to produce insulin and

Type 2, or Non-insulin-dependent diabetes mellitus (NIDDM) - in which there is resistance to the effects of insulin or a defect in insulin secretion.

Type 2 diabetes commonly occurs in adults associated with obesity. There are many underlying factors that contribute to the high blood glucose levels in these individuals. An important factor is the resistance to insulin in the body essentially ignoring its insulin secretions. A second factor is the decreased production of insulin by the cells of the pancreas. Therefore, an individual with Type 2 diabetes may have a combination of deficient secretion and deficient action of insulin. In contrast to Type 2 diabetes, Type 1 diabetes most commonly occurs in children and is a result of the body's immune system attacking and destroying the beta cells. The trigger for this autoimmune attack is not clear, but the result is the end of insulin production².

Multiple risk factors for the development of Type 2 diabetes mellitus³:

- Family history (parents with diabetes).
- Obesity (i.e., $\geq 20\%$ over ideal body weight or body mass index $\geq 25\text{kg/m}^2$).
- Habitual physical inactivity.
- Impaired glucose tolerance.
- Hypertension ($\geq 140/90\text{mm Hg}$ in adults).
- High density lipoprotein (HDL) cholesterol $\leq 35\text{mg/dl}$ and/or triglyceride level $\geq 250\text{mg/dl}$.

Epidemiology⁶

Present status projects that incidence of diabetes is on rise. Present number of diabetics worldwide is 150 million and according to new estimates from researchers at the World Health Organization (WHO), there will be an increase of about 300 million or more by the year 2030 (Warner, 2004). Only in year 2001, about 441,004 deaths were registered and 49,855 of them provoked by diabetes, representing 11.2% of the total population. In United States, diabetes is the sixth leading cause of death. The prevalence of diabetes mellitus is rapidly increasing worldwide and India is estimated to have 31 million diabetics from the total population of the world. Diabetes is predicted to become one of the most common diseases in the world within a couple of decades, affecting at least half a billion people.

The driving force behind the high prevalence of diabetes is the rise of obesity, sedentary lifestyle, consumption of energy rich diet, etc. The diabetes epidemic is accelerating in the developing world, with an increasing proportion of affected people in younger age groups.

The prevalence of Type 2 diabetes is now at epidemic proportions. Type 2 diabetes has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. Type 2 diabetes accounts for about 90-95 % of population while Type 1 diabetes accounts for about 5 -10% of the total population. In the

past, Type 2 was rarely seen in the young, but recent reports describe Type 2 diabetes being diagnosed even in children and adolescent⁷.

Sugar Regulation: Carbohydrate, protein and lipid metabolism⁸

Glucose is an essential fuel for the body and is the main source of energy for the tissue cell. The amount of glucose in the blood is controlled mainly by the hormones insulin and glucagon. The rise in blood glucose following a meal is detected by the pancreatic beta cells, which respond by releasing insulin. Glucose is transported into the beta cell by Type 2 glucose transporters (GLUT2). As glucose metabolism proceeds, ATP is produced which closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell. The rise in positive charge inside the beta cell causes depolarization thereby opening the voltage-gated calcium channels and allowing calcium ions (Ca^{2+}) to flood into the cell. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis.

Insulin increases the uptake and use of glucose by tissues such as skeletal muscle and fat cells. Once inside the cell, some of the glucose is used immediately via glycolysis. Any glucose that is not used immediately is taken up by the liver and muscle where it can be converted into glycogen (glycogenesis). When glycogen stores are fully replenished, excess glucose is converted into fat in a process called lipogenesis by increasing the number of glucose transporters (GLUT4) expressed on the surface of the fat cell, causing a rapid uptake of glucose. Glucose is converted into fatty acids that are stored as triglycerides for storage⁹.

The rise in glucose also inhibits the release of glucagon, inhibiting the production of glucose from other sources, e.g., glycogen break down. Glucose may also indirectly contribute to protein synthesis by synthesis of amino acids. Glucagon is the main hormone opposing the action of insulin and is released when food is scarce. Glucagon also helps the body to switch to using resources other than glucose, such as fat and protein during starvation¹⁰.

MATERIALS AND METHODS

Alloxan was gift sample from Quali Kems Fine Chem Pvt, Ltd, Vadodara, Methanol was gift sample from ChangshuYangyuan Chemicals, China., Alcohol was gift sample from ChangshuYangyuan Chemicals, China and Gliben clamide was gift sample from Orchid Pharma Ltd, Chennai.

RESULTS

Phytochemical screening of *Zingiber officinale*.

The present investigation concluded that the isolated compounds from the plant *Zingiber officinale* shows the various Pharmacological effects was determined due to the presence of different phytochemical compounds. Further study is needed for the isolation of the constituents present in the plant and its individual pharmacological activity

should need to consider and ultimately it should be implemented for the benefit to human beings.

Table 1. Phytochemical screening of *Zingiber officinale*.

S.No.	Phytoconstituents	Aqueous	Alcoholic
1.	Alkaloids	-	+
2.	Tannins	+	-
3.	Anthraquinones	+	+
4.	Flavonoids	-	+
5.	Saponins	-	-
6.	Triterpenes	+	-
7.	Sterols	+	+
8.	Coumarin	-	+

Acute toxicity testing

Acute toxicity studies revealed that the alcoholic extracts of *Zingiber officinale* were safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

Hypoglycemic activity in normal rats

Fasting Blood Glucose Levels (FBGL) were within the range of 90-105 mg/dl in all the groups at 0day. Repeated treatment with the doses of aqueous and alcoholic extract (100 and 200 mg/kg) significantly decrease the blood glucose level on 7th, 14th and 21st day, indicating that the extract produce significant hypoglycemic activity after

repeated administration. Glibenclamide (10mg/kg) also significantly reduced Fasting Blood Glucose Level (FBGL) after repeated administration as compare to normal control group. Changes in FBGL in different groups after repeated dose administration are summarized in Table No: 10

Repeated administration of both aqueous and alcoholic extracts had significantly ($p < 0.005$) reduced the FBGL on 7th, 15th and 21st day, indicating these extracts can produce hypoglycemia on repeated administration. However hypoglycemic activity was more significant on 7th, 14th and 21st day for Glibenclamide treated as compare with other groups. The results suggest that the both aqueous and alcoholic extracts possess significant hypoglycemic activity after repeated dose administration. The detailed results are summarized in TableNo: 10

Effect of extracts of *Zingiber officinale* on fasting blood glucose level (FBGL) in normal rats

Table No: 2 Effect of extracts of *Zingiber officinale* on fasting blood glucose level (FBGL) in normal rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		7 th day	14 th day	21 st day
Normal control	-	87.18±2.63	76.59±1.89	71.18±2.63
Glibenclamide	10	82.75±4.28	74.35±1.96	70.12±1.53
AQZO1	20	89.17±1.60	82.58±2.15	78.86±5.46
AQZO2	30	83.52±4.98	76.17±2.95	71.15±3.75
ALZO1	20	76.7±2.91	65.85±1.38	60.17±6.78
ALZO2	30	86.2±9.70	76.14±6.18	70.18±5.35

Values are expressed as mean± S.E.M. n=6. Significant values were compared with $p < 0.005$, normal control Vs all groups. Parent thesis indicates % reduction in BGL.

Oral glucose tolerance test (OGTT)

Both the aqueous and alcoholic extracts of *Zingiber officinale* significantly ($P < 0.005$) suppress the rise in FBGL after glucose load (2g/kg) in rats, at first half-an-hour and up

to 2hr time period as compare with other groups extract Glibenclamide on 8th, 15th and 22nd day. While aqueous and alcoholic extracts produced significant reduction in FBGL. Glibenclamide(10mg/kg) showed ($P < 0.005$) significant suppression in FBGL rise at first half-an-hour, 1hr and normalized FBGL within 2hr. The detailed results are summarized in Table No: 3

Table No: 3- Effect of extracts of *Zingiber officinale* on 8th, 15th and 22nd day in normal rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		8 th day	15 th day	22 st day
Normal control	-	89.12±5.15	82.75±1.14	75.36±2.79
Glibenclamide	10	85.17±3.58	75.14±2.93	70.96±4.35
AQZO1	20	86.35±5.18	78.15±1.72	68.52±4.14
AQZO2	30	83.12±3.79	77.37±2.83	65.12±3.65
ALZO1	20	90.35±2.56	82.12±3.96	72.43±2.34

ALZO2	30	75.86±2.42	65.28±2.75	58.99±2.10
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Values are expressed as mean \pm S.E.M. n=6. Significant values were compared with P<0.005. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

Anti-diabetic activity in alloxan induced diabetic rats

Fasting blood glucose levels (FBGL) in normal rats were in range of 90-100 mg/dl. Treatment with alloxan (120 mg/kg, I.P.) had increased the FBGL to range of 252-266 mg/dl after 72 hours. These values on subsequent days got stabilized by day seven on an average between 255 mg/dl.

Changes in the fasting blood glucose levels in different groups are tabulated in Table No: 11. This data shown that blood glucose level of normal control animals has maintained throughout the study period.

The diabetic control group has shown significant increase in fasting blood glucose levels during this 21st day study period. Glibenclamide(10mg/kg) treated group has shown (p<0.05) significant decrease in fasting blood glucose level during 7th, 14th and 21st day of study period.

Effect of Zingiber officinale extracts on antidiabetic activity in alloxan induced diabetic rats

The animals treated with 100 and 200mg/kg of aqueous and alcoholic of different extracts shown significant decrease (P<0.05) in FBGL on 7th, 14th and 21st day of treatment when compare to other groups of animals. The aqueous extracts have reduced more (%) in FBGL when compared to alcoholic extracts except standard group. The detailed results are summarized in TableNo: 4

Table No: 4- Effect of extracts of Zingiber officinale on fasting blood glucose level (FBGL) in Alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		7 th day	14 th day	21 st day
Normal control	-	85.70±6.90	75.82±5.15	68.36±2.98
Diabetic control	10	296.15±21.53	272.52±12.15	265.58±24.39
Glibenclamide	10	263.10±25.19	246.14±85.28	228.14±52.76
AQZO1	20	375.29±68.17	360.35±15.85	332.74±12.63
AQZO2	30	381.26±15.89	368.24±19.85	351.23±21.96
ALZO1	20	292.15±86.75	271.15±59.13	221.85±36.15
ALZO2	30	223.52±16.85	185.15±02.61	153.75±55.89

Values are expressed as mean \pm S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

Oral glucose tolerance test (OGTT) on 8th, 15th and 22nd day

Both the aqueous and alcoholic extracts of *Zingiber officinale* are significantly (P<0.05) suppress the rise in

FBGL after glucose load (2g/kg) in rats, at first half-an-hour and upto 2hr time period as compare with other groups extract Glibenclamideon 8th,15th and 22nd day. While aqueous and alcoholic extracts produced significant reduction in FBGL. Glibenclamide(10mg/kg) showed (P<0.05) significant suppression in FBGL rise at first half-an-hour, 1hr and normalized FBGL within 2hr. The detailed results are summarized in TableNo: 5.

Table No: 5- Effect of extracts of Zingiber officinale on 8th,15th and 22nd day in Diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		8 th day	15 th day	22 st day
Normal control	-	85.12±2.85	75.28±1.91	63.14±2.89
Diabetic control	10	283.25±11.71	251.14±20.95	221.39±19.86
Glibenclamide	10	365.89±75.50	286.15±39.52	275.93±15.78
AQZO1	20	262.13±72.89	232.71±25.53	198.17±13.99
AQZO2	30	283.82±10.27	262.13±10.78	242.89±15.32
ALZO1	20	264.18±93.56	221.80±96.15	186.55±11.89
ALZO2	30	363.12±10.28	321.18±25.98	282.15±19.12

Values are expressed as mean \pm S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

DISCUSSION

Despite the fact that diabetes has high prevalence, morbidity and mortality globally, it is regarded as non

curable but controllable disease. Different synthetic drugs, plant remedies and dietary modification play an effective role in the reduction of the suffering that it causes. The potential role of medicinal plants as antidiabetic agents has been reviewed by several authors. In order to identify the plants with antidiabetic properties various plants have been tested *in-vivo* using animal models, for example rats, against the complications caused by inducers of diabetes, and it has

been established that many plants possess the potential to lower the fasting blood glucose levels and besides help in improving other diabetic complications. The sustained reduction in hyperglycemia automatically decreases the risk of other major complications of diabetes. Effective glucose control is the key for preventing or reversing the diabetic complications and improving the quality of life of the diabetics.

Many natural active compounds have been isolated from plants of different species. These active principles are complex Alkaloids, Tannins, Anthraquinones, Flavonoids, Saponins, Triterpenes, Sterols, Coumarin and others. These compounds have been shown to produce potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities. These effects might be achieved by facilitating insulin release from pancreatic β -cells, inhibiting glucose absorption in gut, stimulating glycogenesis in liver and/ or increasing glucose utilization by the body. These compounds may also exhibit Anti-Inflammatory, Antibacterial, Antifungal and Cardioprotective activities, and restore enzymatic functions, repair and regeneration of pancreatic islets and the alleviation of liver and renal damage.

Crude aqueous and alcoholic extracts of rhizome of *Zingiber officinale* at a dose of 20 and 30mg/kg showed significant effect on the glucose tolerance of rats and it also showed reduction in the fasting blood glucose levels of the normoglycaemic rats, thus revealing the hypoglycaemic nature of the extracts. The effect was more pronounced for both extracts. These findings indicate that the extracts might be producing hypoglycaemic effect by a mechanism independent from the insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption.

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β -cells of islets of langerhans. It is a generator of free radicals of oxygen which cause extensive DNA damage. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. As the hyperglycemia induced by alloxan falls under category of mild diabetes and may reverse after a few weeks, the hypoglycemic effect of the plant in hyperglycemic rats was studied during 22 days treatment. The difference observed between the initial and final fasting serum glucose levels of extract treated hyperglycemic rat's revealed antihyperglycemic effect of rhizome of *Zingiber officinale* throughout the period of study. The effect of the extracts was compared to that of reference standard, Glibenclamide and was found to be significant.

Phytochemical analysis of extracts of rhizome of *Zingiber officinale* revealed the presence of secondary metabolites that have been shown to possess antidiabetic effect in other plants. Flavonoids, alkaloids and Steroids which were responsible for the antidiabetic effect in other plants were also detected in the extracts of this plant. The presence of phenols in the plant could also be responsible for the antidiabetic effect have been shown to prevent the destruction of β -cells by inhibiting the peroxidation chain

reaction and thus they may provide protection against the development of diabetes. Extracts of rhizome of *Zingiber officinale* appear to be attractive materials for further studies leading to possible drug development for diabetes. Development of phytomedicines is relatively inexpensive and less time consuming; it is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years.

CONCLUSION

The study was performed to find out the beneficial effects of two different extracts of rhizome of *Zingiber officinale* in normoglycaemic rats and alloxan induced diabetic rats and the results reveal that the plant has beneficial effects on blood glucose levels.

In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of rhizome of *Zingiber officinale*. Alkaloids, Tannins, Anthraquinones, Flavonoids, Saponins, Triterpenes, Sterols, Coumarin.

The aqueous and alcoholic extracts had hypoglycemic activity because the presence of flavonoids which are rich in treatment of hypoglycaemia with less side effects. Flavonoids might be producing hypoglycaemic effect by a mechanism independent from insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. The present study *Zingiber officinale* of both aqueous and alcoholic extracts was showed significant effect on glucose tolerance and also showed reduction in fasting blood glucose levels in normal diabetic rats.

The data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed. It was observed that significant lowering of sugar in aqueous and alcoholic extract. The administration of different extracts at a dose of 20 and 30 mg/kg showed significant anti-hyperglycaemic effect at 22nd day which was evident from the 7th day onwards as compared to standard. The aqueous and alcoholic extract of *Zingiber officinale* has showed better anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats are shown in table. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Glibenclamide. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 22nd Day compare to the initial. The aqueous and alcoholic extracts of 20 and 30mg/kg body weight shows significant ($P < 0.05$), effect.

Results of anti-diabetic activity in normal and alloxan induced rats the extracts established the scientific basis for the utility of these plants in the treatment of diabetes. The extracts have shown significant reduction in blood glucose levels in normal and alloxan induced diabetic rats and produced maximum anti-diabetic activity and are higher than the hypoglycemic activity of Glibenclamide in the diabetic rats. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic

secretion or increasing the glucose uptake. In conclusion, these extract showed significant anti-diabetic effect in normal and diabetic rats after administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of these plants in the treatment of diabetes stands confirms.

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