

## Research Article

## Evaluation of the Anti-Diabetic Activity of Glibenclamide in Combination with Ginger in Streptozotocin Induced Diabetes

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### ABSTRACT

Diabetes is a common and very prevalent disease and a major health problem worldwide. It has been reported to be the major cause of blindness, kidney failure, lower-extremity amputation cardiovascular diseases and premature mortality. Diabetes has increasing cases in rural and poor populations throughout the world. The possibility of its management by the oral administration of hypoglycemic agents has stimulated great research interest in over the years. Considering that diabetes mellitus is a heterogeneous disorder with multiple causes, the combination of therapeutic agents aimed at specific patho-biological pathways of diabetes and its complications may result in a better and more effective management of this disorder. The present study was conducted to evaluate the anti-diabetic activity of Glibenclamide in combination with Ginger. Diabetes was induced by administration of Streptozotocin (i.p 65 mg/Kg). Biochemical parameters were evaluated in the study. When Glibenclamide is used in combination with Ginger, even at sub therapeutic level of Glibenclamide showed similar effects as that of therapeutic dose of Glibenclamide.

**Keywords:** Diabetes, Glibenclamide, Streptozotocin, Biochemical.

### INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia (high blood sugar) with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both<sup>1</sup>. Diabetes is a common and very prevalent disease affecting the citizens of both developed and developing countries. The greatest increase in prevalence is however expected to occur in Asia and Africa, where more patients will likely be found by 2030. 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. The possibility of its management by the oral administration of hypoglycemic agents has stimulated great research interest in over the years. Though different types of oral hypoglycemic 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 hypoglycemic activity<sup>2</sup>. The increasing prevalence of this disorder not only poses severe medical implications but also has financial consequences due to the costs of managing this disorder and its associated complications. Considering that diabetes mellitus is a heterogeneous disorder with multiple causes, the combination of

therapeutic agents aimed at specific patho-biological pathways of diabetes and its complications may result in a better and more effective management of this disorder. Even though the currently available drugs may be valuable in the management of diabetes mellitus, these drugs have limitations due to undesirable adverse effects such as hypoglycemia, weight gain, secondary failure, and inability to arrest pancreas degeneration or diabetic complications which have been linked to oxidative stress<sup>3</sup>.

Several classes of allopathic drugs namely Sulphonylureas, Biguanides, Thiazolidinediones,  $\alpha$ -glucosidase inhibitors and semi synthetic Insulin are extensively used to control the hyperglycemia. These drugs exert different modes of action to reduce the elevated blood glucose, but none of them were found to be effective in providing complete glycaemic control in diabetic patients, moreover these drugs also exert their individual adverse effects such as pregnancy related complications and hypoglycemic episodes when administered to the diabetic patients<sup>4</sup>. Hence there is a need for an anti-diabetic drug/formulation that can provide optimal glycaemic control without producing unwanted side effects in the host system<sup>5</sup>.

### MATERIALS AND METHODS

#### Experimental Animals

Healthy Wistar albino rats (150–200 g) of either sex were used for the experiments.

They were maintained under standard conditions (temperature  $22 \pm 2^\circ\text{C}$ , relative humidity  $60 \pm 5\%$  and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. Experiments were conducted between 10:00 to 15:00 h. All experimental protocols will be reviewed and approved by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment and the care of the laboratory animals will be taken as per the CPCSEA regulations.

### Chemicals

All the chemicals and reagents used were of analytical grade and were purchased from YARROW CHEM, LOBA CHEM, HIMEDIA and Agappe diagnostics.

### Preparation of aqueous extract of Ginger

Aqueous Ginger extract was prepared from locally available Ginger rhizomes. Ginger rhizomes (500g) were peeled on crushed ice and were cut in to small pieces and homogenized in 750ml cold, sterile 0.9% NaCl solution and 250ml ice cold water to make the volume 1000ml. The homogenization was carried out in a blender for 12 minutes. The homogenized mixture was filtered three times through cheese cloth. The filtrate was centrifuged at 2000rpm for 10 min and the clear supernatant fraction was separated and volume made up to 1000ml with normal saline. The concentration of this Ginger preparation was considered to have 500mg/ml on the basis of the weight of the starting material according to the formula. The extract was stored in sample tubes at  $-4^\circ\text{C}$  until fed to rats<sup>6</sup>.

### Anti-diabetic Activity

#### Streptozotocin induced anti-diabetic activity<sup>7,8</sup>

Fasting blood glucose was determined after depriving food for 16 h with free access to drinking water. Hyperglycemia was induced by single i.p injection of 65 mg/kg of STZ in citrate buffer, freshly prepared and injected within 5 minute of preparation to prevent degradation. After administration of STZ the animals had free access to feed and water *ad libitum*. The development of hyperglycemia in rats was confirmed by fasting blood glucose estimation 48 h post STZ injection wherein animal were fasted overnight again for blood collection from tail vein. The rats with fasting blood glucose level of above 200 mg/dl at 48 h after STZ

injection were considered diabetic and included in the study.

### Citrate buffer (pH4.4, 0.1M)

Citric acid monohydrate 0.6306 g was dissolved in 50 ml of distilled water. Trisodium citrate 0.7352 g was dissolved in 25 ml of distilled water. 28 ml of Citric acid monohydrate and 22 ml solution were taken and mixed together. It is made up to a volume of 1000 ml with distilled water. The pH of the solution was adjusted to pH 4.4.

### Experimental design

Animals will be randomly divided into 4 groups of 6 each and assigned as below.

Group I: Vehicle control (Citrate buffer).

Group II: Diabetic control (Streptozotocin 65mg/Kg).

Group III: Diabetic + Glibenclamide (1mg/Kg)

Group IV: Diabetic + Glibenclamide (0.5 mg/Kg) + Ginger (500mg/Kg)

### The parameters studied were as follows

- Biochemical parameters such as
  - i. Fasting blood glucose
  - ii. Serum glutamic pyruvate transaminase (SGPT)
  - iii. Serum glutamic oxaloacetate transaminase (SGOT)
- Endogenous antioxidant parameters includes
  - i. Superoxide dismutase (SOD)
  - ii. Catalase (CAT)

### Estimation of Biochemical Parameters

The above treatment was carried out in each group of animals for both models for 21 days. Fasting blood glucose was measured using select single touch glucometer. Blood samples were withdrawn under mild anesthesia from tail tip of the overnight fasted animals on 1<sup>st</sup>, 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> day. On 21<sup>st</sup> day the blood was collected by retro orbital puncture and the serum was obtained by centrifuging the blood samples at 3000 rpm for 10 min and they were used for estimation of SGPT, SGOT by using a corresponding kit from Agappe Diagnostics Pvt. Ltd and the intensity of the coloured complex formed after treating with these reagents were estimated in semi-auto analyzer.

### Estimation of Antioxidant parameters

#### Tissue preparation

- Animals were sacrificed by cervical dislocation and were perfused transcardially with an ice-cold saline.
- The whole liver was perfused in situ with ice-cold saline, dissected out,

blotted dry and immediately weighed.

- A liver homogenate was prepared with ice-cold saline-EDTA.
- The homogenate was centrifuged at 10,000 rpm for 10 min and the pellet discarded. The supernatant was again centrifuged at 20,000 rpm for 1 hour at 40°C.

### Superoxide Dismutase<sup>9</sup>

2.8 ml of sodium carbonate buffer (0.05 mM) and 0.1 ml of tissue homogenate or sucrose (Blank) was incubated at 30°C for 45 minutes. Then, the absorbance was adjusted to 0 to sample. Thereafter, the reaction was initiated by adding 10µl of adrenaline solution (9mM). The change in absorbance was recorded at 480nm for 8-12 minutes. Throughout the assay, the temperature was maintained at 30°C. The results are expressed as unit (U) of SOD activity per mg of protein.

### Catalase<sup>10</sup>

The liver homogenates containing 5µg total protein was mixed separately with 700µl, 5mM hydrogen peroxide and incubated at 37°C. The disappearance of peroxide was observed at 240nm for 15min. One unit of catalase activity is that which reduces 1µmol of hydrogen peroxide per minute.

### Statistical analysis

Results of biochemical estimation were reported as mean ± S.E.M. The total variation present in a data was analyzed by one way analysis of variance (ANOVA). P value less than 0.05 was considered as statistically significant.

## RESULTS AND DISCUSSION

### Effect on Fasting Blood Glucose Level

Fasting blood glucose (FBG) level was within the range of 80-90 mg/dl in all the groups prior to STZ administration. Treatment with STZ in normal saline (65 mg/kg, i.p) had increased the FBG level above 290 mg/dl after 48 h. Treatment with Glibenclamide and Glibenclamide in combination with Ginger significantly normalized the elevated blood glucose level as shown in Table 1.

### Estimation of Serum biomarkers

After 21 days of experiment, serum biomarkers such as SGPT and SGOT level were significantly elevated in diabetic control group. In animals treated with Glibenclamide and Glibenclamide in combination with Ginger, SGPT and SGOT levels were decreased significantly ( $p < 0.001$ ,  $p < 0.01$  respectively) as compared to the diabetic control (Table 2).

### Antioxidant parameters

From antioxidant studies, it was found that STZ induced diabetic control animals showed a significant decrease in the levels of SOD and CAT as compared to normal control. Standard group treated with Glibenclamide and test group treated with Glibenclamide+ginger combination showed significant increase in CAT and SOD as compared to diabetic control (Table 3)

The present study was undertaken to evaluate the anti-diabetic activity of a standard synthetic anti-diabetic drug Glibenclamide in combination with an herbal anti-diabetic drug Ginger in diabetic rats.

In the study, the blood glucose levels in STZ treated rats were significantly increased as compared to normal rats. Where as the group treated with standard drug Glibenclamide (1mg/Kg) and those treated with Glibenclamide(0.5mg/Kg)+ginger combination showed significant reduction in glucose levels. The results indicate that the combination Glibenclamide and Ginger could lead to increase in the effect of Glibenclamide that may be helpful to reduce the dose of Glibenclamide and to minimize the adverse effects as well as maintain enhanced therapeutic hypoglycemic effect.

Insulin deficiency leads to various metabolic aberrations in the rats; the rise in blood glucose level is accompanied by increase in SGPT and SGOT level. In this present study, Glibenclamide and Glibenclamide+ginger combination treated serum reports showed significant reduction in SGPT and SGOT level which indicate that combination therapy with sub therapeutic dose of Glibenclamide and Ginger is comparable to effect of Glibenclamide at its therapeutic dose.

Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed in diabetes by glucose oxidation, protein glycation, and the subsequent degradation of glycated proteins. High levels of free radicals and the simultaneously declined antioxidant enzyme levels lead to cell damage, inactivation of enzymes and lipid peroxidation. Superoxide dismutase and catalase play an important role in the detoxification of super oxide anion and H<sub>2</sub>O<sub>2</sub> respectively. In present study, Catalase and SOD which are most important antioxidant enzymes were found to be decreased in diabetic control group with induction of diabetes, but treatment with Glibenclamide and Glibenclamide+ginger combination level of both enzymes were found to be increased. Glibenclamide+Ginger combination treatment

showed more increased levels of SOD and Catalase than that of Glibenclamide treatment.

### CONCLUSION

The findings of the study suggest that even sub therapeutic dose of Glibenclamide (0.5mg/Kg) in combination with Ginger (500mg/Kg) showed antidiabetic activity comparable to that of Glibenclamide alone at its therapeutic dose (1mg/Kg). So the dose of Glibenclamide can be reduced when using in combination with Ginger and side effects due to Glibenclamide can be minimized. Hence it

can be concluded that when Glibenclamide is used in combination with Ginger, the dosage of Glibenclamide can be reduced up to half of the dose than it is used alone. So it can be considered as a safe supplementary in management of diabetes mellitus.

### ACKNOWLEDGEMENTS

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**Table 1.0: Serum glucose in STZ induced diabetic rats**

GROUPS	Blood glucose level (mg/dl)				
	Before diabetic induction	Day 0	Day 7	Day 14	Day 21
Normal Control	85.62±1.38	86.10±1.86	86.34±1.92	87.37±2.56	85.25±3.16
Diabetic control	85.08±4.58	298.45±2.26	315.72±5.367	311.87±4.56	321.91±5.23
Glibenclamide (1 mg/Kg)	84.93±1.132	295.16±2.56	244.21±6.54**	189.59±4.89**	127.36±3.45**
Glibenclamide (0.5mg/Kg) + Ginger	85.406±4.67	304.08±7.56	268.56±4.32**	192.45±5.92**	140.46±4.67**

Values are mean ± SEM (n=6 except in control) one way ANOVA followed by Dunette's test. Where \*\* represents highly significant at  $p < 0.01$  and \*\*\* represents very significant at  $p < 0.001$  as compared to diabetic control.

**Table 2: SGPT and SGOT levels in diabetic rats**

Group	SGPT	SGOT
Normal	58.61±2.65	62.63±3.75
Diabetic	139.48±2.38	148.72±1.05
Glibenclamide (1mg/Kg)	79.52±3.54***	76.57±1.95**
Glibenclamide (0.5mg/Kg) + Ginger	92.80±2.11***	84.64±1.69**

Values are mean ± SEM (n=6 except in control) one way ANOVA followed by Dunette's test. Where, \*\* represents highly significant at  $p < 0.01$ , \*\*\* represents very significant at  $p < 0.001$  as compared to diabetic control.

**Table 3: Serum antioxidant parameters SOD and CAT in diabetic rats**

Group	SOD	CAT
Normal	11.36±3.25	6.45±2.57
Diabetic	3.28±1.78	2.35±1.15
Glibenclamide (1mg/Kg)	9.52±2.54***	4.46±2.75**
Glibenclamide (0.5mg/Kg) + Ginger	9.98±1.32***	5.02±2.96***

Values are mean ± SEM (n=6 except in control) one way ANOVA followed by Dunette's test. Where, \*\* represents highly significant at  $p < 0.01$ , \*\*\* represents very significant at  $p < 0.001$  as compared to diabetic control

## ANNEXURE

## 1. Institutional Animal Ethical Committee Certificate

**SRINIVAS COLLEGE OF PHARMACY**

(A UNIT OF SRINIVAS INSTITUTE OF HEALTH SCIENCES)

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Ref: SCP/CPCSEA/P11/F150/2011

Date: 13/12/2011

To,

Mr.Arshad Mohamed.M,  
P G Student,  
Department of Pharmacology,  
Srinivas College of Pharmacy,  
Valachil, Mangalore – 574143.

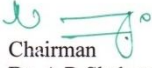
Dear Mr.Arshad Mohamed.M,

We have received your below mentioned research proposal for the consideration for approval of the ethical committee. After careful securitization of the same, I hereby inform you of the decision of the committee to sanction approval to carry out the said work.

Kindly note that any changes in the proposed work should be brought to the notice of the committee and necessary approval should be obtained.

Thanking You.

Yours faithfully,

  
Chairman  
Dr. A R Shabaraya



Topic: Evaluation of Anti – diabetic activity of glibenclamide in combination with Ginger.

Investigators :. Mr.Arshad Mohamed.M., Mr.Ashok Shenoy M..

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## REFERENCES

1. Erasto P, Adebola PO, Grierson DS and Afolayan AJ. An ethanobotanical study of plants used for the treatment of diabetes in the eastern cape province, South Africa. *African J Bio tech.* 2005;4(2):1458-60.
2. Osinubi AA, Ajayi OG and Adesiyun AE. Evaluation of the anti-diabetic effect of aqueous leaf extracts of *Tripinanthus butungil* in male spragne dawley rats. *Med J Islamic World Academy. Sci* 2006;16(1):41-7.
3. Erejuwa OO, Sulaiman SA, Wahab MS, Sirajudeen KNS, Salleh MS and Gurtu S. Effect of glibenclamide alone versus glibenclamide and honey on oxidative stress in pancreas of streptozotocin- induced diabetic rats. *Int J Applied Research in Natural products.* 2011; 4(2):1-10.
4. Nolte MS and Karam JH. Pancreatic hormones and antidiabetic drugs. In: Katzung BG, editor. *Basic and clinical pharmacology.* 10<sup>th</sup> ed. New Delhi: Mc Graw Hill Lange; 2007;683-4.
5. [http://www.worlddiabetescongress.org/files/docs/WDC\\_programme\\_book.pdf](http://www.worlddiabetescongress.org/files/docs/WDC_programme_book.pdf).
6. Sharma N and Garg V. Anti-hyperglycemic and anti-oxidative potential of hydro alcoholic extract of *Butea monosperma* Lam flowers in alloxan-induced diabetic mice. *Indian J Exp Biol.* 2009;47:571-6.
7. Gangadevi T, Babu V and Subramoniam A. Antidiabetic activity of ethanol extract of *Cassia kleinii* leaf in streptozotocin induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian J Pharmacol.* 2003;35:290-6.
8. Anand P, Murali KY and Tandon V. Preliminary studies on anti-hyperglycemic effect of aqueous extract of *Brassia nigra* in streptozotocin induced diabetic rats. *Indian J Exp Biol.* 2007;45:696-701.
9. Esterbauer H and Cheeseman KH. Determination of aldehydic lipid peroxidation products: Malondialdehyde and 4-hydroxynonenal. *Meth Enzymol.* 1990;186:407-21.
10. Aebi H. Catalase in *Methods of enzymatic analysis.* 2<sup>nd</sup> ed. New York: Verlag Chemie Weinhei Academic Press, Inc; 1974;673-84