



## Antidiabetic and Antihyperlipidemic Effects of Aqueous Extract of Polyherbal Formulation(Ziabeetein Powder) in Experimental Animals

Dr. Sameena Alam<sup>1\*</sup>, Abdullah Baig<sup>1</sup>, Surabhi Kavya Reddy<sup>1</sup>, Madhavi Kolan Reddy<sup>1</sup>, Mohammed Mohiuddin<sup>1</sup>, Dr. M. Venkata Reddy<sup>1</sup>, Dr. Rajesh Kumar Gupta<sup>2</sup>

<sup>1</sup> Sree Dattha Institute of Pharmacy, Sheriguda, Hyderabad, A.P, India

<sup>2</sup> Gautam Buddha University, Greater Noida, U.P, India

Received on: 05/02/2013

Accepted on: 21/02/2013

### ABSTRACT

Ayurveda and other traditional medicinal system describe a number of plants used as herbal drugs for the treatment of diabetes. These herbal drugs play an important role as alternative medicine due to low cost and less or no side effects. The aim of our present study was to investigate the polyherbal formulation (Ziabeetein powder) for antidiabetic effect in streptozotocin induced diabetes in wistar rats. The polyherbal formulation (Ziabeetein powder) was also investigated for its effect on serum lipid profile. Streptozotocin toxicant cause a symptomatic increase in serum glucose, cholesterol, triglycerides, HDL and creatinine levels and this abnormal growth was found to get decrease when aqueous extract of Ziabeetein powder was administered to the mild diabetic animals for the 14 days of study at the dose of 250 and 500 mg/kg body weight. In case of diabetic animals fasting blood glucose (FBG) levels of treated animals reduced by 25.7% after 14 days of treatment with the aqueous extract of Ziabeetein. A fall of 23% of total cholesterol (TC) and 38% in triglycerides (TG) levels were also observed in diabetic rats, respectively. Continuously feeding the extract for 14 days also increased the HDL cholesterol level by 37% in diabetic rats as compared with control animal group. The serum creatinine levels in diabetic animals was found to be in normal range after 14 day of treatment. The phytochemical estimation of various extracts of Ziabeetein powder showed the presence of alkaloids, flavonoids, glycosides and tannis which serve to be good antioxidants in nature. All these results clearly indicate that aqueous extract of Ziabeetein formulation has favorable hypoglycemic effect along with significant hypolipidemic effects. Therefore, we conclude that this polyherbal formulation is having antidiabetic, antilipidemic activity and can be used as a therapeutic agent.

**Key Words:** Polyherbal formulation, Streptozotocin, Antidiabetic, Antihyperlipidemia, Glibenclamide.

### INTRODUCTION

Diabetes mellitus is an endocrine disorder that is characterized by hyperglycemia<sup>1</sup>. Type II diabetes is the commonest form of diabetes constituting 90% of the diabetic population<sup>2</sup>. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin.<sup>3</sup> The countries with the largest number of diabetic patients in the year 2025 will be India, China and United States<sup>4</sup>. Reasons for this increase include increase in sedentary lifestyle, lack of exercise, obesity, stress, etc. Clinically, the disease is associated with a number of chronic complications including nephropathy, neuropathy, retinopathy and cardiovascular diseases.<sup>5</sup> Diabetes can lead to cardiovascular diseases like stroke, high levels of cholesterol in blood etc. Hyperlipidemia in association with insulin resistance is common in patients with type 2 diabetes mellitus (DM). Many oral hypoglycemic agents, such as biguanides and sulfonylureas are available along with insulin for the treatment of diabetes mellitus, but these

synthetic agents can produce serious side effects, and in addition, they are not suitable for use during pregnancy<sup>6-9</sup>. Therefore the search for the safe and more effective agents has contributed to be an important area of active research. The World Health Organisation (WHO) has also recommended the evaluation of the plant's effectiveness in conditions where we lack safe modern drugs<sup>10</sup>. This has led an increasing demand of research on antidiabetic natural products which produces minimal or no side effects<sup>11</sup>. India is a country with a vast reserve of natural resources and a rich history of traditional medicine<sup>12</sup>. Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity<sup>13-15</sup>. The hypoglycemic activity of a large number of these plants/plant products has been evaluated and confirmed in animal models as well as in human beings<sup>16-23</sup>. Plants often contain substantial amounts of antioxidants, flavonoids and tannins.<sup>24</sup> The active principles present in medicinal plants have been reported to possess pancreatic beta cells regenerating, insulin releasing and fighting the problem of

insulin resistance.<sup>25</sup> Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues and inhibit glucose absorption from intestine and glucose production from liver.<sup>26</sup>

By considering the information in view, Ziabateen powder an indigenous polyherbal formulation was used in present study to evaluate its hypoglycemic and hypolipidemic activities in male wistar rats.

## MATERIALS AND METHODS

### Drugs

The polyherbal formulation Ziabateen powder was procured from Ahmed and Company Unani Pharmacy, Gulzar Houz, Hyderabad, India. Streptozotocin was purchased from Sigma Aldrich.

### Composition of Ziabateen Powder

Each 100 gm of formulation contains i) Methi(12.8gm), ii) Hing (4gm), iii) Jamoon (12.8gm), iv) Kavita (12.8gm), v) Neem (6.4gm), vi) Karela (12.8gm), vii) Gudmarg (12.8gm), viii) Peekha (12.8gm), ix) Filfil siyah (6.4gm), x) Pambadana (6.4gm).

### Preparation of Extracts

The coarse powder was macerated for 48 hours using different solvents. The filtrate obtained was evaporated under reduced pressure to dryness. The aqueous extract was selected and used for subsequent experimentation.

### Pytochemical Study for Polyherbal Drug

The different extracts obtained were subjected to phytochemical screening for the presence of flavanoids, tannins, alkaloids, carbohydrates, phytosterols, triterpenoids, saponins according to standard procedures<sup>27</sup>.

### Acute Toxicity Studies

The acute toxicity study was carried out in male wistar rats by the "fix dose" method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. The fixed dose method as in annex 2d, test procedure with a starting dose of 2000 mg/kg body weight, was adopted. The animals in two groups of six each were taken and fasted overnight. The next day the product (suspended in 5% tween 80 solution) was administered orally at a dose level of 2000 mg/kg body weight and 5000 mg/kg body weight. Then the animals were observed continuously for 3 hours for general behavioral, neurological, and autonomic profiles and then every 30 minutes for next 3 hours and finally for mortality after 24 hours till 14 days. No mortality was observed at the end of 14 day.

### Experimental Design

In the investigation, a total of 30 rats (24 diabetic surviving rats and 6 normal rats) were taken and divided into five groups of 6 rats each. The group I was normal rats, group II serves as a diabetic control, group III receiving aqueous extract of polyherbal formulation (250 mg/kg of body weight), group IV receiving aqueous extract of polyherbal formulation (500 mg/kg of body weight) and group V serves as a positive control receiving the standard drug (Glibenclamide) at the dose of (10mg/kg of body weight). The experiments were carried out according to guidelines of

'Committee for Prevention and Control of Scientific Experimentation on Animals'(CPCSEA) New Delhi, numbered 1636/PO/a/12/CPCSEA and the procedures were approved by Institutional Animal Ethics Committee (IAEC), Sree datta institute of Pharmacy, Sheriguda, Hyderabad, A.P, India.

### Induction of Diabetes

Animals were fasted for 24 hours then a single intra peritoneal injection of freshly prepared streptozotocin (40 mg/kg dissolved in 0.9% in citrate buffer) was injected.

### Determination of Antidiabetic Activity

The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 1<sup>st</sup>, 7<sup>th</sup>, and 14<sup>th</sup> day of the experiment.

### Assessment of Antidiabetic Activity on Glucose Tolerance in Mildly Diabetic Rats

The anti-diabetic effect of aqueous extract of polyherbal formulation (Ziabateen powder) in mild-diabetic rats was assessed by improvement in glucose tolerance. The rats were divided into six groups. Group I control, received vehicle (distilled water) only, whereas variable doses of 250, and 500 mg/kg b wt of extract was given orally to group II and III respectively. Blood glucose levels were first checked after 90 min of treatment, considered as "0" h value, and then 2g/ kg b wt glucose was given orally to all the groups. Blood glucose levels were further checked up to 3 h at regular intervals of 1 h each, considered as 1, 2, and 3 h values. The results were compared with group V rats, which were treated with 10 mg/kg b wt of Glibenclamide (synthetic hypoglycemic agent).

### Biochemical Determination

After the 14<sup>th</sup> day of treatment, blood was collected from the orbital plexus of overnight fasted rats. The serum was separated and creatinine, triglycerides, HDL and cholesterol level were determined by using creatinine mono reagent test kit, triglycerides test kit, and HDL and cholesterol test kit (Span diagnostic Ltd, Surat), respectively.

### Statistical Analysis

All the group data were statistically evaluated using student's t-test, expressed as the mean  $\pm$  S.D. from six rats in each group. P-value of 0.05 or less was considered to be significant.

## RESULTS AND DISCUSSION

The preliminary photochemical study of aqueous extract of Ziabateen contains a range of active phytochemical agents, which include alkaloids, flavonoids and tannins as reported in table 1. These principles are known to be bioactive for the management of diabetes.<sup>28-30</sup> It is known that certain alkaloids and flavonoids exhibit hypoglycemic activity<sup>31,32</sup> and are also known for their ability of beta cell regeneration of pancreas<sup>33,34</sup>. Tannins have also shown to decrease blood sugar in experimental animal models<sup>35,36</sup>. Thus, the significant antidiabetic effect of aqueous extract of polyherbal formulation may be due to the presence of more than one antihyperglycemic principle and/or their synergistic effects.

Treatment of streptozotocin induced diabetic rats with aqueous extract of Ziabateen showed improvement in many parameters. Fasting blood glucose levels, blood glucose levels and lipid profiles were near normal, reversing pathological changes known to occur in diabetes. The effect on FBG on repeated oral administration of aqueous extract of Ziabateen in streptozotocin induced diabetes is shown in table 2. Administration of most effective dose 500 mg/kg body weight of aqueous extract of Ziabateen produced a marked antihyperglycemic effect in treated diabetic rats. There was a fall of 25.7% in FBG at the 14<sup>th</sup> day of treatment. CV diseases are listed as the cause of death in 65% people suffering from diabetes<sup>37</sup>. High levels of TC and more importantly LDL cholesterol are major coronary risk factors<sup>38,39</sup>. Administration of aqueous extract of Ziabateen at a dose of 500 mg/kg body weight to diabetic rats for 14 days lowered TC by 23% as shown in table 3. This is an important finding of this experiment as diabetes is

associated with coronary complications, which is the major cause of morbidity and deaths in diabetic subjects<sup>40,41</sup>. Several studies have shown that an increase in HDL-cholesterol is associated with a decrease in coronary risk<sup>42</sup> and most of the drugs that decrease total cholesterol also decrease HDL cholesterol.<sup>43</sup> In the present study the aqueous extract of Ziabateen at a dose of 500mg/kg body weight enhances the cardio protective lipid HDL by 27%. According to the recent studies which suggest that TG itself is independently related to coronary heart disease<sup>44,45</sup> and most of the antihypercholesterolemic drugs do not decrease TG levels but aqueous extract of Ziabateen lowered it by 38% after 14 days of treatment. Its effect on diabetic hypertriglyceridemia could be through its control of hyperglycemia. The above studies indicates that the aqueous extract of Ziabateen has favorable effect in bringing down the severity of diabetes.

**Table-1:** Phytochemical study of different extracts of polyherbal formulation (Ziabateen powder)

S. No.	Test	Type of Extract				
		Chloroform	Petroleum Ether	Ethyl Acetate	Ethanol	Aqueous Extract
1	Alkaloids	-ve	+ve	-ve	+ve	+ve
2	Coumarines	+ve	+ve	+ve	+ve	+ve
3	Carbohydrates	+ve	+ve	+ve	+ve	+ve
4	Flavonoids	+ve	+ve	+ve	+ve	+ve
5	Glycosides	-ve	-ve	-ve	-ve	-ve
6	Saponins	+ve	+ve	+ve	+ve	+ve
7	Steroids and Pytosteroids	-ve	-ve	-ve	-ve	-ve
8	Tannins	-ve	-ve	-ve	+ve	+ve
9	Terpinoids	-ve	-ve	-ve	-ve	-ve

**Table 2:** Effect of administration of Ziabateen powder for 14 days on serum fasting glucose levels in diabetic rats.

Treatment	Fasting Blood Glucose Level (FBG)
Normal (Group 1)	76 ± 2.0
Control Diabetic rats (Group 2)	101 ± 1.9
Diabetic rats + aqueous extract 250mg (Group 3)	81 ± 2.1*
Diabetic rats + aqueous extract 500mg (Group 4)	75 ± 3.8*
Diabetic rats + Std. (Glibenclamide)(Group 5)	70 ± 4.0*

\*  $P < 0.01$  as compared to control group

**Table 3:** Effect of administration of Ziabateen for 14 days on serum cholesterol levels, serum triglycerides, serum HDL and serum creatinine levels in diabetic rats

Experimental Groups	Serum Cholesterol	Serum Triglycerides	Serum HDL	Serum Creatinine
Normal Group 1	90 ± 0.6	49 ± 0.9	45 ± 2.9	53 ± 1.9
Control Group 2	137 ± 0.9	97 ± 0.8	27 ± 1.9	46 ± 4.3
Diabetic rats + Aqueous extract 250 mg Group 3	125 ± 0.7	74 ± 0.7	28 ± 1.8*	45 ± 1.9
Diabetic rats + Aqueous extract 500 mg Group 4	105 ± 0.5	60 ± 1.3*	37 ± 1.9*	50 ± 1.8*
Diabetic rats + Std. (Glibenclamide) Group 5	96 ± 0.8	53 ± 1.6	43 ± 0.9*	40 ± 1.5*

$P < 0.01$  as compared to control group

**CONCLUSION**

Type II diabetes mellitus was induced in male Wistar rats by administering streptozotocin (40 mg/kg, i.p). The selected animals were then administered with Ziabateen powder extract (250mg/kg, body weight, p.o, 500mg/kg, body weight, p.o) and glibenclamide (10 mg /kg, body weight, p.o) for a period of 14 days. The animals were then sacrificed after 14 days and the blood was collected for various biochemical estimations. Diabetic control rats exhibited elevated serum glucose, serum cholesterol, serum triglycerides and decreased serum HDL-cholesterol, whereas the extract administered rats exhibited decreased serum glucose, serum cholesterol, serum triglycerides and increased serum HDL-cholesterol.

**ACKNOWLEDGEMENT**

The authors are grateful to the Chairman Mr. G. Panduranga Reddy, Sree Dattha group of Institutions, Hyderabad, India for providing financial assistance and facilities for the above work.

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**\*Corresponding Author:**

*Dr. Sameena Alam,*

M.Sc , Ph.D, Associate Professor,

Sree Dattha Institute of Pharmacy, Sheriguda(v),  
Ibraimpatnam (M), Nagarjuna Sagar Road,  
Dist.Rangareddy, Pin-501510 (A.P), India

*Email:* samina121@gmail.com

*Mobile No.:* 08414-320919, 9346090280, 9247098884/83