

EVALUATION OF ANTI-DIABETIC AND ANTI-LIPIDIMIC POTENTIAL OF KALONJI SUGAR POWDER WATER EXTRACT IN STZ INDUCED DIABETIC RATS

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ABSTRACT

Natural therapies from the medicinal plants are known for their safe and alternative therapies in the treatment of diabetes mellitus. The aim of our present study was to investigate the polyherbal formulation (kalonji sugar powder) for antidiabetic effect in streptozotocin induced diabetes in wistar rats. The polyherbal formulation (kalonji sugar powder) was also investigated for its effect on serum lipid profile. Two doses of the water extract were chosen viz. 250 mg/kg bwt and 500 mg/kg bwt in wistar rats. The aqueous extract at a dose of 500 mg/kg bwt showed significant ($p < 0.01$) reduction in fasting blood glucose level to 61.3% after 14 days of treatment. A preliminary phytochemical analysis revealed the presence of various phytoconstituents which were believed to be responsible for pharmacological activity. It brought about fall in the level of total cholesterol by 21% with increase of 45% in high density lipoprotein (HDL) cholesterol including 53.6% decrease in triglyceride level (TG) as compared to diabetic control animals. The present study reveals the fact that polyherbal formulation (kalonji sugar powder) has antidiabetic effect. It reverses the abnormal lipid profile observed in diabetic animals. Therefore the water extract of this formulation is useful in maintaining healthy glucose levels and cholesterol levels.

Keywords: Antidiabetic, Antihyperlipidemic, Polyherbal extract, Streptozotocin

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease caused by an absolute or relative lack of insulin or reduced insulin activity, which results in hyperglycemia and abnormalities in carbohydrate, protein and fat metabolism [1,2]. Diabetes mellitus (DM) is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) [3]. It is estimated that 143 million people worldwide are suffering from diabetes, almost five times more than the estimates ten years ago. This number may probably be double by the year 2030 [4]. Though different therapies are available for the treatment of diabetes mellitus but there is a growing interest in herbal remedies, due to less or no side effects associated with these therapeutic agents. Because of their perceived effectiveness, minimal side effects in clinical experience and relatively low costs, herbal drugs are prescribed widely even when their biologically active compounds are unknown. Ayurveda is the oldest healing system of medicine for the treatment of diabetes. So an attempt has been made to evaluate the effectiveness of an ayurvedic medicine- Kalonji Sugar Powder (a polyherbal formulation containing leaves and seed of *Trigonella foenumgraecum*, seed of *nigella sativa*, seed of *syzygium cumini*, seed of *cinchorium intybus*, leaves of *gymnium sylvestre*) in treatment of diabetes in wistar rats.

The constituents of polyherbal formulation are- *Nigella sativa* linn. known as black cumin and kalonji is a small medicinal herb and its different parts have been reported as therapeutic agents in traditional system of medicines [5,6]. *Trigonella foenum graecum* seeds decrease blood glucose concentration [7]. *Syzygium cumini* seed powder exhibits normoglycemia and better glucose tolerance [8]. *Syzygium cumini* seed decrease blood glucose level and also inhibit alpha glucosidase enzyme [9]. The seeds are used in diabetes treatment [10]. *Cichorium intybus* roots and seeds are used in the treatment of diabetic and hyperlipidemic disorders [11]. *Gymnema sylvestre* leaves lowers plasma glucose level [12].

MATERIALS AND METHODS

Drugs, Chemicals, Reagents

The polyherbal formulation, kalonji sugar powder was procured from Mohammedia products, Shah Sahab Mohalla, Karimnagar, Andhra Pradesh, India, streptozotocin, glibenclamide and all other chemicals were purchased from sigma aldrich.

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.

Phytochemical screening of polyherbal formulation

The different extracts obtained were subjected to phytochemical screening for the presence of flavanoids, tannins, alkaloids, carbohydrates, phytosterols, triterpenoids, saponins according to standard procedures [13].

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 [14]. The fixed dose method as in annex 2d, test procedure with a starting dose of 5000 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 bwt and 5000 mg/kg bwt. 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.

Antidiabetic Activity

Induction of diabetes

Animals were fasted for 24 hours then a single intraperitoneal injection of freshly prepared streptozotocin (40 mg/kg dissolved in 0.9% in citrate buffer) was injected [15]

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 (250 mg/kg of body weight) formulation, group IV receiving aqueous extract of polyherbal (500 mg/kg of body weight) formulation and group V serves as a positive control receiving the standard drug (glibenclamide) at the dose of (10mg/kg of body weight).

Determination of antidiabetic activity

The fasting blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 1st, 7th, and 14th 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(kalonji sugar powder) in mild-diabetic rats was assessed by studying improvement in glucose tolerance(OGTT). The rats were divided into six groups- group I control, received vehicle (distilled water) only, group II served as diabetic control whereas variable doses of 250, and 500 mg/ kg bwt of leaf extract was given orally to group III and IV respectively. Blood glucose levels were first checked after 90 min of treatment, considered as "0" h value, and then 2g/ kg bwt 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 bwt of glibenclamide (synthetic hypoglycemic agent).

Biochemical determination

After the 14th day of treatment, blood was collected from the orbital plexus of overnight fasted rats. The serum was separated, glucose, triglycerides, LDL, HDL and cholesterol level were determined by

using glucose, triglycerides test kit ,LDL, and HDL[16] and cholesterol test kits (Span diagnostic Ltd, Surat), respectively.

Statistical analysis

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

RESULTS AND DISCUSSION

Our study reveals a well define role in suppressing the high blood glucose levels in mild diabetic rats of the polyherbal formulation(kalonji sugar powder) prepared from plants like *Nigella sativa*, *Trigonella foenum graecu*, *Syzygium cumini*, *Cichorium intybus*, *Gymnema Silvestre*. It also showed hypolipidemic effects in rats after 14 day of treatment.

The OGTT studies of the mild diabetic animals reveal a maximum fall of 40% in 1 h by the dose of 500 mg/kg bwt whereas, the doses of 250 mg/kg bwt produced fall of about 36.5% as compared to the control.(fig 1). Moreover, the dose of 500 mg/kg bwt of the aqueous extract showed nearly similar effect as that of synthetic drug glibenclamide. The OGTT studies also confirm 500 mg/kg bwt to be the most effective dose. This dose was therefore, selected for further studies in the case of mildly diabetic animals and a fall of 42% and 61% was observed in FBG after 7 and 14 days of treatment, respectively (Table. 1).

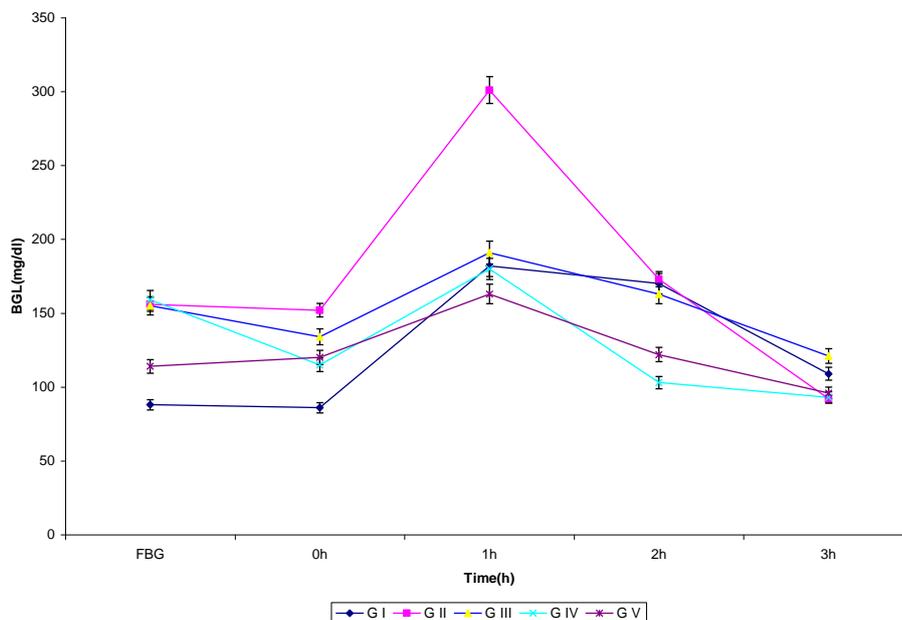


Fig. 1: Antidiabetic effect of aqueous extract of polyherbal formulation(kalonji sugar powder) on glucose tolerance in mild diabetic rats. *p<0.01 as compared to control.

Table 1: Effect of administration of aqueous extract of polyherbal formulation(kalonji sugar powder) for 14 days on serum fasting glucose levels in diabetic rats(mean±S.D.)

Treatment	Blood Glucose Level (BGL) in mg /dl		
	1 st day	7 th day	14 th day
Normal Rats	96±1.12	95±2.67	94±2.43
Control Diabetic Rats	201±1.89	259±2.43	290±2.67
Diabetic Rats +aqueous extract 250mg	221±2.1*	179±3.22*	151±2.98**
Diabetic Rats +aqueous extract 500mg	214±2.61*	150±2.3**	112±3.21*
Diabetic Rats + SD (Glibenclamide)	198±2.93*	160±2.82*	119±3.11*

*P < 0.01 as compared to control; **P < 0.05 as compared to control

Hyperlipidemia is mostly coupled with hyperglycemia. High levels of triglycerides and LDL cholesterol are associated with high risk of coronary dysfunction. Whereas increase in HDL cholesterol is associated with decrease in coronary risk. It is interesting to note that the dose of 500 mg/kg bwt of the aqueous extract lowered the TG,LDL and total cholesterol by

53.6%, 53% and 21% and simultaneously an increase in HDL cholesterol level by 45% after 14 day treatment was observed (Table 2) in our study. This would cause a decrease in mortality in individuals suffering from hyperglycemia accompanied by hyperlipidemia where the cause of deaths is coronary dysfunction

Table 2: Effect of administration of aqueous extract of polyherbal formulation(kalonji sugar powder) for 14 days on serum cholesterol levels, Serum triglycerides, Serum HDL and Serum LDL levels in diabetic rats. (mean±S.D.)

Treatment	Serum cholesterol	Serum triglycerides	Serum HDL	Serum LDL
Normal Rats	90±2.21	49±2.87	45±1.12	10.92±1.22
Control Diabetic Rats	137±2.87	97±2.92	28±3.12	51.4±3.21
Diabetic Rats +aqueous extract 250mg	127±3.23*	52±2.15*	39±2.45**	32.1±1.23*
Diabetic Rats +aqueous extract 500mg	108±3.54**	45±2.12*	51±2.54*	23.71±2.98*
Diabetic Rats + SD (Glibenclamide)	96±2.34*	53±2.12**	43±3.22*	21.05±3.54*

*P < 0.01 as compared to control; **P < 0.05 as compared to control

The phytochemical investigation revealed the fact that the hypoglycemic and hypolipidemic activity could be due to the presence of flavonoids, terpenoids or alkaloids (Table 3).

Table 3: Pytochemical study for polyherbal formulation (kalonji sugar powder)

Tests	Chloroform	Petether	Ethylacetate	Ethanol	Aqueous extract
Alkaloids	-	+	-	+	+
Coumarines	+	+	+	+	+
Carbohydrate	+	+	+	+	+
Flavonoids	+	+	+	+	+
Glycosides	-	-	-	-	-
Saponins	+	+	+	+	+
Steroids and pytosteroids	-	-	-	-	-
Tannins	-	-	-	+	+
Terpinoids	-	-	-	-	-

For the present study we conclude that the aqueous extract of the polyherbal formulation (kalonji sugar powder) showed remarkable antidiabetic and hypolipidemic effect in streptozotocin induced diabetic rats. The extract showed no toxicity at significantly higher doses. Furthermore the above results could be due to the synergism exhibited by different components of the polyherbal formulation (kalonji sugar powder)

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