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Research Article

EFFECT OF "ILOGEN EXCEL" ON LIPIDS IN EXPERIMENTALLY INDUCED DIABETES MELLITUS

LUCIA JANSI RANI S*

Department of Biochemistry, Annamalai University, Chidambaram, Tamil Nadu, India. Email: sadam.success@gmail.com

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ABSTRACT

The present study was used to evaluate the anti-hyper lipidemic effect and anti-oxidant effect of "Ilogen-Excel" and Ayurvedic herbal formulation in experimentally induced diabetic rats. Increase in the level of blood glucose, decrease in the level of plasma insulin, increase in the concentration of lipids in the heart and brain were registered in streptozotocin (STZ)-induced diabetic rats as compared with normal rats. Oral administration of Ilogen-Excel 100 mg/kg daily for a period of 2 months to diabetic rats decreased the levels of cholesterol, triglycerides, free fatty acids, phospholipids in heart and brain of diabetic rats. Thus, our study shows anti-hyper lipedimic effect of Ilogen-Excel in STZ-induced diabetic rats.

Keywords: Antihyperlipidemic, Anti-oxidant, Free fatty acids, Phospholipids, Streptozotocin.

INTRODUCTION

Diabetes mellitus (DM) is a potentially morbid condition characterized by a loss of glucose homeostasis (elevated glycemia) and frequently by concomitant glycosuria [1]. The major cause of hyperglycemia is insufficient secretion of insulin or its insufficient action on the level of peripheral tissues. Metabolic consequences of this are reflected not only in altered metabolism of saccharides, but quite often in the proteins or lipids, the pathogenesis of which is however more difficult [1].

In Type 1 diabetes, the pancreas undergoes an autoimmune attack by the body itself, and is rendered incapable of making insulin [2]. The immunologic markers, which are auto antibodies directed to islet cells molecules, are glutamic acid decarboxylase, insulin, tyrosine phosphatase IA2 and IIB [3].

In Type 2 diabetes, there are two metabolic defects, insulin resistance, and impaired insulin secretion [4]. Insulin resistance is defined as decreased sensitivity of tissues such as liver, skeletal muscle, and adipose tissues to the action of the hormone.

The insulin receptor in these tissue are normal, but a defect in the glucose uptake pathway slows down the entry of glucose into the cells, (i.e.,) post receptor defect are believed to play a prominent role in insulin resistance. Polymorphism in insulin receptor substrate-1 and various post-receptor molecules may combine to create insulin resistance. The pathogenesis of insulin is focused on phosphoinositide-3 kinase signaling defect, which reduces translocation of glut-4 to the plasma membrane [5].

Features of the metabolic syndrome include: Insulin resistance, central obesity, hypertension (over 160/90 mmHg), dyslipidemia, triglycerides (TGL) over 1.7 mmol/L, high-density lipoprotein (HDL) cholesterol <0.9 mmol/L, microalbuminuria over 20 mg/minutes.

Impaired glucose tolerance is defined as fasting plasma glucose under 7 mmol/L and a 2 hrs oral glucose tolerance test value of 7.8-11.1 mmol/L [6]. Lack of sensitivity to insulin in adipose tissue and skeletal muscle results in free fatty acids (FFAs) being released into the blood stream. These stimulate the liver to produce extremely very low-density lipoprotein (VLDL) triglyceride and VLDL particles. Increased VLDL triglyceride acts on HDL rendering it more easily cleared from the circulation and hence that HDL cholesterol concentration falls. Raised TGL and low HDL cholesterol is a very atherogenic mix and a major risk factor for cardiovascular disease [7] of heart and brain.

METHODS

Animals

Male albino Wistar rats, body weight of 180-200 g bred in the central animal house, Department of Experimental medicine, Rajah Muthiah Medical College, Annamalai University was used in the present study. The animals were housed in polypropylene cages (47 cm × 34 cm × 18 cm) lined with husk. It was renewed for every 24 hrs. They were fed with standard pellet diet (Pranav Agro Industries Ltd., Maharastra, India) and water was freely available. The animals were maintained in a controlled environment. All the experimental protocol was approved by the Ethical Committee of Annamalai University.

Ilogen-Excel

"Ilogen-Excel" was Purchased from a local pharmacy, Cuddalore District, Tamil Nadu, India, (Manufactured by Pankaja Kasthuri Herbals India (P) Ltd. Poovachal, Thiruvananthapuram - 75, Kerala, India) (Table 1).

Induction of DM in rats

A freshly prepared solution of streptozotocin (STZ) (45 mg/kg) in 0.1 M citrate buffer, (PH 4.5) was injected intraperitoneally in a volume of 1 ml/kg [7]. After 72 hrs of STZ administration, the rats with moderate diabetes having glycosuria and hyperglycemia (i.e., with a blood glucose range of (230-280 mg/dl) were taken for the experiment.

Table 1: "Ilogen-Excel," an ayurvedic herbal formulation consists of the following plant constituents

Composition of "Ilogen-Excel' tablet	
Name	**Concentration (mg/tablet)**
C. longa	8.33
S. potatorum	8.33
S. oblonga	8.33
T. cordifolia	8.33
V. zizaniodes	4.16
C. fenestratum	4.16
A. paniculata	8.33
M. pudica	4.16

C. longa: Curcuma longa, S. potatorum: Strychnos potatorum, S. oblonga: Salacia oblonga, T. cordifolia: Tinospora cordifolia, V. zizaniodes: Vetiveria zizaniodes, C. fenestratum: Coscinium fenestratum, A. paniculata: Ancrographis paniculata, M. pudica: Mimosa pudica

Experimental design

A total of 42 rats were used in the study. Each group consists of six rats.

Group I: Normal untreated rats.

Group II: Normal rats treated with "ilogen-excel" (50 mg/kg body weight) in physiological saline using an intragastric tube daily for 2 months.

Group III: Normal rats treated with "ilogen-excel" (100 mg/kg body weight) in physiological saline using an intragastric tube daily for 2 months.

Group IV: STZ induced diabetic rats (45 mg/kg body weight).

Group V: STZ treated diabetic rats given "ilogen-excel" (50 mg/kg body weight) In physiological saline using an intragastric tube daily for 2 months.

Group VI: STZ treated diabetic rats given "ilogen-excel" (100 mg/kg body weight) in physiological saline using an intragastric tube daily for 2 months.

Group VII: STZ treated diabetic rats given insulin (6 units/kg body weight) intra peritoneally for 2 months.

After 60 days of treatment, all the rats were decapitated after an overnight fast. Blood was collected in potassium oxalate and sodium fluoride tubes for the estimation of fasting blood glucose and plasma insulin.

The tissue lipids were extracted according to the method of Folch *et al.* [7]. The heart and brain tissues were rinsed in cold physiological saline thoroughly and dried by pressing between the folds of the filter paper. The tissues were homogenized in cold chloroform-methanol (2:1 V/V) mixture and the contents were extracted after 24 hrs. The extraction was repeated 4 times. The combined filtrate was swashed with 0.7% KCL and the aqueous layer discarded. The organic layer was made up to a known volume with chloroform and used for various biochemical estimations such as cholesterol, TGL phospholipids (PLS), proteins and FFAs.

Statistical analysis

Statistical analysis was performed using SPSS Software package version 12.0 (Lead technologies , Chicago 1991-2000). The values were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). All the results were expressed as mean = Standard deviation (SD) for six rats in each group. $p<0.05$ were considered to be significant.

RESULT

The effect of "ilogen-excel" on blood glucose and changes in blood insulin in normal and experimental rats. STZ induced diabetic rats showed an increase in the levels of blood glucose when compared with normal control rats. Oral administration of "ilogen-excel" for a period of 2 months decreased the levels of blood glucose in diabetic rats when compared with STZ induced diabetic rats. The level of plasma insulin was significantly decreased in diabetic rats when compared with normal control rats. Oral administration of "Ilogen-Excel" (50 mg/kg and 100 mg/kg) daily for a period of 2 months to diabetic rats showed a significant increase in the levels of plasma insulin when compared to STZ-induced diabetic rats.

Fig. 1 shows the levels of cholesterol, TGL, FFAs, PLS in heart in normal and STZ-induced diabetic rats. The concentration of Total Cholesterol, FFAs, PLS, and TGLs was significantly elevated during diabetes, when compared to normal group. Oral administration of "Ilogen-Excel" (50 mg/kg and 100 mg/kg) daily for a period of 2 months showed a significant decrease in the concentration of all parameters in diabetic rats.

Each column is mean SD for 6 rats in each group. Columns that have a different letter (a, b, c, d) differ significantly with each other ($p< 0.05$, DMRT).

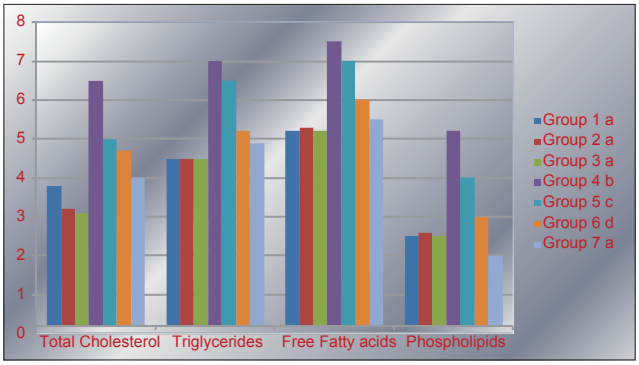

Fig. 1: Effect of "Ilogen-Excel" on the concentration of cholesterol, free fatty acids, triglycerides, phospholipids in the heart in normal and streptozotocin induced diabetic rats

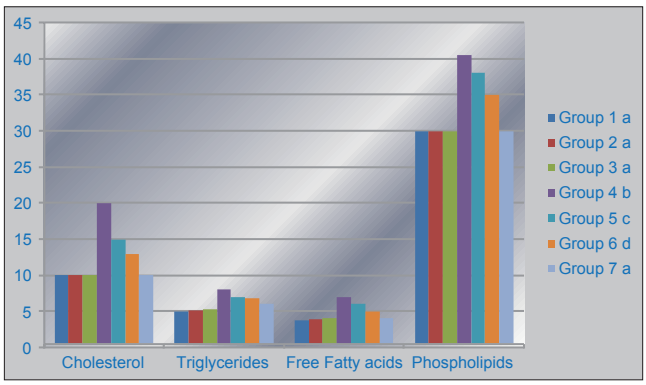

Fig. 2: Effect of "Ilogen-Excel" on the concentration of cholesterol, free fatty acids, triglycerides, phospholipids in the brain in normal and streptozotocin induced diabetic rats

Fig. 2 shows the levels of cholesterol, TGLs, FFAs, PLS in brain in normal and STZ-induced diabetic rats. The concentration of total cholesterol, FFAs, PLS, and TGLs was significantly elevated during diabetes, when compared to normal group. Oral administration of "Ilogen-Excel" (50 mg/kg and 100 mg/kg) daily for a period of 2 months showed a significant decrease in the concentration of all parameters in diabetic rats.

Each column is mean SD for six rats in each group. Columns that have a different letter (a, b, c, d) differ significantly with each other ($p<0.05$, DMRT).

DISCUSSION

STZ induced hyperglycemia has been described as a useful experimental model to study DM [8]. We have studied the effects of 'Ilogen-Excel' an ayurvedic herbal formulation in STZ induced albino Wister rats. Oral administrations of Ilogen-Excel to STZ induced diabetic rats showed anti hyperlipidimic effect.

Diabetic mellitus causes disturbance in the uptake of glucose as well as glucose metabolism. The use of a lower dose of STZ (45 mg/kg) produced and incomplete destruction of pancreatic β cells even though the rats became permanently diabetic [9]. After treatment with a low dose of STZ, there should be many surviving β cells, and regeneration is also possible [10]. The increased levels of blood glucose in STZ induced diabetic rats were lowered by Ilogen-Excel administration. The anti-hyperglycemic actions of the "Ilogen-Excel" results from the potentiating of insulin from existing β cells of the islets of Langerhans. This is evidenced by a significant increase in plasma insulin levels in STZ induced diabetic rats.

Cholesterol concentration in biological system is associated with membrane fluidity [6]. The rise in cholesterol levels observed in the diabetic heart of rats may mead to decreased membrane fluidity. This is in agreement with the observation made by Shijin *et al*. [7]. WHO suggested that elevated membrane cholesterol in diabetes reduces the fluidity of lymphocyte membranes of diabetic mice.

Under normal conditions the myocardium depends on fatty acids for its energy metabolism [7]. However, under stress conditions myocardial fatty acid utilization is depressed [11]. During diabetes, the heart is under stress and may therefore preferentially utilize glucose for its energy production, resulting in a rise in the concentration of fatty acids in the myocardium. Chattaopadhay *et al*. [12] have reported elevated myocardial non esterified fatty acid diabetic rat's heart. This raised level of fatty acids in the diabetic heart may also be responsible to increased concentration n of triacylglycerol [13,14].

PLS are vital components of biomembranes and cholesterol is responsible for the increased ordering of ischemic PLS [15]. In this context, higher levels of PLS were observed in diabetic heart.

DM alters the normal metabolism of brain [16]. Brain cholesterol and PLS showed an increase in diabetic rats. Lipid metabolism and membrane composition are altered in HT brains of non-insulin dependent diabetic mice [17]. The elevated cholesterol may be associated with relatively large increase in molecular ordering of residual PLS resulting in a decrease in membrane fluidity rendering lipid-dependent membrane bound enzymes nonfunctional [18]. The fluidity of membranes is also altered in both the types of diabetes. Rabini *et al*., [19] have reported an increase in PLS content in the platelet membranes from insulin dependent and non-insulin dependent DM. Recently, Yang *et al*., [20] have suggested erythrocyte membrane fatty acid components may contribute to alterations in membrane fluidity in Type II diabetes. In this context, other workers have reported an increase in brain cholesterol and PLS in experimentally induced diabetic rats [21].

The levels of FFAs increased in the diabetic brain. The brain can extract fatty acids from the plasma and this may be responsible for the higher levels of FFAs in brain during diabetes. In this context, Suresh Kumar and Menon [22] reported an increase in FFAs in diabetic brain.

Oral administration of Ilogen-Excel decreased the concentrations of cholesterol PLS and FFAs in diabetic heart and brain [23]. The observed results show the anti-hyperlipidemic effect of Ilogen-Excel in diabetic rats. The effect of Ilogen-Excel is due to the presence of antihyperlipidemic plants such as *Curcuma longa*, *Salacia oblonga* and *Tinospora cordifolia* in the Ilogen-Excel [24,25].

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