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Original Article

Evaluation of the effect of tigogenin on the activities of certain key enzymes of carbohydrate metabolism in streptozotocin induced diabetic rats

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ABSTRACT

This study was carried out to evaluate the role of key enzymes of carbohydrate metabolism in the antidiabetic action of Tigogenin. The Streptozotocin induced diabetic rats were treated with tigogenin at a dose of 10, 20 and 30 mg/kg body mass for 15 days. After 15 days, the blood samples were collected from each group of rats and the blood glucose level was estimated. The liver samples of rats were subjected to estimate the glycogen content, metabolic enzymes such as glycogen phosphorylase, glucose-6-phosphatase, fructose-1, 6 bisphosphatase and Hexokinase. A significant increase in blood glucose level was observed in diabetic rats. After treatment with tigogenin, the blood glucose level was found to be normal. Glycogen phosphorylase activity was greatly decreased in tigogenin treated rats. Similarly glucose-6-phosphatase and fructose-1,6 bisphosphatase activities were found to be reduced in tigogenin treated rats. In diabetic rats, the amount of glycogen, and Hexokinase activities were decreased, but they were normal in the tigogenin treated rats. The study indicates that tigogenin shows potent antihyperglycemic activity by the modulatory effect of key enzymes of carbohydrate metabolism and it is more pronounced when the dose of tigogenin is at 30mg/kg body weight.

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1. Introduction

Diabetes is a chronic metabolic disease that is showing an alarming increase in prevalence in developing countries such as India. It is also a major endocrine disorder affecting nearly 10% of the population all over the world [1]. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems [2]. Many traditional plants are used in the treatment of diabetes. But most of the evidences for their beneficial effects is anecdotal [3]. In the recent past, many hypoglycemic agents were introduced, still the diabetes and the related complications continue to be a major medical

problem not only in developed countries, but also in developing countries. Many Indian medicinal plants are reported to be useful in diabetes 1 and 2.

Tigogenin is a crystalline steroid sapogenin $C_{27}H_{44}O_3$ obtained especially by hydrolysis of Tigonin. It can also be used as pharmaceutical intermediate. Tigogenin is an aglycon which has a 3- β hydroxyl group and flat steroid nucleus. Tigogenin is extracted from the waste residues of sisal fibres from *Agave sisalana* and *Agave americana* [4]. Tigogenin is an important raw material in the synthesis of steroid hormones. It is reported to be a potential drug preventing the development of osteoporosis and the related disorders [5]. Review of literature reveals that the research works on anti diabetic activity and role of metabolic enzymes on tigogenin is scarce and hence the present study was undertaken to determine the effect of tigogenin on the activities of certain key enzymes of carbohydrate metabolism in diabetic rats.

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2. Materials and Methods

2.1. Chemicals

The chemicals used for the study were of Analytical grade. Streptozotocin, Tigogenin and Glibenclamide were purchased from Sigma Chemical Co. (USA). All other chemicals were purchased from Hi-media Laboratories Private Ltd., Mumbai.

2.2. Experimental animals

Healthy adult male wistar albino rats weighing (200-250g) were used for the study. The rats were housed individually in polypropylene cages, maintained under standard conditions (12h light and 12h dark cycle; 35-60% humidity), fed with standard pellet diet (Hindustan Lever Ltd., India) and water ad libitum. The experiments were designed and conducted in accordance with the ethical norms of Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakamangalam, Kanyakumari District, Tamilnadu.

2.3. Tigogenin

The authentic Tigogenin was purchased from Sigma Chemical Co. (USA) and it was used for the antidiabetic study.

2.4. Induction of diabetes

Streptozotocin (STZ) is a naturally occurring nitrosourea product of *Streptomyces achromogenes*. Diabetes was induced by a single intraperitoneal injection of 60mg STZ/kg body mass. STZ was prepared fresh in 0.1 M cold citrate buffer, pH 4.5, whereas the control animals were injected with citrate buffer alone. The rats were allowed to drink 5% glucose solution overnight to overcome drug induced hypoglycemia. The blood glucose level above 250mg/dl on the third day after STZ injection was considered as diabetic rat.

2.5. Experimental design

The rats were divided into seven groups with six rats in each group.

Group I: control rats

Group II: diabetic control rats

Group III: tigogenin control (normal rats treated with tigogenin (20 mg/kg body mass) in 0.1M phosphate buffered saline (pH-7.4) orally, daily for 15 days.

Group IV: diabetic rats treated with tigogenin (10mg/kg body mass) in 0.1M phosphate buffered saline (pH-7.4) orally, daily for 15 days.

Group V: diabetic rats treated with Tigogenin (20 mg/kg body mass) in 0.1M phosphate buffered saline (pH-7.4) orally, daily for 15 days.

Group VI: diabetic rats treated with tigogenin (30mg/kg body mass) in 0.1M phosphate buffered saline (pH-7.4) orally, daily for 15 days.

Group VII: diabetic rats treated with glibenclamide (600 µg /kg body mass) in 0.1M phosphate buffered saline (pH-7.4) orally, daily for 15 days.

Tigogenin was given by oral intragastric tube. After 15 days of tigogenin treatment, experiments were terminated and observations were made. Body weight was taken before and after experiment with the help of mono pan balance. Blood glucose level was estimated on 3rd and 15th day of experiment by glucose-oxidase method. The animals were deprived of food over night and sacrificed by cervical dislocation the next day. The liver tissue was excised and rinsed in ice-cold saline. The liver tissue was homogenized and the extract was used for the estimation of hepatic glycogen [6], metabolic enzymes such as Hexokinase [7], glycogen phosphorylase [8], glucose-6-phosphatase [9] and fructose-1, 6 bisphosphatase [10].

3. Results

The present investigation was carried out to establish the role of metabolic enzymes on the antidiabetic effect of tigogenin.

3.1. Effect of tigogenin and Glibenclamide on blood glucose level

Changes in fasting blood glucose level in diabetic rats treated with tigogenin and glibenclamide is presented in Table 1. Change in glucose was calculated as (Glucose level on day 15 after treatment- Glucose level on day 3 after Streptozotocin injection). No significant change in fasting blood glucose level was observed in the normal controls during the treatment period. The fasting blood glucose level ranged from 88.07 ± 1.29 to 90.0 ± 1.41 mg/dl. Significant ($p < 0.05$) increase in blood glucose level from 278 ± 5.29 to 315.51 ± 5.29 mg/dl with a net change of 32.50 ± 5.21 mg/dl was observed in diabetic rats. Oral administration of tigogenin in diabetic rats at doses 20mg/kg and 30 mg/kg body mass for 15 days revealed a significant reduction (-101.33 ± 8.89 and -154.83 ± 14.09 mg/dl, respectively) in blood glucose level. The reduction observed in Group VI (30 mg/kg body mass) was nearer to that of glibenclamide (-152.33 ± 8.641 mg/dl).

3.2. Effect of tigogenin and glibenclamide on body weight

Effect of Tigogenin and glibenclamide on body weight of normal and STZ-induced diabetic rats is given in Table 2. Change in body weight was calculated as Body weight 15th day - Body weight 3rd day. The normal control rats showed a significant ($p < 0.05$) elevation in their body weight (25.00 ± 4.60 g) when compared to STZ-induced diabetic rats which has shown significant ($p < 0.05$) reduction in their body weight (-38.17 ± 1.94 g) after the experimental period. The reduction was significantly less in the tigogenin treated groups (-29.00 ± 4.24 g and -6.33 ± 3.45 g for group IV and group V respectively). Weight gain also been observed on the diabetic rats treated with tigogenin (30mg/kg) was found to 6.17 ± 4.31 g and was comparable with that of Glibenclamide (8.50 ± 1.64 g).

Table 1: Effect of Tigogenin and Glibenclamide on blood glucose level in control and experimental rats.

Groups	Blood glucose(mg/dl) after induction of diabetes		
	Day 3 after streptozotocin induction	Day 15 after tigogenin treatment	Change in glucose
Control	88.07 ± 1.29	90.0 ± 1.41 ^a	-0.500 ± 4.41 ^b
Diabetic control	278.0 ± 5.29	315.51 ± 5.29 ^b	32.50 ± 5.21 ^a
Tigogenin control	90.37 ± 1.21	86.32 ± 1.52 ^a	-3.00 ± 9.25 ^b
Diabetic+ tigogenin (10mg/kg)	260.46 ± 3.81	220.30 ± 4.74 ^c	-57.00 ± 8.43 ^c
Diabetic+tigogenin (20 mg/kg)	267.78 ± 3.49	187.29 ± 3.26 ^d	-101.33 ± 8.89 ^d
Diabetic+tigogenin (30mg/kg)	266.33 ± 5.39	143.41 ± 4.06 ^e	-154.83 ± 14.09 ^e
Diabetic+glibenclamide (600µg/kg)	265.16 ± 4.79	112.99 ± 5.68 ^f	-152.33 ± 8.64 ^e

Each value is a mean ± SD of six samples.

Values not sharing a common superscript differ significantly (p < 0.05; DMRT).

Table 2: Effect of tigogenin and Glibenclamide on body weight in control and experimental rats.

Groups	Body weight(g)		
	Day 3 after streptozotocin induction	Day 15 after tigogenin treatment	Change in body weight
Control	220.17 ± 2.86	245.17 ± 2.86 ^a	25.00 ± 4.60 ^a
Diabetic control	223.00 ± 2.45	184.83 ± 2.14 ^e	-38.17 ± 1.94 ^e
Tigogenin control	213.17 ± 4.02	236.00 ± 1.41 ^b	22.83 ± 3.37 ^a
Diabetic+ tigogenin (10mg/kg)	229.83 ± 3.31	200.83 ± 3.31 ^d	-29.00 ± 4.24 ^d
Diabetic+tigogenin (20 mg/kg)	232.00 ± 3.16	225.67 ± 1.63 ^c	-6.33 ± 3.45 ^c
Diabetic+tigogenin (30mg/kg)	229.67 ± 3.44	235.83 ± 1.94 ^b	6.17 ± 4.31 ^b
Diabetic+glibenclamide (600µg/kg)	236.50 ± 3.51	245.00 ± 2.53 ^a	8.50 ± 1.64 ^b

Each value is a mean ± SD of six samples.

Values not sharing a common superscript differ significantly (p < 0.05; DMRT).

3.3.Effect of tigogenin and Glibenclamide on hepatic glycogen and glycogen phosphorylase

The effect of tigogenin and glibenclamide on hepatic glycogen and glycogen phosphorylase in normal and STZ-induced diabetic rats are shown in Table 3. STZ-induced diabetic rats showed a significant (p<0.05) reduction in the level of hepatic glycogen content (21.18 ± 2.64mg glycogen/100g of liver), while the activity of glycogen phosphorylase was increased to 821.83 ± 10.03U/mg of protein in STZ-induced diabetic rats when compared with normal control rats (83.92 ± 3.37mg/100g of liver and 634.17 ± 9.91U/mg of protein for glycogen and glycogen phosphorylase respectively). Oral administration of tigogenin (30 mg/kg) in STZ-induced diabetic rats significantly increased the level of hepatic glycogen (80.47 ± 2.72mg/100g of liver) and decreased the activity of glycogen phosphorylase (678.50 ± 6.95U/mg of protein) when compared with diabetic control rats.

Table 3: Effect of Tigogenin and Glibenclamide on hepatic glycogen and glycogen phosphorylase

Groups	Blood glucose(mg/dl) after induction of diabetes	
	Glycogen(mg glycogen/100g of liver)	Glycogen phosphorylase (U*/mg of protein)
Control	83.92 ± 3.37 ^a	634.17 ± 9.91 ^e
Diabetic control	21.18 ± 2.64 ^e	821.83 ± 10.03 ^a
Tigogenin control	85.61 ± 3.20 ^a	630.67 ± 7.42 ^e
Diabetic+tigogenin (10mg/kg)	63.85 ± 2.57 ^d	735.50 ± 7.97 ^b
Diabetic+tigogenin (20 mg/kg)	73.14 ± 2.91 ^c	710.17 ± 10.83 ^c
Diabetic+tigogenin (30mg/kg)	80.47 ± 2.72 ^b	678.50 ± 6.95 ^d
Diabetic+glibenclamide (600µg/kg)	78.45 ± 2.87 ^b	672.83 ± 8.06 ^d

U* = µmole phosphate liberated in hr

VII). Finally below the fourth table should be add following mistakes.

U* = µmole of inorganic phosphorous liberated

U** = µmole of glucose phosphorylated

3.4. Effect of tigogenin and Glibenclamide on the activity of glucose-6-phosphatase, fructose-1, 6 bisphosphatase and Hexokinase

The effect of tigogenin and glibenclamide on the activity of glucose-6-phosphatase, fructose-1,6 bisphosphatase and hexokinase in normal and STZ-induced diabetic rats are presented in Table 4. STZ-induced diabetic rats showed a significant elevation in the activity of glucose-6-phosphatase (0.501 ± 0.005 U/ min/mg protein) and fructose-1,6 bisphosphatase (0.612 ± 0.003 U/h/mg protein) and decrease in the activity of hexokinase (0.072 ± 0.002 U/h/mg protein), when compared to normal control rats. Oral administration of tigogenin (30 mg/kg) in diabetic rats significantly reduced the activity of glucose-6-phosphatase (0.241 ± 0.003 U/ min/mg protein) and fructose-1,6 bisphosphatase (0.430 ± 0.005 U/h/mg protein) and elevated the Hexokinase activity (0.190 ± 0.002 U/h/mg protein) as compared to diabetic control rats. The effects were comparable with that of the standard drug glibenclamide. (0.430 ± 0.005 U/h/mg protein) and elevated the Hexokinase activity (0.190 ± 0.002 U/h/mg protein) as compared to diabetic control rats. The effects were comparable with that of the standard drug glibenclamide.

Table 4: Effect of tigogenin and Glibenclamide on the activity of glucose-6-phosphatase, fructose-1, 6 bisphosphatase and Hexokinase

Groups	Blood glucose(mg/dl) after induction of diabetes		
	Glucose-6- phosphatase (U*/min/mg protein)	Fructose-1,6 bisphosphatase (U*/h/mg protein)	Hexokinase U**/h/ mg protein
Control	0.178 ± 0.004 ^f	0.412 ± 0.003 ^f	0.201 ± 0.002 ^a
Diabetic control	0.501 ± 0.005 ^a	0.612 ± 0.003 ^a	0.072 ± 0.002 ^f
Tigogenin control	0.176 ± 0.002 ^f	0.409 ± 0.002 ^f	0.198 ± 0.003 ^b
Diabetic+ Tigogenin (10mg/kg)	0.278 ± 0.003 ^b	0.460 ± 0.004 ^b	0.180 ± 0.004 ^e
Diabetic+ Tigogenin (20 mg/kg)	0.264 ± 0.002 ^c	0.440 ± 0.003 ^c	0.188 ± 0.003 ^d
Diabetic+tigogenin (30mg/kg)	0.241 ± 0.003 ^d	0.430 ± 0.005 ^d	0.190 ± 0.002 ^d
Diabetic+glibenclamide µg/kg) (600)	0.231 ± 0.003 ^e	0.424 ± 0.003 ^e	0.194 ± 0.002 ^c

U* = moles of inorganic phosphorous liberated

U** = moles of glucose phosphorylated

Each value is a mean ± SD of six samples.

Values not sharing a common superscript differ significantly (p < 0.05; DMRT).

4. Discussion

Streptozotocin is known for its selective β -cell toxicity. It breaks the nuclear strand of the islet cells and brings an increase in blood glucose levels [11]. Glibenclamide is often used as a standard antidiabetic drug in streptozotocin induced diabetic rats to compare the efficacy of variety of hypoglycemic compounds [12].

The present study was undertaken to assess the antihyperglycemic activity of Tigogenin in streptozotocin induced diabetic rats. The level of blood glucose was increased as expected in streptozotocin injected rats, since streptozotocin causes a massive reduction in insulin release, by the destruction of the β -cells of the islets of langerhans and inducing hyperglycemia. Tigogenin treated normal rats (Tigogenin control) did not show any significant ($p>0.05$) changes in the blood glucose level when compared to control rats. The administration of tigogenin resulted in a significant reduction in the blood glucose level, when compared to diabetic animals. The dose containing 30 mg/kg body weight showed a better glucose reduction than 20mg and 10mg/kg body mass. The reduction may be due to the stimulation of beta cell for elevated secretion of insulin, thereby increasing the oxidation of glucose in various tissues [12].

Body mass was increased in the control rats when compared to the initial body weight, whereas in the diabetic control rats, there was a significant reduction in the body weight. It indicates the degradation of structural proteins due to diabetes [13]. Tigogenin and Glibenclamide treatment significantly prevented the reduction in the body weight. This shows that tigogenin has the ability to reduce hyperglycemia.

Liver plays a vital role in regulation of blood glucose level and hence it is of interest to study the role of tigogenin on key enzymes of carbohydrate metabolism in liver. In the present study, there was a marked reduction in the level of liver glycogen and increased activity of glycogen phosphorylated in diabetic control rats. The conversion of glucose to glycogen in the liver cells is dependent on the extracellular glucose concentration and the availability of insulin which stimulate glycogenesis over a wide range of glucose concentration [14]. The reduction of glycogen in diabetic rats has been attributed to increased activity of glycogen phosphorylase [15]. Tigogenin treated groups restored the level of hepatic glycogen by means of decreasing the activity of glycogen phosphorylase.

Glucose-6-phosphatase and fructose-1, 6 biphosphatase are the key enzymes in gluconeogenesis. The enzyme levels were observed to be increased in diabetic rats [16]. The increased activity may be due to insulin insufficiency. In tigogenin treated rats, the activity of glucose-6-phosphatase and fructose-1,6 biphosphatase were found to be decreased and it may be due to the modulation and regulation of the activities of these two gluconeogenic enzymes either through regulation of cAMP or inhibition of gluconeogenesis [17].

Hexokinase is the key enzyme catalyzing the conversion of glucose to glucose-6-phosphate and also hexokinase activity is severely impaired during diabetes [18,19]. Impairment is due to

impaired oxidation of glucose resulting in hyperglycemia. Insulin increases hepatic glycolysis by increasing the activity of hexokinase. The hexokinase activity was found to be decreased in diabetic rats which may be due to insulin deficiency. Treatment with tigogenin elevated the activity of hexokinase and it is more pronounced in the group VI which is treated with tigogenin (30mg/kg body weight). Tigogenin may stimulate insulin secretion, which may activate hexokinase.

5. Conclusion

The result obtained suggest that the tigogenin exhibits its antihyperglycemic effect mediated through inhibition of the activity of key hepatic gluconeogenic enzymes such as glucose-6-phosphatase, fructose-1,6 biphosphatase and glycogenolytic enzyme glycogen phosphorylase, through an accelerated rate of glycolytic enzyme Hexokinase and generation of glycogen. Although the exact mechanism responsible for the antihyperglycemic effects of tigogenin still remain speculative, experimental evidence obtained from this study indicates that tigogenin has the potential antidiabetic activity. Therefore it is recommended that tigogenin is very promising to develop standardized medicine for diabetes mellitus.

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