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Research Article

ANTI-HYPERGLYCAEMIC POTENTIAL OF TRIPHALA ONSTREPTOZOTOCIN-INDUCED DIABETES IN RATS

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Abstract

Present study was undertaken to evaluate the anti-hyperglycaemic potential of the herbal formulation Triphala in diabetic rat models. Diabetes was induced by a single intra-peritoneal injection of streptozotocin(50 mg/kg b.w.) in the experimental animals. The diabetic rats were orally administered with Triphala, once a dayfor 30 days at a dosage of 500 mg/kg b.w. and the efficacy of the same was compared with that of the Glibenclamide (600 µg/kg b.w.) treated diabetic rats. The estimation of biochemical parameters were carried out in the experimental animals and compared with that of the normal rats. It was found that Triphala-treated rats showed significant decrease in the levels of blood glucose, serum liver and kidney function markers. There was significant increase in serum total protein and albumin levels and also oral glucose tolerance on treatment with Triphala. Our results indicate that Triphala possesses anti-hyperglycaemic activity in streptozotocin-induced diabetes in Wistar albino rats.

Key Words

Diabetes, hyperglycaemia, Triphala, antioxidant

1. Introduction

Diabetes is a complex metabolic disorder that needs considerable attention related to its early diagnosis, prevention, treatment and management in developing countries [1], [2]. Though present day anti-diabetic drugs are effective anti-hyperglycaemic agents, their efficacy in preventing chronic diabetic complications is poor. This has led to the search of herbal drugs that possess anti-diabetic activity and show less toxic effects as compared to present day oral anti-hyperglycaemic drugs. Therefore, a large volume of work has been carried out by researchers from different parts of the world in screening and validating the anti-hyperglycaemic potential of herbal drugs, plant extracts and phytochemicals [3], [4], [5], [6].

Triphala is a well-known Indian Ayurvedic herbal formulation which is prescribed for anaemia, fatigue, jaundice, inflammation, constipation, asthma and tuberculosis. Its components, *Terminaliabellerica*, *Terminaliachebula* and *Emblicaofficinalis* fruits have been proven to contain several active components and possess antioxidant potential [7], [8], [9]. Studies have shown that Triphala possesses anti-inflammatory, anti-hyperlipidemic and anti-microbial properties [10], [11], [12]. These properties may be beneficial for diabetic patients in maintaining normal blood glucose and cholesterol levels thereby preventing diabetic complications and infections.

Present study was aimed to evaluate the anti-diabetic potential of Triphala in streptozotocin-induced diabetes in Wistar albino rats.

2. Materials and methods

2.1) Drugs and chemicals:

Streptozotocin used in the present study was obtained from Sigma Aldrich, India. Triphala powder was purchased from Indian Medical Practitioners Co-operative Stores and Society (IMCOPS), Mylapore, Chennai, India. The standard drug glibenclamide used in this study was purchased locally. Commercial diagnostic kits used for the estimation of the biochemical parameters were obtained from AutoSpan, India. All the other chemicals and reagents used in the present study were of analytical grade and purchased locally.

2.2) Animals:

A total of 24 female Wistar albino rats weighing about 148.86±3.45 g were procured from VIT Animal House, VIT University, Vellore, Tamilnadu, India. The animals were housed six per cage and maintained in a light and temperature controlled room. The animals were acclimatized for 1 week and provided with standard pelleted rat feed (Hindustan Lever Ltd, India) and water *ad libitum*.

2.3) Experimental design:

The animals were divided into 4 groups of 6 rats each. The study duration was 30 days. Group I was normal control group and the rats were allowed free access to normal rat feed and water. No drug was administered to this group. Group II was the diabetic control group administered with a single dose of STZ (50 mg/kg b.w.) in 0.1 M citrate buffer (i.p.). Group III rats were given a single dose of STZ (50 mg/kg b.w.) in 0.1 M citrate buffer (i.p.) and Triphala (500 mg/kg b.w.) was given orally on daily basis until the end of the study duration. Group IV was the STZ-induced diabetic rats treated orally with glibenclamide(600 µg/kg b.w.) once every day. Fasting blood glucose was measured in all experimental rats using a clinical glucometer (OneTouch Ultra) on day 1 after which diabetes was induced in

groups II, III and IV. Following this the animals were kept in their cages provided with 5% glucose solution for 24 hours to prevent drug-induced hypoglycaemia. The administration of Triphala or glibenclamide was started once the hyperglycaemic state of the rats was established and the presence of diabetes proven.

The animals were sacrificed by ether anaesthesia at the end of the study and blood was collected from the trunk for evaluation of biochemical parameters.

2.4) Estimation of blood glucose and urine glucose and ketones:

Blood glucose was estimated in the experimental rats on day 0, 5, 10, 15, 20, 25 and 30 using a glucometer (OneTouch Ultra). Urine glucose and ketones were also measured semi-quantitatively at same intervals using Ketodiastix strips.

2.5) Estimation of Biochemical Parameters:

Serum biochemical parameters such as total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea, creatinine and uric acid were estimated using commercial diagnostic kits (AutoSpan, India).

2.6) Glucose tolerance Test:

On day 26, oral glucose tolerance test was carried out in the experimental rats. 2 g/kg b.w. of glucose was given orally and the blood glucose levels were measured at 30, 60 and 90 min.

3. Results and discussion

3.1) Effect of Triphala on blood and urine glucose levels: The levels of blood glucose were significantly ($P < 0.05$) elevated in the diabetic rats while treatment with Triphala showed to decrease the elevated levels of blood glucose to near normal levels (Figure 1). This was comparable with that of the standard drug glibenclamide-treated rats. The diabetic rats presented with glycosuria and ketonuria which was normalised on treatment with Triphala(Table-1).

Table-1: Effect of Triphala on urine glucose and Ketones.

Groups	Urine glucose							Urine ketones						
	0	5	10	15	20	25	30	0	5	10	15	20	25	30
I	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
II	Nil	++	++	+++	++++	++++	++++	Nil	Nil	Trace	Trace	+	++	+++
III	Nil	Trace	++	++	Trace	Trace	Nil	Nil	Nil	Trace	Trace	Nil	Nil	Nil
IV	Nil	++	++	+	+	Trace	Nil	Nil	Nil	Trace	+	Trace	Nil	Nil

The above mentioned effects could be due to the antioxidant rich components of Triphala which might have either reversed the pancreatic beta cell damage or increased the secretion of insulin by the existing pancreatic beta cells [13], [14]. Also, Triphala might have increased pancreatic beta cell regeneration either by stem cell differention or self-duplication of pre-existing pancreatic cells. Apart from insulin insufficiency, diabetes also involves hyperglycaemia due to increased rates of gluconeogenesis and glycogenolysis and reduced utilization of glucose by the cells [15], [16]. Triphala may have reduced the rate of hepatic glycogenolysis and gluconeogenesis. This would have reduced the blood glucose levels thereby preventing the urinary excretion of glucose. Diabetic ketoacidosis is a serious complication seen in diabetic patients due to the production of ketone bodies from fatty acids [17]. The efficacy of Triphala in restoring normal blood glucose levels has reduced the extent of ketogenesis which is evident from the urine ketone semi-quantitative estimation in the Triphala-treated diabetic rats. This was comparable to that of the glibenclamide-treated rats.

3.2) Effect of Triphala on biochemical parameters: The levels of total protein and albumin were significantly ($P < 0.05$) reduced in the diabetic rats (Table 2). The oral administration of Triphala was able to restore normal levels of total protein and albumin in the group III rats. The levels of liver function maker enzymes such as ALT, AST and ALP were significantly ($P < 0.05$) elevated in the diabetic rats which were normalized on treatment with Triphala (Figure 2). There was also significant ($P < 0.05$) elevation of renal functional markers such as creatinine, urea and uric acid in the serum of the diabetic rats which were reversed to near normal levels on the administration of Triphala (Table 2).

Table-2: Effect of Triphala on oral glucose tolerance in STZ-induced diabetes.

Groups	Fasting blood glucose (mg/dL)	30 min blood glucose (mg/dL)	60 min blood glucose (mg/dL)	90 min blood glucose (mg/dL)
Group I (normal control)	81.34±2.15	150.41±3.87	183.08±3.73	118.23±2.56
Group II (diabetic control)	146.27±3.48	262.15±4.26	391.84±3.96	244.34±4.12
Group III (Triphala 500 mg/kg b. w.)	94.87±2.85	158.52±3.62	191.06±3.81	117.21±3.71
Group IV (Glibenclamide 600 µg/kg b. w.)	98.18±2.91	167.31±3.82	221.79±3.01	124.36±2.73

The role of reactive oxygen species (ROS) in the disease progression and pathogenesis of diabetes has been studied extensively [18]. The elevation of liver marker enzymes in diabetes may be due to cellular damage caused by ROS eventually leading to the leakage of these intracellular enzymes into circulation. Triphala being an antioxidant rich formulation may have reduced the extent of cellular damage thereby reducing the levels of serum ALT, AST and ALP. The secretory and excretory functions of the system might have been normalized by Triphala in the STZ-induced diabetic rats thereby significantly ($P < 0.05$) increasing the reduced levels of serum total protein and albumin. The elevated serum urea, creatinine and uric acid levels in the diabetic group are indicative of renal dysfunction. This was reversed by the oral administration of Triphala in STZ-induced diabetic rats.

3.3) Effect of Triphala on oral glucose tolerance: The oral glucose tolerance test revealed that the diabetic rats had reduced tolerance to oral glucose and increased insulin resistance while the Triphala treated rats showed normal tolerance to glucose and the glucose levels were normal at 90 min (Table 3). This was comparable with that of the glibenclamide treated group. It is therefore evident that Triphala was able to restore normal tolerance to oral glucose challenge and increase the secretion and release of insulin. Oral glucose intolerance has been found to be associated with peripheral neuropathy in diabetic patients [19]. Therefore, Triphala might be useful in ameliorating glucose intolerance in such patients.

Table-3: Effect of Triphala on renal functional markers in STZ-induced diabetes.

Parameters	Group I (Normal control)	Group II (Diabetic control)	Group III (Triphala 500 mg/kg b.w)	Group IV (Glinbenclamide 600µg/kg b.w)
Total protein	6.52±0.731	4.42±0.418 a*	6.45±0.705 b*	6.29±0.729 b*
albumin	4.06±0.40	2.20±0.146 a*	3.90±0.452 b*	3.93±0.43 b*
Creatinine	0.55±0.14	2.54±0.19 a*	0.81±0.10 a*b*	0.78±0.11 b*
Uric acid	0.68±0.43	2.46±0.39 a*	0.81±0.41 b*	0.98±0.50 b*
Urea	26.77±4.82	47.40±6.12 a*	31.44±3.977 b*	30.65±4.303 b*

Conclusion

Present study indicates the anti-diabetic effect of Triphala in STZ-induced diabetic rats in accordance with the results thus obtained. The efficacy of Triphala in restoring normal blood glucose levels and renal function indicate that this formulation would be helpful in the management of diabetes. However, further studies need to be done in

investigating the effect of thisformulation on the key regulatory enzymes of glucose metabolism which would provide insight into the specific effects of Triphala in diabetes.

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