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## Research Article

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### Insulin Secretagogue Activity of *Orthosiphon Thymiflorus* Leaves

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#### ABSTRACT

In the present study, the effect of hydroalcoholic extract of *Orthosiphon thymiflorus* leaves (HAEOT) was studied on glucose induced insulin secretion in rat pancreas with an evaluation of their mechanism of action. Isolation and preparation of pancreas by collagenase digestion method and Insulin secretion from islets were observed with graduate concentrations of HAEOT. There was a concentration-dependent increase in insulin release when islets were incubated with various concentrations of HAEOT at 10 and 60 min. For inhibition of insulin from pancreas, the pancreas islets were pre incubated with diazoxide along with and without 100 mg of HAEOT. Incubation of pancreatic islets with glucose and 100mg of HAEOT in the presence of diazoxide caused a 24.76% (10 min) and 26.73 % (60 min) inhibition in insulin release. This study clearly indicated that *Orthosiphon thymiflorus* reverse the effect of diazoxide. It can be concluded that the insulin secretagogue effect of hydroalcoholic extract of *Orthosiphon thymiflorus* leaves regulates the insulin secretion of islets of pancreas because of its antioxidant property.

**Keywords:** *Orthosiphon thymiflorus*; diazoxide; Insulin secretagogue; Glucose induced insulin secretion.

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## 1. Introduction

Diabetes mellitus, one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications (Patel DK *et al* 2011). Due to deficiency of the insulin people suffering from diabetes have high blood glucose level (Ponnusamy S *et al* 2011). Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes (Malviya N *et al* 2010). In 2007, Eidi *et al* studied again the hypoglycemic effect of the fenugreek seeds which was previously found to inhibit  $\alpha$ -glucosidase, and they reported that the hydroalcoholic extract significantly decreased serum glucose, triacylglycerol, cholesterol, urea, uric acid, AST, and ALT, whereas it increased serum insulin levels in treated STZ-induced diabetic rats similar to that observed for glibenclamide used as a standard drug. Hence the present study was evaluated for insulin secretagogue effect of *Orthosiphon thymiflorus* on glucose induced insulin release from rat pancreatic islets model.

*Orthosiphon thymiflorus* (Family: lamiaceae) is a medicinal plant, slightly aromatic subshrub commonly seen in India, It is grown in Hills above 600m on the slopes, in crevices of rocks; more numerous by arable lands, etc (Mathew K.M *et al.*, 1983) . Many species of this genus possesses several pharmacological properties like antidiabetic, diuretic, antihepatotoxic antibacterial, hypertensive and antitumor activity. *Orthosiphon thymiflorus* has good antioxidant activity enriched with terpenoids (Sundarammal *et al.*, 2012) and shows inhibitory effect on skeletal muscle contraction (Kavimani S *et al.*, 1998).

## 2. Materials and method

### Plant material

The leaf of *Orthosiphon thymiflorus* were collected from Tirumala hills, Tirumala, Chittoor DT, A.P, India. The plant was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P, and India.

### Preparation of extract

After shade drying leaves of *Orthosiphon thymiflorus* were then blended in to fine powder with a blender and used for the preparation of hydroalcoholic extract. Hydroalcoholic extract was prepared by maceration method. The extract obtained, was concentrated and dried under reduced pressure at controlled temperature (40-50 C) (Sukhdev Swami Handa *et al* 2008).

### Selection of experimental animals

Wistar Albino rats of either sex (200-230 gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient International Journal of Pharmacy and Natural Medicines

temperature of  $22 \pm 2$  C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized for at least 7days to the laboratory conditions prior to experimentation .Tap water and food pellets were provided ad libitum. Food pellets was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of "Guide for the care and Use of Laboratory animals". (Institute of Laboratory Animals Resources, National Academic Press 1996: NIH Publication number # 85-23, revised 1996).

### Isolation and preparation of pancreas by collagenase digestion method

Albino wistar male rats (160-180 g) were used for this study. They were sacrificed by cervical dislocation and abdomen part was opened. The upper part of the bile duct should be free by displacement of stomach and dissection of liver. Total blood was bleed by heart puncture. Inject the collagenase solution in to pancreas via common bile duct. Collagenase solution was used for the disaggregation of pancreatic tissues and isolation of pancreatic cells. The pancreas was swallowed due to Collagenase administration. These pancreas was removed and remain pancreases were transferred to 10 cm petridish (Rangachari balamurugan *et al.*, 2011).

### Insulin secretion from islets of pancreas

All isolated pancreas were pre incubated with 0.5 ml of incubation buffer containing 11.1mM glucose alone and combination with different concentrations of hydroalcoholic extract of leaves of *Orthosiphon thymiflorus* (HAEOT) (5, 10, 20, 40, 80 and 100 mg) at 37 °C for 30 min in a shaking water bath under 95% O<sub>2</sub> and 5% CO<sub>2</sub> atm. Insulin secretion from islets were observed with graduate doses of HAEOT (dose dependent activity) (Moskalewski, S *et al.*, 1965).

For inhibition of insulin from pancreas, the pancreas islets were pre incubated with 0.25mM diazoxide (potassium channel activator) along with and without 100 mg of hydroalcoholic extract of *Orthosiphon thymiflorus* (HAEOT) and 11.1 mM glucose for 30 min. After the pre incubation the buffer was removed, new additions were made in a similar fashion and incubated under similar conditions. Among total parts, 50 $\mu$ l was taken at different time interval of 0, 10 and 60 min and it was kept in frozen immediately up to insulin assay was performed by ELISA method.

## 3. Results and Discussion

### Effect of hydroalcoholic extract of *Orthosiphon thymiflorus* on glucose-induced insulin release

When pancreatic islets were incubated with 11.1 mM glucose in the presence of hydroalcoholic extract of *Orthosiphon thymiflorus*, a significant increase in insulin release was observed. There was a dose-dependent increase in insulin release when islets were incubated with various concentrations of HAEOT at 10 and 60 min when compared with control group without the addition of HAEOT (Table 1 & Figure 1). Effects of Diazoxide on glucose and HAEOT -induced insulin release was studied. Diazoxide, a potent K<sup>+</sup>-ATP channel opener, is a well-

known inhibitor of glucose-induced insulin release. Incubation of pancreatic islets with 11.1 mM glucose and 100mg of HAEOT in the presence of 0.25 Mm diazoxide caused a 24.76% (10 min) and 26.73 % (60 min) inhibition in insulin release when compared with 100 mg of HAEOT treated islets without diazoxide. Decreased secretion of insulin was observed in the absence of HAEOT (Table 2 & Figure 2).

**Discussion**

The currently using drug treatment for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs. Previously, studies on anti-diabetic effect of hydroalcoholic extract of HAEOT in high fat diet (HFD)-streptozotocin (STZ)-induced diabetic rats has been reported. To understand the exact mechanism of action of *Orthosiphon thymiflorus*, the present study was performed with incubating pancreatic islets. This study suggested that *Orthosiphon thymiflorus* possess protective nature and

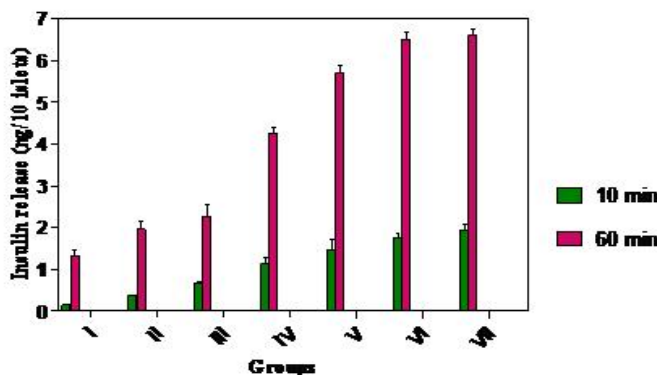
stimulate the beta cells of islets of pancreases dose dependently. Glucose inhibiting effect of *Orthosiphon thymiflorus* might be due to stimulation of surviving  $\beta$ -cells of islets of Langerhans leading to more insulin release (Li, W.L., Zheng et al., 2004).

Additionally, insulin secretagogue effect of *Orthosiphon thymiflorus* was evaluated by pancreatic islets were incubated with diazoxide, a known inhibitor of insulin release via opening the  $K^+$ -ATP channel. Since diazoxide stimulates the reactive oxygen species (ROS) production by opening of mitochondrial  $K^+$  ATP channel leads to inhibit the release of insulin from pancreas (Dickinson, K et al., 1997 & Nagy, K., et al., 2004). HAEOT might be produced insulin-mimetic effect by closing the potassium channel due to prevent the generation of ROS with anti-oxidant activity. This study clearly indicated that *Orthosiphon thymiflorus* reverse the effect of diazoxide on glucose induced insulin release at 10 min and 60 min.

**Table 1:** Dose dependent effect of HAEOT on glucose induced insulin release from rat pancreatic islets.

Groups & Treatment	Insulin release ng/10 islets	
	10min	60min
I – Control 11.1mM glucose	0.18±0.01	1.42±0.15
II HAEOT 5 mg + 11.1mM glucose	0.35±0.02	1.97±0.19
III - HAEOT 10 mg + 11.1mM glucose	0.67±0.02*	2.29±0.26**
IV - HAEOT 20 mg + 11.1mM glucose	1.14±0.17***	4.27±0.14***
V - HAEOT 40 mg + 11.1mM glucose	1.49±0.24***	5.73±0.18***
VI - HAEOT 80 mg + 11.1mM glucose	1.75±0.08***	6.53±0.17***
VII HAEOT 100 mg + 11.1mM glucose	1.93±0.15***	6.60±0.17***

Values are mean ± S.E.M. (n=6). \* p < 0.05 ; \*\* p < 0.01; \*\*\*p<0.001. All groups compared with untreated control group



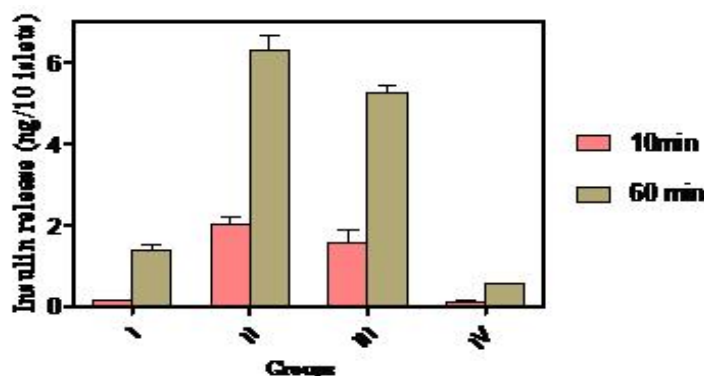
**Figure 1:** Comparative effect of HAEOT on glucose induced insulin release from rat pancreatic islets at 10min & 60 min.

**Table 2:** Effect of HAEOT (alone and with diazoxide) on glucose induced insulin release from rat pancreatic islets

Groups & Treatment	Insulin release ng/10 islets	
	10min	60min
I – Control 11.1mM glucose	0.15±0.02	1.39±0.15
II - HAEOT 100 mg + 11.1mM glucose	2.08±0.17 <sup>a***</sup>	6.34±0.38 <sup>a***</sup>
III - HAEOT 100 mg + 11.1mM glucose + 0.25mM diazoxide	1.60±0.20 <sup>a***, b ns</sup>	5.28±0.14 <sup>a***, b**</sup>
IV - 0.25mM diazoxide + 11.1mM glucose	0.12±0.02 <sup>a ns</sup>	0.55±0.02 <sup>a ns</sup>

Effect of HAEOT on (alone and with diazoxide) glucose induced insulin release from rat pancreatic islets. Values are mean ± S.E.M. (n=6). \* p < 0.05 ; \*\* p < 0.01;\*\*\* p<0.001. All groups compared with untreated control group.

a – Control group I Vs Group II-IV. b–Group II Vs Group III.



**Figure 2:** Comparative Effect of HAEOT (alone and with diazoxide) on glucose induced insulin release from rat pancreatic islets at 10min & 60 min.

#### 4. Conclusion

The present study concluded that *Orthosiphon thymiflorus* exhibit potent insulin secretagogue effect by regulates the insulin secretion of islets of pancreases because of its antioxidant property.

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