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Insulin Secretagogue Effect of *Bombax Ceiba* on Glucose Induced Insulin Release from Rat Pancreatic Islets

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A B S T R A C T

In the present study, the effect of methanolic leaf extract of *Bombax ceiba* (MEBC) was studied on glucose induced insulin secretion in rat pancreas with an evaluated 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 MEBC. There was a concentration-dependent increase in insulin release when islets were incubated with various concentrations of MEBC 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 MEBC. Incubation of pancreatic islets with glucose and 100mg of MEBC in the presence of diazoxide caused a 22.16% (10 min) and 16.64 % (60 min) inhibition in insulin release. This study clearly indicated that *Bombax ceiba* reverse the effect of diazoxide. It can be concluded that the insulin secretagogue effect of methanolic extract of leaf of *Bombax ceiba* regulates the insulin secretion of islets of pancreas because of its antioxidant property.

Keywords: *Bombax ceiba*, diazoxide, Insulin secretagogue, Glucose induced insulin secretion.

A R T I C L E I N F O

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1. Introduction

Bombax ceiba is one of the well known medicinal plant belonging to the family malvaceae. It is found in moist evergreen and deciduous forests. It is also in dry forests and in gallery forests. It is also referred as silent doctor. Every part of this tree is used to treat various ailments. In Ayurveda it is referred as aphrodisiac, astringent, antidiarrheal, antidysenteric, antimicrobial diuretic, alterative, antipyretic and tonic [1, 2, 3]. The phytochemical screening revealed the presence of steroid, alkaloid, tannin, phenols, flavonoid, in methanol soluble fractions [4]. Hence the present study was evaluated for its insulin secretagogue effect on glucose induced insulin release from rat pancreatic islets model.

2. Materials and Methods

Plant extraction

The leaves of *B. ceiba* (Illavam panju) was collected and dried. The leaves are dried in shade for more than 1 week. The leaf materials were dried under shade and grinded to coarse powder. Powdered leaf materials (50g) were extracted with methanol (500ml) and then filtered. The methanolic extract of Bombax ceiba leaf powder was collected by using Soxhlet apparatus [5].

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 temperature of 22 ± 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 (200-230 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 [6].

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 methanolic extract of leaf of *Bombax ceiba* (MEBC) (5, 10, 20, 40, 80 and 100 mg) at 37 °C for 30 min in a shaking water bath under 95% O_2 and 5% CO_2 atm. Insulin secretion from islets were observed with graduate doses of MEBC (dose dependent activity) [6]. 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 methanolic leaf extract of *Bombax ceiba* (MEBC) 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, 50µl 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 methanolic extract of *bombax ceiba* on glucose-induced insulin release

When pancreatic islets were incubated with 11.1 mM glucose in the presence of methanolic leaf extract of *Bombax ceiba*, 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 methanolic extract of *Bombax ceiba* at 10 and 60 min when compared with control group without the addition of MEBC (**Table 1 & Figure 1**). Effects of Diazoxide on glucose and MEBC-induced insulin release was studied. Diazoxide, a potent K^+-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 MEBC in the presence of 0.25 Mm diazoxide caused a 22.16% (10 min) and 16.64 % (60 min) inhibition in insulin release when compared with 100 mg of MEBC treated islets without diazoxide. Decreased secretion of insulin was observed in the absence of MEBC (Table 2 & Figure 2).

Discussion and conclusion

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 methanolic extract of MEBC in streptozotocin (STZ)-induced diabetic rats has been reported. To understand the exact mechanism of action of *Bombax ceiba,* the present study was performed with incubating pancreatic islets. This study suggested that *Bombax ceiba* possess protective nature and stimulate the beta cells of islets of pancreases dose dependently. Glucose inhibiting effect of *Bombax ceiba* might be due to stimulation of surviving β-cells of islets of Langerhans leading to more insulin release [7].

Additionally, insulin secretagogue effect of *Bombax ceiba* 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 [8, 9]. MEBC 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 *Bombax ceiba* reverse the effect of diazoxide on glucose induced insulin release at 10 min and 60 min.

Table 1: Dose dependent effect of MEBC on glucose induced insulin release from rat pancreatic beta cells

Groups & Treatment	Insulin release ng/10 islets	
	10min	**60min**
I - Control 11.1mM glucose	0.15±0.01	1.33±0.15
II MEBC 5 mg + 11.1mM glucose	0.33±0.01	1.95±0.17
III - MEBC 10 mg + 11.1mM glucose	0.67±0.02*	2.27±0.26**
IV - MEBC 20 mg + 11.1mM glucose	1.14±0.15***	4.25±0.14***
V - MEBC 40 mg + 11.1mM glucose	1.47±0.24***	5.69±0.18***
VI - MEBC 80 mg + 11.1mM glucose	1.75±0.08***	6.52±0.15***
VII MEBC 100 mg + 11.1mM glucose	1.94±0.14***	6.62±0.16***

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

Table 2: Effect of MEBC (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	0.13±0.02	1.39±0.15
II - MEBC 100 mg + 11.1mM glucose	2.04±0.18[a]***	6.32±0.35[a]***
III - MEBC 100 mg + 11.1mM glucose + 0.25mM diazoxide	1.57±0.31[a]***, [b] ns	5.27±0.14[a]***,[b]**
IV - 0.25mM diazoxide + 11.1mM glucose	0.11±0.02[a] ns	0.53±0.02[a] ns

Effect of MEBC 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.

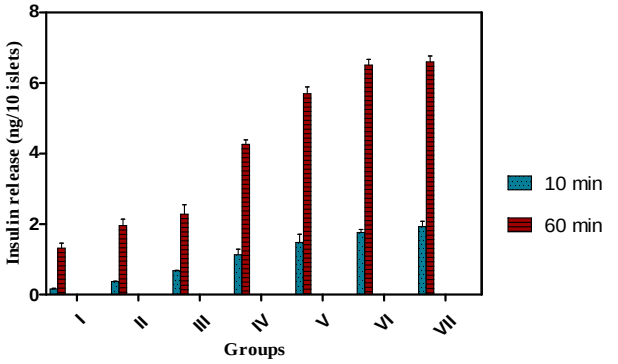

Figure 1: Comparative effect of MEBC on glucose induced insulin release from rat pancreatic islets at 10min & 60 min.

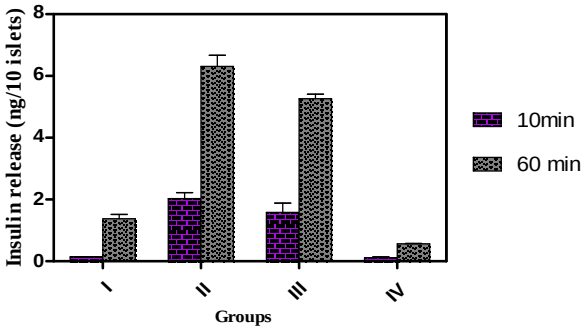

Figure 2: Comparative Effect of MEBC (alone and with diazoxide) on glucose induced insulin release from rat pancreatic islets at 10min & 60 min.

4. Conclusion

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

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