ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 2, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

INSULIN SECRETAGOGUE EFFECT OF ROOTS OF *RAVENALA MADAGASCARIENSIS* SONN. - AN *IN VITRO* STUDY

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Received: 09 September 2018, Revised and Accepted: 11 October 2018

ABSTRACT

Objective: The objective of this study was to establish the cytotoxicity profile and to evaluate the insulin secretagogue effect of ethanolic root extract of *Ravenala madagascariensis* Sonn.

Methods: The cell viability of rat insulinoma 5F (RIN5F) cell lines over the treatment of plant extract was assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide assay. The insulin-releasing effect was evaluated by insulin secretion assay over RIN5F cell lines by enzyme-linked immunosorbent assay.

Results: The ethanolic extract of the roots of *R. madagascariensis* Sonn. showed negligible cytotoxicity at 20–40 µg/ml, and hence, concentrations up to 40 µg/ml were used in insulin secretion assay. The ethanolic root extract at 20 and 40 µg/ml significantly ($p<0.05$ compared to control) stimulated the insulin release in a dose-dependent manner even in the presence of glucose at lower and higher concentrations (5 and 10 mM).

Conclusion: Thus, our results validate its traditional claim in the treatment of diabetes by stimulating the secretion of insulin, thereby suggesting a possible mechanism of its antidiabetic effect.

Keywords: Insulin secretagogue, Rat insulinoma 5F, Diabetes, *In vitro, Ravenala*, Insulin secretion assay, Cytotoxicity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i2.29011

INTRODUCTION

Diabetes mellitus is a metabolic disorder showing levels of high blood glucose due to improper insulin secretion or insulin activity or both [1]. In 2013, it was found to be 382 million people affected with this chronic disorder and the data estimated will be 592 million by 2035 [2]. The current treatment demands a chronic management with oral hypoglycemic agents that carry a burden of adverse effects and drug resistance [3,4]. Market available peroxisome proliferator-activated receptor-gamma agonists to minimize insulin resistance and meglitinides that stimulate insulin secretion, and glucagon-like peptide-1 analog exenatide and dipeptidyl peptidase-IV inhibitors have been nowadays preferred by the diabeticians [5].

The mechanism of antidiabetic activity of the most drugs of natural origin suggests the stimulation of insulin release from pancreatic beta-cells [6]. In the reported literature, various plant extracts including *Capparis zeylanica* [6], *Gymnema sylvestre* [7], *Caulerpa lentillifera* [8], *Gynura procumbens* [9], *Ficus deltoidea* [3], and *Abutilon indicum* [4] were explored for their insulin secretagogue activity in beta-cell lines.

Ravenala madagascariensis Sonn. of family Strelitziaceae commonly known as Traveller's palm has been traditionally used for diabetes [10]. The successive ethanolic extract of the leaves of *R. madagascariensis* Sonn. was reported to exhibit significant antidiabetic [11], hypolipidemic [12], renoprotective [12], and antioxidant [13] activity against alloxan-induced diabetic rats. The aim of the present study is to identify whether the ethanolic extract of the roots of *R. madagascariensis* Sonn. has the potential to elevate the insulin secretion without exhibiting the deleterious effects on beta-cell viability.

METHODS

Cell lines

Rat insulinoma 5F (RIN5F) cell lines were used in the study. RIN5F cell lines were obtained from the National Centre for Cell Science, Pune. The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C.

Collection and authentication of plant material

Roots of *R. madagascariensis* Sonn. were collected from Kochi, Kerala (Fig. 1), during a fine dry weather and dried under shade for 3 weeks. The plant was identified and authenticated by Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen (PARC/2017/3572) has been reserved in the Department of Pharmacognosy, SRM College of Pharmacy, Chennai.

Extraction of plant material

The shade dried and coarsely powdered roots of *R. madagascariensis* Sonn. were macerated with ethanol for 5 days with intermittent shaking. The ethanolic extract obtained on maceration is then filtered, concentrated to dryness and the percentage yield was calculated. Preliminary phytochemical screening was carried out to detect the phytoconstituents present in the extract using standard phytochemical methods [14].

Cell viability assay

The cytotoxic effect of *R. madagascariensis* Sonn., root extract on RIN5F cell lines, was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay [15]. The cells were seeded in a 96-well plate at a density of 1×10^5/well and incubated at

37°C in the presence of 5% CO_2. A fresh medium was replaced at the end of 24 h. Varying concentrations of ethanolic root extract were prepared and transferred to the cells in the 96-well plate. After 48 h of incubation, 100 µl of MTT solution containing 5 mg/ml MTT bromide in physiologically balanced solution was added and the mixture was incubated for 4 h at 37°C after which the medium was removed, and the formazan crystals formed by the living cells were dissolved in 100 µl of dimethyl sulfoxide. The absorbance was measured at 570 nm. The assay was carried out in triplicate. The percentage of cell viability was calculated using the following formula:

$$\%\,\text{Cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

Graphs are plotted using the percentage of cell viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control were included in each assay to compare the complete cell viability.

Insulin secretion assay

RIN5F cell lines were seeded in 24-well plates at 2×10^5 cells/well and incubated at 37°C and 5% CO_2. After 24 h of incubation, the cells were washed twice with excess medium containing glucose at lower and higher concentrations. The cells were incubated at 37°C for 3 h. Glucose-induced insulin secretion assay of ethanolic root extract at 20 and 40 mg/ml was also studied in the presence of glucose at lower (5 mM) and higher (10 mM) concentrations. The aliquots in all wells were collected to determine the concentration of insulin in the media by enzyme-linked immunosorbent assay kit [16].

Statistical analysis

Data are expressed as mean ± standard error of the mean of triplicates. Statistical comparison between groups was done by one-way ANOVA followed by Tukey-Kramer multiple comparison tests to analyze the difference. Statistical significance was considered when $p<0.05$.

Fig. 1: Root - *Ravenala madagascariensis* Sonn.

RESULTS

The maceration of the powdered roots of *R. madagascariensis* Sonn., with ethanol yielded a semisolid yellowish extract, and the percentage yield was found to be 12.8% w/w. The preliminary phytochemical screening of the root extract showed the presence of carbohydrates, flavonoids, alkaloids, glycosides, phenols, tannins, steroids, saponins, and proteins (Table 1).

To assess the non-cytotoxic concentration of *R. madagascariensis* Sonn., the viability of RIN-5F cells was evaluated by treating increasing concentrations of root extract at dose ranging from 0 to 10,000 µg/ml using MTT assay.

Within the tested concentration, the root extract showed a negligible cytotoxicity between 20 and 40 µg/ml (Figs. 2 and 3), and hence, concentrations up to 40 µg/ml were used for the further insulin secretion assay.

The treatment of RIN-5F cells at 20 and 40 µg/ml with ethanolic root extract of *R. madagascariensis* Sonn. significantly increased the secretion of insulin as compared to the control (Table 2). Furthermore, a marked glucose-stimulated insulin secretary (GSIS) effect was observed in RIN-5F cells. We observed that GSIS was directly proportional to the concentration of glucose and dose dependently, a significant GSIS was observed at 20 and 40 µg/ml in low (5 ml) as well as high glucose medium (10 ml).

DISCUSSION

Secondary metabolites from natural sources were proven as leads combating and treating various disease ailments [17]. A major chunk of the traditional medicinal system remains unorganized as people rely more on the local medicine men or Vaids in spite of the enormous commercially available herbals [18].

The current study has demonstrated that the ethanolic root extract of *R. madagascariensis* Sonn. possesses insulin secretagogue effect in the presence of glucose. The results in Table 2 exhibit the effect of ethanolic extract in stimulating the insulin release *in vitro* in a dose-dependent manner. The insulin secretagogue effect of the ethanolic root extract of *R. madagascariensis* Sonn. was significantly higher at hyperglycemic conditions, and this, in turn, explains the role of β-cell glucose metabolism in insulin secretagogue activity of the extract [19].

Investigating the mode of action of glucose lowering agents by *in vivo* approaches is challenging. Hence *in vitro* insulin secretion assay on RIN 5F cell lines, where the potential of the extract to stimulate the insulin secretion can be studied by its direct action on β cells are carried out [7].

Table 1: Qualitative phytochemical screening of the ethanolic extract of the root of *Ravenala madagascariensis* Sonn.

S. No.	Plant constituents	Powdered plant material	Ethanolic root extract of *Ravenala madagascariensis* Sonn.
1	Carbohydrate	+	+
2	Flavonoids	+	+
3	Glycosides	+	+
4	Alkaloids	+	–
5	Phenols	+	+
6	Tannins	+	+
7	Terpenoids	–	–
8	Saponins	+	+
9	Proteins	+	+
10	Lipids	–	–
11	Steroids	+	+
12	Anthraquinones	–	–
13	Iridoid glycosides	–	–

+: Denotes the presence of phytoconstituent, –: Denotes the absence of phytoconstituent

Table 2: Insulin release of the ethanolic root extract of *Ravenala madagascariensis* Sonn. on RIN5F cell line

Concentration of the extract	0 mM glucose (pg/ml)	5 mM glucose (pg/ml)	10 mM glucose (pg/ml)
Control	60.83±0.059	103.54±0.052	135.59±0.088
20 µg/ml	164.45±0.197*	207.20±0.047*	257.52±0.216*
40 µg/ml	196.43±0.121*	282.38±0.190*	307.28±0.072*

Data are expressed as mean±SEM of triplicates (n=3). Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparison test. *p<0.05 compared to control. SEM: Standard error of the mean

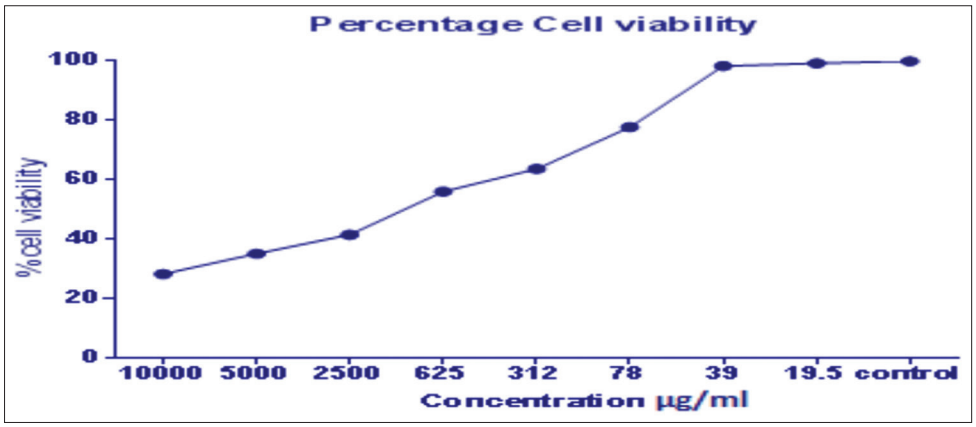

Fig. 2: Cell viability effect of ethanolic root extract of *Ravenala madagascariensis* Sonn. Data are expressed as mean ± standard error of the mean of triplicates (n=3). Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparison test. p<0.001 compared to control

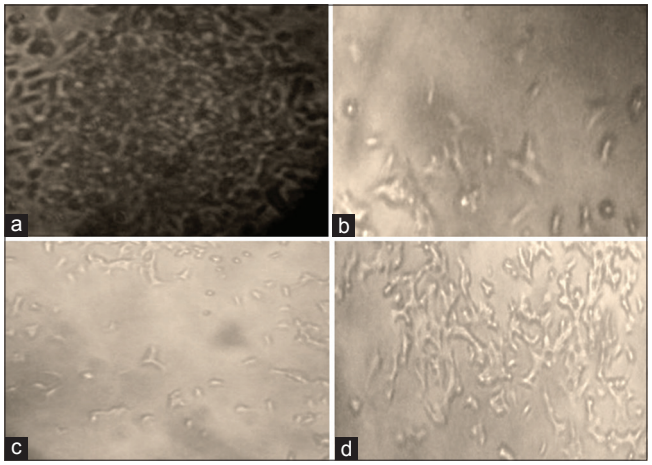

Fig. 3: Cell viability profile of ethanolic root extract of *Ravenala madagascariensis* Sonn. (a) Normal rat insulinoma 5F, (b) viability - 10,000 µg/ml, (c) viability - 1250 µg/ml, (d) viability - 39 µg/ml

Studies on the insulin secretion stimulating effect of *G. sylvestre* extract prepared by ethanol-sulfuric acid extraction and reported its insulinotropic effect from mouse insulinoma 6β cells at 0.25 mg/ml [20]. Band *et al.* have studied the role of arachidonic acid as an effective insulin secretagogue formed in β-cells as a result of phospholipase A-2-mediated hydrolysis of membrane phospholipids in the presence of voltage-operated calcium channel inhibitor [21]. Sharma and Rhyu have reported the stimulated secretion of insulin in RIN cells and an enhanced glucose transporter expression and glucose uptake in 3T3 L1 adipocytes (derived from mouse embryonic fibroblast) [8].

The aqueous extract of *G. procumbens* as a hypoglycemic agent and the mechanisms involved were investigated by Hassan *et al*. and found that it can be due to reduced intestinal glucose absorption as a result of its high-fiber content [9,22,23]. Studies have been reported that the stimulation of insulin secretion and glucose absorption in a dose-dependent manner by hydroalcoholic extract of saffron and the effect was even more pronounced at higher doses [24].

CONCLUSION

The findings of the present *in vitro* studies revealed that *R. madagascariensis* Sonn. ethanolic root extract showed antidiabetic activity by stimulating the insulin secretion from RIN5F cell lines. Further, investigations are under progress to identify the proper dose and role of the phytoconstituents of *R. madagascariensis* Sonn. on diabetic parameters at the molecular level.

AUTHORS' CONTRIBUTION

All the authors have contributed equally to the conductance of study, writing, and editing the article.

CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to be declared.

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