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

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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 (RINSF) 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 RINSF 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.

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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 detoidea [3], and Abutilon indicum [4] were explored for their insulin secretagogue activity in beta-cell lines. Ravenala madagascariensis Sonn. of family Strelitziaeae 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 (RINSF) cell lines were used in the study. RINSF 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 CO₂ 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 RINSF cell lines, was determined by 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT) assay [15]. The cells were seeded in a 96-well plate at a density of 1×10⁵/well and incubated at
37°C in the presence of 5% CO₂. 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:

\[
\% \text{ Cell viability} = \frac{A_{570 \text{ of treated cells}}}{A_{570 \text{ of control cells}}} \times 100
\]

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 2×10⁴ cells/well and incubated at 37°C and 5% CO₂. 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 μg/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.

**RESULTS**

The maceration of the powdered roots of *R. madagascariensis* Sonn., with ethanol yielded a semi-solid 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 secretory (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 herbs [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].

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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Plant constituents</th>
<th>Powdered plant material</th>
<th>Ethanolic root extract of <em>Ravenala madagascariensis</em> Sonn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Terpenoids</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Saponins</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Proteins</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Lipids</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Steroids</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Anthraquinones</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Iridoid glycosides</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

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

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