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ORIGINAL ARTICLE

Insulinotropic and Cytoprotective Effect of L-theanine: An *In vitro* Dose Dependent Study

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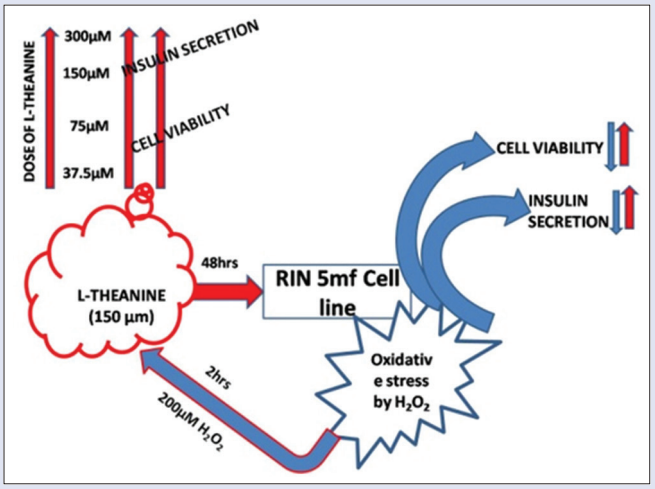
ABSTRACT

Background: L-theanine is a natural bioactive amino acid present in tea (*Camellia sinensis* [L.] O. Kuntze) and consumed worldwide through tea decoction. **Objective:** The present work aims at evaluation of the insulin secretion and cytoprotective efficacy of L-theanine, a bioactive amino acid present in tea, on pancreatic β-cell line, RIN-m5F. **Methods:** RIN m5F cell were treated with L-theanine alone or prior to hydrogen peroxide (200 um) treatment and subsequently cell viability, cellular morphology, insulin secretion and insulin gene expression were analyzed. **Results:** Studies have shown that L-theanine dose dependently (0–300 μm) increases the β-cell mass as well as increases insulin production by RIN-m5F cell. It was also observed that pretreatment of the cell with L-theanine partially protected the oxidative stress of β-cells that were evident from cell viability, cellular morphology, and restoration of insulin-secreting ability. **Conclusion:** Results suggest that L theanine is an insulinotropic agent as well as effective in giving partial protection to pancreatic β-cells in oxidatively stressed condition. L-theanine can be used in the prevention or treatment of diabetes.

Key words: β-cell, diabetes, hydrogen peroxide, insulinotropic, L-theanine, oxidative stress

SUMMARY

- L-theanine is consumed worldwide through tea decoction
- L-theanine dose dependently increases the beta cell mass and insulin secretion
- L-theanine has cytoprotective property against peroxide-induced stress.



Abbreviations used: EGCG: Epigallocatechin gallate; FBS: Fetal Bovine Serum; DMEM Dulbecco's Modified Eagle Medium

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INTRODUCTION

Diabetes is a major noncommunicable disease worldwide. The disease diabetes was first reported in the Egyptian manuscript in the year 1936.[1] The incidence of this ailment is increased rapidly due to urbanization, stress, food habit, and obesity and is becoming a major health problem worldwide.[2] According to a study published in Lancet, China, India, and the USA are the top three countries that have a large number of populations affected with the disease.[3] India ranks 3 among countries with the diabetic population, and it is expected within the year 2025 that India will be the diabetic capital of the world.[4] Hyperglycemia is the hallmark of diabetes.[5] Imbalance of glucose homeostasis and increased blood sugar level cause different diabetic complications including cardiovascular disease, nephropathy, retinopathy, and neuropathy.[6] Type I diabetes is an autoimmune disorder whereas Type II is resulting from increased insulin resistance and insufficient insulin secretion.[7,8] The current mode of treatment of diabetes is also type dependent. Insulin therapy is the main treatment of Type I whereas Type II diabetic patients are treated with agents that increase the sensitivity of target organs to insulin.[9] Therefore, restoration of pancreatic β-cell function is the best strategy to treat Type I and II diabetes.[10] Due to the toxicity of the drugs in use, search for biomolecules having antidiabetic activity is initiated.[11]

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In this backdrop, searching for new drugs with less toxicity has been initiated that has simultaneously insulinotropic and cytoprotective activity such that it can be used in both Type I and II diabetes.

Tea contains abundant polyphenols and caffeine which showed antidiabetic activity, so the development of antidiabetic medications from tea and its extracts is increasingly receiving attention.[5,12] A clinical study by our group has shown that daily 5 cups of tea consumption decrease the different markers of Type II diabetes.[13] Biochemical analysis of tea highlighted that it contains L-theanine, a unique nonprotein-forming amino acid, in considerable quantity with the other known bioactive components such as epigallocatechin gallate (EGCG) (green tea) and theaflavins (black tea).[14] Several studies suggest that L-theanine plays a pivotal role in the health-promoting effect of tea.[15,16] Therefore, the present study aims at deciphering the insulinotropic and cytoprotective activity of L-theanine, if any.

MATERIALS AND METHODS

Chemicals

Fetal bovine serum (FBS) was purchased from Gibco (Auckland, New Zealand); cell culture media Dulbecco's Modified Eagle Medium (DMEM), pen strep solution, and L-theanine were supplied by MP-BIOMEDICALS (USA); cell proliferation reagents (WST-1) and hydrogen peroxide (H_2O_2) were purchased from Sigma (India); Insulin Elisa kit was procured from Bioassay Technology Laboratory (Shanghai, China); and Polymerase Chain Reaction (PCR) kit was purchased from Thermo Fishers Scientific.

Cell culture

RIN-m5F was cultured in DMEM with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mmol/L in a humidified atmosphere (5% CO_2, 37°C).

Effects of L-theanine on RIN-m5F cells

Cells (1×10^5) were seeded in a 96-well plate for five different experimental groups. After 24 h incubation, different groups, for example, Control (C), L-1, L-2, L-3, and L-4 groups were treated with different doses of L-theanine (0, 37.5, 75, 150, and 300 μm) and again incubated for 24 and 48 h. After the incubation period, the cell viability was measured by the WST-1 reagent. Whole experiments were performed in triplicate. Cellular morphology of all the groups was investigated by inverted phase contrast microscope.

Effects of hydrogen peroxide on L-theanine-treated RIN-m5F cells

As stated above, Control (C), L-1, L-2, L-3, and L-4 cells were preincubated with 0–300 μm doses of L-theanine for 48 h, then cells were challenged with a previously standardized dose of 200 μm H_2O_2 for 2 h. After the incubation period, the cell viability was measured by the WST-1 assay. Whole experiments were performed in triplicate. Cellular morphology of all the groups was investigated by inverted phase contrast microscope.

Quantification of insulin secretion and insulin gene expression

Cells were divided into two sets of experimental groups, for example, Set-I (Cont, L-1, L-2, L-3, L-4) and Set-II (C, L, H, L + H). Secreted insulin was determined using ELISA kit-based method, and insulin gene expression was studied using PCR-based assay.

RESULTS AND DISCUSSION

L-theanine is a nonprotein amino acid.[15] This amino acid is naturally produced in a tea plant and contributes to the umami test of tea.[17] Darjeeling tea contains a considerable amount of L-theanine.[18] L-theanine is not only responsible for umami taste but also has several health-promoting activities.[15,19] In our laboratory, we have already determined NSAID-induced gastric ulcer healing effects of L-theanine on experimental mice model.[20] A recent study by our group revealed that daily intake of 5 cups of tea can play an important role in reducing surrogate markers of insulin resistance.[13] Another study revealed that tea catechins, especially EGCG have antiobesity and antidiabetic effects such as other antioxidants.[21,22] Recent study showed that black tea brew contains a good quantity of L-theanine with its unique beneficial antioxidants, for example, theaflavins.[18] L-theanine has also tissue-healing property as evident from its ulcer-healing activity.[20] In this backdrop, the present study aims at the determination of the antidiabetic effects of L-theanine. As it was already proven that one of the major causes of diabetes is the loss of β-cell mass due to oxidative stress,[23,24] in this study, we have tried to find out the insulinotropic and cytoprotective activity of L-theanine.

Effects of L-theanine on cellular (RIN-m5F cell) growth

In this context, RIN-m5F cells were treated with different doses of L-theanine (0-300 μm). The result showed that at the higher dose, L-theanine increased β-cell masses significantly [Figure 1].

The study highlighted that 150 μm (L-3) and 300 μm (L-4) of L-theanine increased the cell mass that was statistically significant compared with the control group. However, there is no significant difference observed between L-3 and L-4 groups. Hence, from this study, we have concluded that 150 μm (L-3) was the optimum dose. Most interesting part of this study was that L-theanine did not only increase cell masses but also increase insulin secretion dose dependently [Figure 1]. Phase contrast microscopic data also expressed that cell masses were increased with the doses [Figure 2].

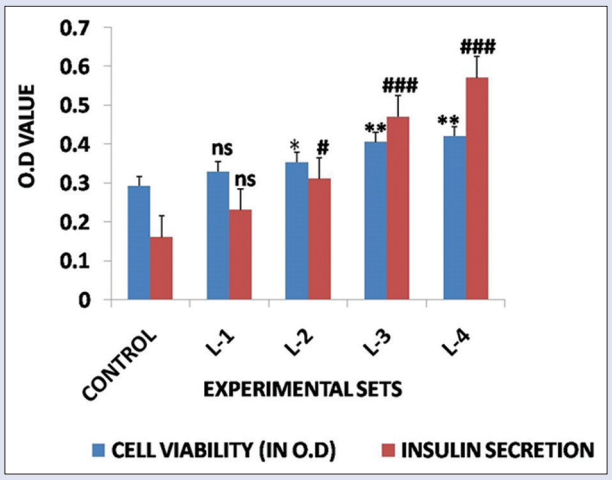

Figure 1: Effects of L-theanine on cell viability and insulin secretion. In this study, we have used four different doses of L-theanine and this is represented as L-1, L-2, L-3, and L-4 (L-1 = 37.5 μm, L-2 = 75 μm, L-3 = 150 μm, and L-4 = 300 μm). Data were presented as means ± standard deviation. ***P < 0.001 versus control group; **P < 0.01 versus control group and *P < 0.05 versus control group for the study of cell viability and ###P < 0.01 versus L3-treated group, *P < 0.05 versus L3-treated group in the study of cell viability and ##P

PCR-based analysis of insulin gene expression study also supports the above facts and can be corroborated with ELISA-based insulin assay [Figure 3].

Protective effects of L-theanine on hydrogen peroxide-induced cytotoxicity on RIN-m5F cell

It was observed that the growth of RIN-m5F cells was effectively inhibited by 200 μm H_2O_2. However, when cells were pretreated with L-theanine (L3 dose), cell death was markedly attenuated as compared with H_2O_2-treated group [Figure 4].

If we try to explain our data, we must say that with respect to control group, maximum number of cell death was visible in the H_2O_2-treated group [Figure 4]. However, when cells were pretreated with L-theanine, percent of cell death was significantly decreased [Figure 4]. 150 μm of L-theanine gave maximum protection against H_2O_2-induced oxidative stress. This dose was also similar with the optimum dose of L-theanine that increases β-cell mass and insulin production [Figure 1]. Phase contrast microscopy also showed that cellular health was improved with treatment of L-theanine on RIN-m5F cell line [Figure 5].

H_2O_2 also inhibited insulin secretion [Figures 4 and 6]. However, when cells were preincubated with L-theanine (150 μm), insulin secretion was improved as compared with the H_2O_2 group.

Hence, from these data, it can be concluded that L-theanine not only protected β-cell mass against oxidative stress but also improved insulin expression in stressed cells.

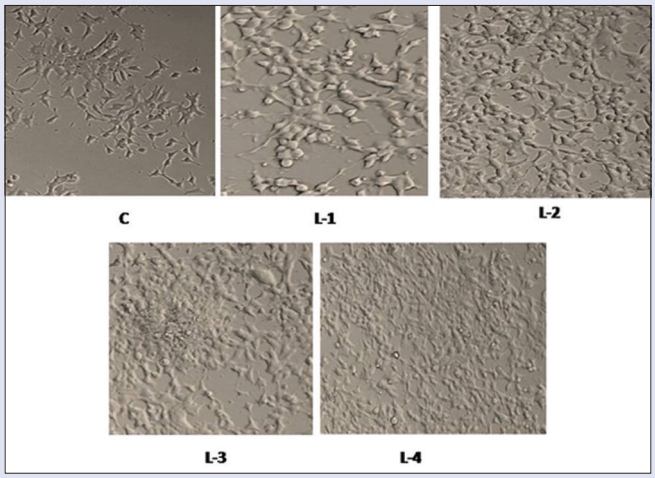

Figure 2: Phase contrast microscopic image of RIN-m5F cell line with 37.5 μm (L1), 75 μm (L2), 150 μm (L3), and 300 μm (L4) doses of L-theanine

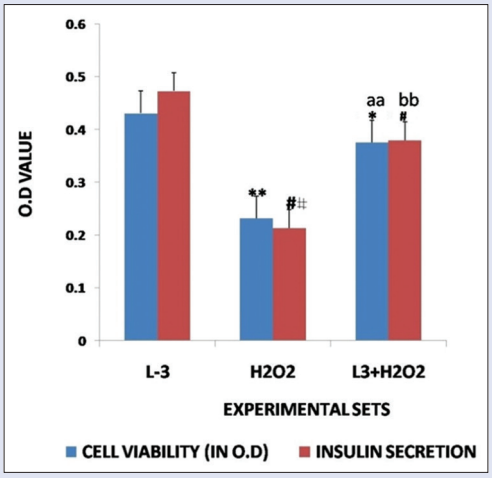

Figure 4: Cell viability and insulin secretion after hydrogen peroxide treatment on RIN-m5F cell line. Value was calculated in respect of optimum dose 150 μm (L-3) of L-theanine. Data were presented as means ± standard deviation. $^{**}P < 0.01$ versus L3-treated group, $^{*}P < 0.05$ versus L3-treated group in the study of cell viability, and $^{\#\#}P < 0.05$ versus L3-treated group, $^{\#}P < 0.05$ versus L3-treated group in the study insulin secretion. $^{aa}P < 0.05$ versus hydrogen peroxide-treated group in the study of cell viability and $^{bb}P < 0.05$ versus hydrogen peroxide-treated group in the study of insulin secretion ($n = 3$)

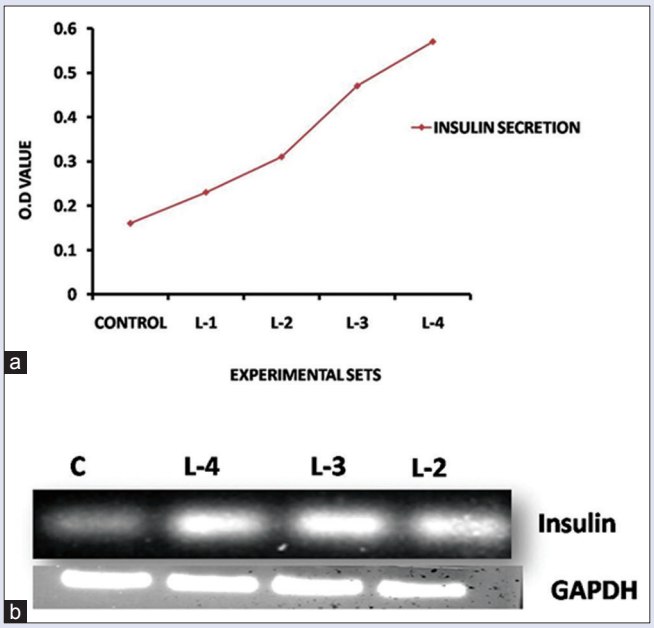

Figure 3: (a) Shows the concentration of secreted insulin in a dose depending manner. (b) shows the Reverse Transcriptase-PCR (RT-PCR) data of insulin gene expression. GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) is used as positive control

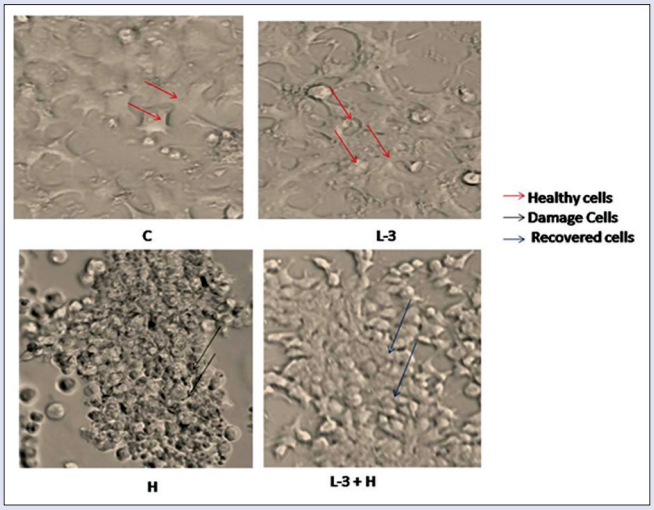

Figure 5: The phase contrast microscopic images of cellular morphology with or without hydrogen peroxide treatment

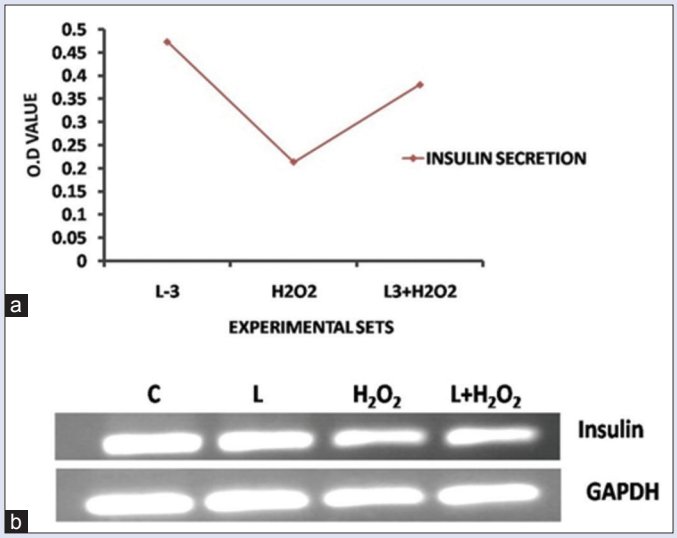

Figure 6: (a) Insulin secretion in the presence or absence of hydrogen peroxide. (b) The reverse transcription-polymerase chain reaction data of insulin gene expression in the presence or absence of hydrogen peroxide. GAPDH is used as positive control

CONCLUSION

From the above study, we may conclude that L-theanine in one hand can give protection to the pancreatic β-cell against oxidative damage, and on the other hand, it can dose dependently increase insulin secretion. This paper for the first time shows the possibility of use of L-theanine as safer biomedicine for prevention and cure of both types of diabetes.

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Conflicts of interest

There are no conflicts of interest.

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