International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.4, pp 489-493, 2017

# Insulin secretagogue activity and inhibitory effects of *Cichorium intybus* on Streptozotocin- induced pancreatic β cell damage *in vitro* in RINm5F cells

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**Abstract :** This study focuses on the preventive effects of aqueous extract of *Cichorium intybus* (CIE) on streptozotocin (STZ) induced β-cell damage. Pancreatic β-cells or islet were treated with STZ in presence and absence of *Cichorium intybus* and the inhibitory effect of it against STZ toxicity were determined in RINm5F (RIN) rat insulinoma cells. Cell viability, Nitric oxide production and Insulin secretion were assayed. RIN cells were treated with STZ which induces cell damage. Treatment of cells with CIE (5µg and 50µg) has protected the pancreatic β cells from STZ mediated toxicity. Viability of RIN cells were checked by MTT assay. After treatment for 24 hours, STZ mediated damage was reduced in the group treated along with CIE. There was a remarkable increase in the viability of RIN cells around 65% was maintained. Nitrite concentration in the cell-free culture supernatant served as a reflection of NO production and was measured. The result implies that decrease in the NO around 50µM/ml from the content in the group treated with STZ along with CIE. Insulin producing capability CIE STZ mediated damaged cells were determined. Cells treated with CIE shows increase in the insulin content of the RIN cells around 77 µIU/ml. CIE has significantly reduced the toxicity of STZ by maintaining viability, reduced nitric oxide production and maintains insulin secretion compared with the group treated with treated only with STZ of pancreatic β cells.

**Keywords:** Insulin secretagogue activity, *Cichorium intybus,* pancreatic β cell damage, RINm5F cells.

## Introduction

Diabetes mellitus is a metabolic disorder in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. There are two main types of diabetes. Type 1 diabetes results from the body's failure to produce insulin and Type 2 diabetes results from insulin resistance, a condition in which cells fail to use insulin properly[1]. Diabetes is now becoming a major threat to health worldwide. Diabetes mellitus is recognized as the fourth most commonly diagnosed chronic condition[2]. Most of the phytochemicals are polyphenols, which are important secondary metabolites in plants and are responsible for antioxidant action[3]. *Cichorium intybus.* L, chicory plant in the family of Asteraceae is being used by the traditional healers for the treatment of diabetes but the mechanism by which *Cichorium intybus* heals diabetes is not yet clear. *Cichorium intybus* is reported for its anti hepatotoxic properties by preventing per oxidation of lipids[4], lowering glucose 6 phosphatase activity[5], can modulate cytokine secretion[6] etc., Streptozotocin (STZ) is a β -cell-specific toxin which induces the formation of

superoxide anions in mitochondria, limits ATP production and causes depletion of nucleotide in β cells[7]. Thus the present study designed to evaluate levels of reduction of cell toxicity of STZ on pancreatic β cell damage in RINm5F cells through evaluating the levels of insulin secretion, cell viability and nitric oxide production.

## Materials and methods

Streptozotocin was obtained from Sigma, St Louis, USA. Dulbecco's Modified Eagles medium (DMEM) was purchased from Thermo Scientific, USA and Fetal bovine serum (FBS) was obtained from GIBCO, USA. 3-4, 5-dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide (MTT). All other chemicals used were of analytical grade.

### Preparation Plant extracts

*Cichorium intybus.* L, a chicory plant locally called as Kasini, leaves were collected from Vaniyampadi Village, Vellore district, Tamil Nadu, India and were authenticated and deposited at Plant Anatomy Research Centre, Tamil Nadu with voucher number PARC/2016/3271. It was shade dried ground and extracted by sequential extraction method from low polar to high polar solvents using Soxhlet apparatus [8]. They were freeze-dried and stored at -80ºC until use.

### Culturing of RINm5F cells and Experimental design

Min6 cells (Mouse insulinoma cells) were purchased from the National Centre for Cell Sciences (NCCS), Pune, India and grown at 37˚C in DMEM medium containing 10% FBS with 2mM L-glutamine, 100 IU/mL of penicillin, 100 U/ml penicillin/streptomycin, 2.5 µg/mL of amphotericin B under a humidified 5% $CO_2$ atmosphere and maintained at a pH 7.4. Streptozotocin (STZ) was used with a final concentration of 5mM was used to induce β cell damage. Treatment with aqueous extract of *Cichorium intybus* was carried out with different concentrations and it has been finalized with two different concentrations (5µg/mL and 50µg/mL) for 24 hours along with 5mM STZ and cells treated with 50µg/mL aqueous extract of *Cichorium intybus* alone was also maintained throughout the study. All the treatments were done in triplicates.

### Assessment of cell viability- MTT assay

$1\times10^4$ RINm5F cells were seeded in 96 well culture plates and were incubated in the $CO_2$ incubator for attaining confluence. After 24 hours of treatment, the reduction of MTT to formazan was determined for cell viability using the available method[9].

### Nitric oxide assay (Griess nitrate assay)

The nitrite scavenging activity of the CIE was determined using the modified method of Ignarro *et al.,*[10]. 100 µl of Griess reagent containing 1% sulphanilamide and 0.1% N-(1-naphthyl) ethylene diamine di hydro chloride in 1 ml of 0.1M HCl was mixed with 100 µl of different concentration of the sample and was incubated for 10 min at room temperature. The absorbance of the mixture was then determined at 540 nm in UV-Visible spectrophotometer (Ultrospec 2100 pro, England). All samples and test standard were performed in triplicates.

### Glucose stimulated insulin secretion

RIN cells were cultured in 6 well plates and after the treatment, the cells were washed three times with Krebs-Ringer bicarbonate buffer and incubated for 1 h at 37°C with 9.9 mM glucose Aliquots were used for insulin assay[11]. Insulin was quantified using Labserv insulin ELISA kit (Thermo scientific, USA).

## Result and Discussions

### Assessment of cell viability

Treatment of RINm5F cells with 5mM STZ for 24 hours resulted in a significant decrease in proliferation of 38% when compared with control cells and treatment with 5µg/ml and 50 µg/ml of CIE along

with 5mM STZ had shown significant increase in viability of up to 48% and 65% respectively by when compared with STZ treatment as shown fig. 2. Our study reveals that treatment with aqueous extract of *Cichorium intybus* in RIN m5F cells showed significant decrease in the cytotoxicity induced by STZ.

**Assessment of glucose-induced insulin secretion**

Increase in the level of insulin secretion in the group treated with CIE along with administration of 5mM STZ up on glucose stimulation was observed up to 77µIU/ml compared with STZ control with around 25µIU/ml. (Fig. 3). It was reported that mitochondrial oxidative stress reduces insulin secretion in pancreatic β cells, similarly in our studies we have shown that STZ treatment decreases insulin secretion which could be due to oxidative stress but further studies are warranted to state the fact whether mitochondrial oxidative stress could have a role[12].

**Assessment of Nitric oxide**

There was significant decrease in the level of nitrate in the group treated with CIE along with administration of 5mM STZ around 50µM/ml compared with the group treated only with STZ showed around 125µM/ml of media. *Cichorium intybus* exhibited significant increased radical scavenging activity against superoxide and nitric oxide as reported in KIOM-4[13]. STZ treated group's showed a significant reduction in nitric oxide production could be due to the report shown by a study [14].

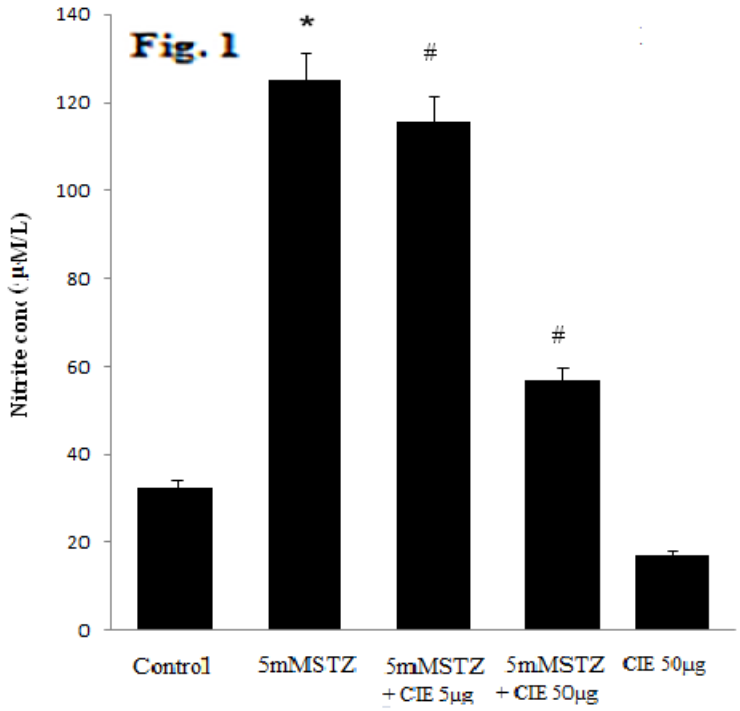


**Fig. 1. Effect of CIE aqueous extracts on pro-oxidant system of RINm5F cells. Levels of Nitric oxide was studied in the untreated control, 5mM STZ , 5mM STZ +CIE 5µg, 5mMSTZ + CIE 50µg and CIE 50µg alone. Column represents the mean ± SD, n = 3. *Represents groups significantly differs from the untreated control group ($p < 0.05$). # Represent groups significantly differs from 5mM STZ control group ($p < 0.05$).**

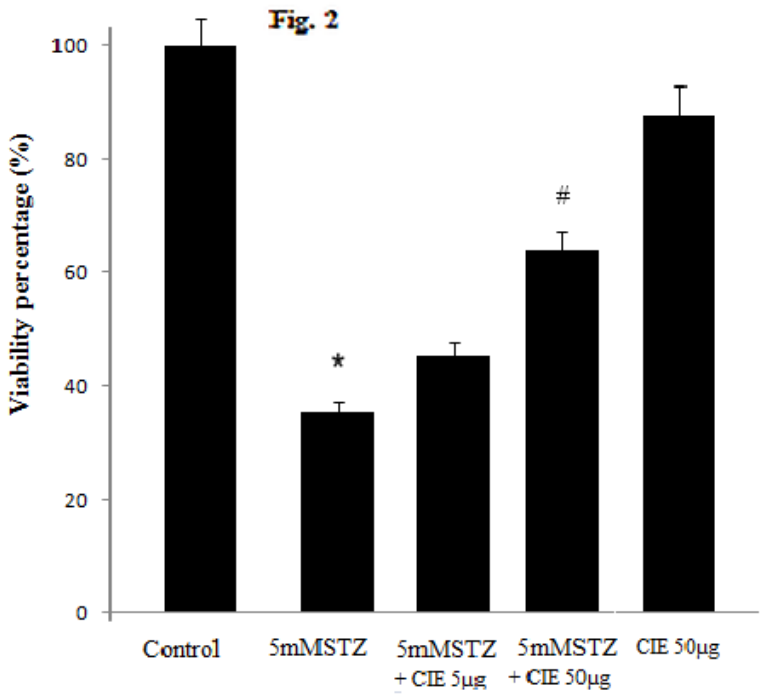


**Fig. 2. Effect of CIE aqueous extract on proliferation of STZ treated Min6 cells a) MTT formazan formation b) BrdU cell proliferation absorbance from control, 5mM STZ, 5mM STZ +CIE 5µg, 5mMSTZ + CIE 50µg and CIE 50µg alone administrated for 24 hours. Column represents the mean ± SD, n = 6. *Represents groups significantly differs from the untreated control group ($p < 0.05$). # Represents groups significantly differs from 5mM STZ control group ($p < 0.05$)**

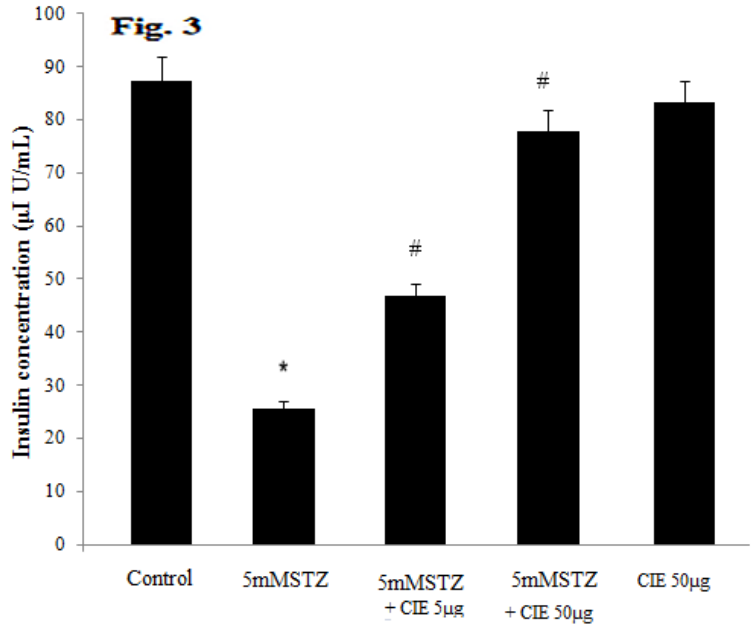


**Fig. 3. Effect of CIE aqueous extracts on glucose-induced insulin secretion. Column represents the mean ± SD, n = 3. *Represents groups significantly differs from the untreated control group ($p < 0.05$). Represent group's significantly differs from 5mM STZ control group ($p < 0.05$).**

## Conclusion

The study implies that aqueous extract of *Cichorium intybus* have insulin secretagogue activity by maintaining cell viability and reducing the oxidative stress through reducing the free radical production such as nitric oxide. CIE did not show any toxicity when treated alone which implies the non toxicity nature of CIE as shown by Manali[15].

## References

1. Fajans SS, Cloutier MC, Crowther RL. Clinical and etiological heterogeneity of idiopathic diabetes mellitus. The banting memorial lecture. Schweiz Med. Wochenschr. 1979; 109(46):1774–85.
2. Hsiao L-C, Muo C-H, Chen Y-C, Chou C-Y *et al.*, Increased risk of coronary heart disease in patients with chronic osteomyelitis: a population-based study in a cohort of 23 million. Heart Br Card Soc. 2014; 100(18):1450–4.
3. Nadia Salem Alrawaiq and, Azman Abdullah. A Review of Flavonoid Quercetin:metabolism, Bioactivity and Antioxidant Properties..Int.J. PharmTech Res.2014, 6(3), pp 933-941.
4. Ahmed B, Al-Howiriny TA, Siddiqui AB. Antihepatotoxic activity of seeds of *Cichorium intybus*. J. Ethnopharmacol. 2003; 87(2-3):237–40.
5. Pushparaj PN, Low HK, Manikandan J, *et al.*,Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. J. Ethnopharmacol. 2007; 111(2):430–4.
6. Karimi MH, Ebrahimnezhad S, Namayandeh M *et al.*, The effects of *Cichorium intybus* extract on the maturation and activity of dendritic cells. Daru J Fac Pharm Tehran Univ. Med. Sci. 2014; 22 (1):28.
7. Kakkar R, Mantha SV, Radhi J *et al.*, Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clin Sci Lond Engl 1979; 94(6):623–32.
8. Muthusamy VS, Anand S, Sangeetha KN *et al.*, Tannins present in Cichorium intybus enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. Chem Biol Interact. 2008; 174(1):69–78.
9. Madesh M, Balasubramanian KA. A microtiter plate assay for superoxide using MTT reduction method. Indian J Biochem Biophys. 1997; 34(6):535–9.
10. Ignarro LJ, Buga GM, Wood KS *et al.*, Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl. Acad. Sci. U S A. 1987; 84(24):9265–9.
11. Latha M, Pari L, Sitasawad S *et al.*, Insulin-secretagogue activity and cytoprotective role of the traditional antidiabetic plant *Scoparia dulcis* (Sweet Broom weed). Life Sci. 2004; 75(16):2003–14.
12. Morita A, Tanimoto K, Murakami *T et al.*, Mitochondria are required for ATM activation by extranuclear oxidative stress in cultured human hepatoblastoma cell line Hep G2 cells. Biochem Biophys Res Commun. 2014; 443(4):1286–90.
13. Adachi H, Kondo T, Koh GY *et al.*, Angptl4 deficiency decreases serum triglyceride levels in low-density lipoprotein receptor knockout mice and streptozotocin-induced diabetic mice. Biochem. Biophys.Res.Commun. 2011; 409(2):177–80.
14. Kang KA, Kim JS, Zhang R *et al.*, KIOM-4 Protects against Oxidative Stress-Induced itochondrial Damage in Pancreatic β-cells via Its Antioxidant Effects. Evidence Based Complement Altern. Med. Ecam. 2011; 2011: 978682.
15. Manalil Jos Jeksy, Puthanveettil Jayaram, Thekkekka ra Devassy Babu and Achuthan C Raghavamenon. Antioxidant and Anti-inflammatory Properties of a Nontoxic Herbal formulation – GSTC. Int.J. PharmTech Res.2014, 6(3), pp 924-932.

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