

Evolution Of Compound Molecules As Potential Drugs To Cure Insulin Resistance

Mr. Prabas Nandan Rout^{1*}, Dr. Prasanna Kumar Purohit²

^{1*}Email: routprabasnandan@gmail.com

²Email: purohit_prasann@yahoo.com

ABSTRACT

The development of NIDDM is mostly due to insulin resistance. This disorder develops when cells in the body do not react normally to insulin, despite having normal blood glucose levels. Type 2 diabetes may be effectively treated with medications that increase insulin receptor (IR) phosphorylation. They aid in enhancing the first stages of insulin signaling. The stem bark of *Cinnamomum zeylanicum* Blume was used to extract a proanthocyanidin called Cinnamtannin B1, which was then utilized in research to determine whether or not it might phosphorylate IR. This chemical is a member of the Lauraceae genus. The chemical makeup of the substance was deduced using spectroscopy. Cinnamtannin B1 (0.11 mM) increases insulin receptor-subunit phosphorylation on 3T3-L1 adipocytes. Inhibition of GLUT4 by cytochalasin B and phosphatidylinositol 3-kinase (PI3K) activity by vanadate. Insulin receptor phosphorylation was unaffected by vanadate. These findings suggest that Cinnamtannin B1 stimulates the PI3K pathway, leading to the phosphorylation of IR.

Keywords: Mitochondrial superoxide dismutase, *Cinnamomum zeylanicum*, phosphorylation, cinnamtannin B1, insulin receptor, adipocyte, diabetes mellitus.

1. INTRODUCTION

Insulin, a hormone secreted by the pancreas, allows glucose to enter muscle, fat, and liver cells, where it is converted into energy. When you eat, your body breaks down the food you consume into glucose, a type of sugar. When you're fasting, the liver can also produce glucose to meet your body's demands. Following a meal, the pancreas discharges insulin into the bloodstream, which functions to decrease the blood glucose concentration. The regulation of blood sugar levels is accomplished by insulin, which facilitates the reduction of blood sugar levels. It is called "insulin resistance" when the insulin-producing cells in your muscles, adipose tissue, or liver are unable to readily absorb glucose from your blood. Pancreatic insulin production rises to help glucose reach your cells. [1] As long as you have enough insulin in your pancreas to overcome your cells' inability to respond, your blood sugar levels will remain in the healthy range. Your pancreas produces extra insulin to compensate. A rise in blood sugar levels is inevitable over time. Insulin resistance (IR) is the term used to describe the condition where cells are unable to reply effectively to the insulin hormone.

Insulin aids glucose's entry into cells, decreasing blood sugar levels. When carbohydrates enter the bloodstream, the pancreas secretes insulin. People with insulin resistance respond differently to insulin, both in terms of glucose transport and blood sugar levels. Sulfate depletion may be crucial to insulin resistance, albeit the mechanism by which this occurs is not well known. [3] Some medications and medical conditions, as well as being overweight or inactive, can increase insulin resistance. Insulin resistance is characteristic of the metabolic syndrome. Doctors seldom employ fasting insulin levels and glucose tolerance testing, despite their usefulness in diagnosing insulin resistance. Changes in diet, exercise, and other elements of everyday life have been linked to better insulin sensitivity and even reversal of insulin resistance.

When insulin binds to insulin receptors in cells, a series of signaling cascades known as the PI3K/Akt/mTOR signalling pathway is activated. Recent research suggests that the insulin response may be a threshold phenomenon, with certain cell types reacting to insulin as if it were a bistable switch under physiological settings. Lipolysis of free fatty acids is only one mechanism by which insulin sensitivity throughout the route might be reduced. However, organisms frequently employ sensitivity tuning (or lowering) to adapt to the dynamic nature of their environment or their metabolic needs. [2] Since both the mother's and the fetus's brains increase their glucose consumption during pregnancy, the mother's muscles become less sensitive to insulin. Insulin sensitivity may be improved, according to the adjustable threshold theory of insulin resistance, by inducing placental growth factor, which blocks the interaction between insulin receptor substrate (IRS) and PI3K.

Insulin resistance has been linked to the antioxidant defence mechanism of superoxide dismutase (SOD) in cell mitochondria, for various reasons. Studies have shown that mitochondrial superoxide dismutase mimics, electron transport chain inhibitors, and uncouplers may rapidly reduce insulin resistance. [4]

2. MATERIALS AND METHODS

• Chemicals

Sigma-Aldrich and Gibco BRL were our go-to spots for all of the research-grade chemicals we needed for our cell culture experiments. Merck-Schuchardt provided the high-quality chemicals used in the analysis. We used Merck (Darmstadt, Germany) chromatography-grade silica gel and thin-layer chromatography-specific silica gel (60 F254) in our experimentation. To supplement this, we ordered Sephadex-LH 20 from Pharmacia in Uppsala, Sweden. Promega (Madison, WI) provided the molecular weight markers used to quantify protein concentrations. Both the main antibody (goat anti-rabbit IgG, HRP conjugated) and the secondary antibody (phosphotyrosine control, EGF-stimulated A431 cell lysate) were

purchased from Upstate Biotechnology (Lake Placid, NY). The Shimadzu UV-100PC was used to capture ultraviolet spectra in methanol. The FT-IR Perkin Elmer 1600 was used to acquire IR spectra from KBr discs. The VG (Micromass) 70-SE mass spectrometer was used to gather the FAB-MS results. One hundred MHz of ^{13}C NMR, three hundred MHz of ^1H NMR, and one hundred and fifty MHz of ^1H - ^1H COSY NMR were all recorded on a Bruker Avance spectrometer.

• Plant-Based Products

C. zeylanicum Blume was collected in West Sumatra, Indonesia. The Herbarium of Universitas Andalas, Padang, Indonesia, has placed a specimen (MT-09) as a specimen of record.

• Substance Purification and Isolation

During an 18-hour period, 750 grams of *C. zeylanicum* Blume stem bark in a dried and powdered form were extracted using n-hexane and acetone with a Soxhlet extractor. The 50g of acetone extract was analyzed by vacuum liquid chromatography on silica gel 60 (230-400 mesh, Merck) and eluted with a mixture of 1:1 methanol and ethyl acetate after the extraction procedure. TLC was employed to analyse each fraction (Silica gel 60 F254, Merck). Column chromatography and elution with ethyl acetate, acetone, and methanol were used to separate the fractions, among other techniques. The combined fractions with the same R_f were mixed, and the peptide fraction 6-20 was separated into 5 fractions, namely 4, 10, 11, 19, and 20-29, through silica gel column chromatography and elution with ethyl acetate and ethyl acetate: methanol (8:2). An amorphous, off-white material was produced after washing with 4-10 acetone fractions. To synthesize Chemical 1, the isolated chemical was purified using Sephadex LH-20 and eluted with methanol (0.6403 g, 0.085 percent). Sigma-Aldrich, Gibco BRL, and Mallinckrodt supplied all research chemicals. Merck (Darmstadt, Germany) supplied chromatography-grade and thin layer chromatography silica gel 60 F254. Promega (Madison, WI) sold protein-size molecular weight indicators. This study used goat anti-rabbit IgG, HRP conjugated, and phosphotyrosine control, EGF-stimulated A431 cell lysate from Upstate Biotechnology (Lake Placid, NY). The Shimadzu UV-100PC recorded ultraviolet spectra in methanol, while the FT-IR Perkin Elmer 1600 ran KBr disc IR spectra. A VG (Micromass) 70-SE mass spectrometer captured FAB-MS data, while a Bruker Avance 300 MHz spectrometer recorded ^{13}C (100 MHz), ^1H NMR (300 MHz), and ^1H - ^1H COSY NMR spectra.

• Acetylation of Compounds

Ac_2O /pyridine was used to acetylate 60 mg of the compound (1:1). It took 24 hours of vigorous stirring at room temperature for the mixture to come together. Ethyl acetate was used to extract the combination from ice-cold distilled water. The light brown solid acetate derivative (58 mg) was obtained by evaporation and Sephadex LH-20 column chromatography, with elution in a 1:1 hexane/ethyl acetate combination.

• Cell Cultures

To culture and maintain 3T3-L1 adipocyte cells, they were grown in DMEM supplemented with 10% fetal bovine serum, 1% penicillin, and 1% streptomycin at 37°C in a humidified 5% CO_2 environment. From day four, cells were incubated with differentiation medium containing 0.25 mM dexamethasone, 0.5 mM 1-isobutyl-3-methylxanthine, and 1 mM insulin, with media changes every two days. After dexamethasone and IBMX removal, cells were maintained in insulin-containing media for two days. In DMEM with 10% fetal bovine serum, differentiation occurred on days 9-14 of the induction phase.

• Assay for Enzyme-Linked Immunosorbent Particles

96-well plates contained 2×10^3 3T3-L1 preadipocytes. 2 days after confluence, adipocyte differentiation was triggered. After 11 days of induction, cells received 100 nM insulin and 0.11 mM cinnamtannoin B1 for 30 minutes. 100 μL of 50:50 methanol and acetic acid fixed cells for 10 minutes. Inverting and tapping the plate eliminated extra PBS after three PBS rinses. Each well was blocked with 200 μL of blocking solution (3 percent skim milk in PBS) for 1 hour at 37°C to avoid non-specific antibody binding. Each well received antiphosphotyrosine antibody diluted in blocking buffer after pressing the plate to remove excess PBS. overnight at 4°C . After three washes with PBS-Tween 0.05%, each well received an HRP-conjugated secondary antibody in blocking buffer (1:5000) for 1 hour at room temperature. After three washings with washing solution, cells were treated with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) reagent (KPL, Gaithersburg, MD) for 30 minutes at room temperature. Each well received 50 μL of 4% sodium dodecyl sulfate (SDS) to terminate the reaction, and a Bio-Rad ELISA plate reader scanned the plate at 410 nm.

3. RESULTS AND ANALYSIS

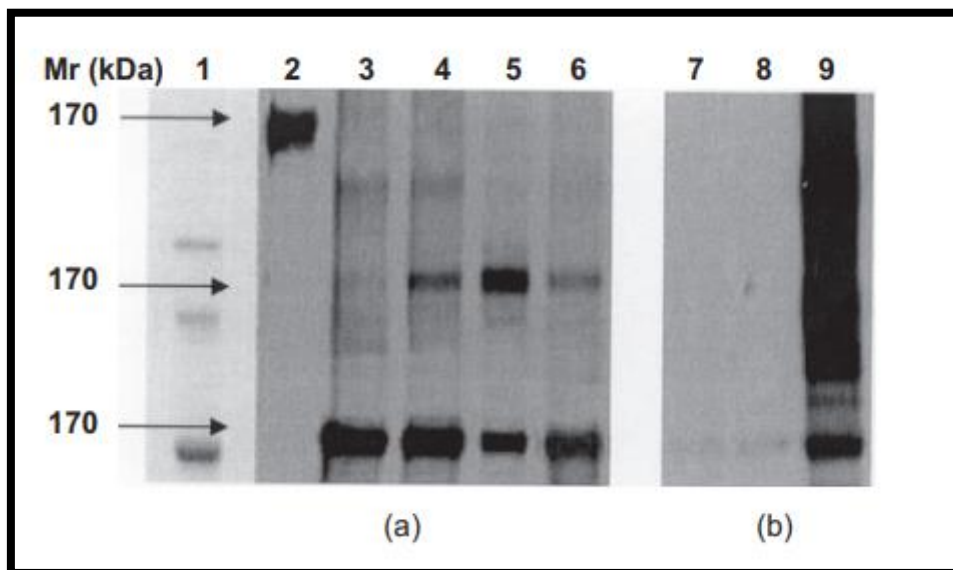


Fig 1. Cinnamtannin B1 has an effect on insulin receptor-subunit phosphorylation.

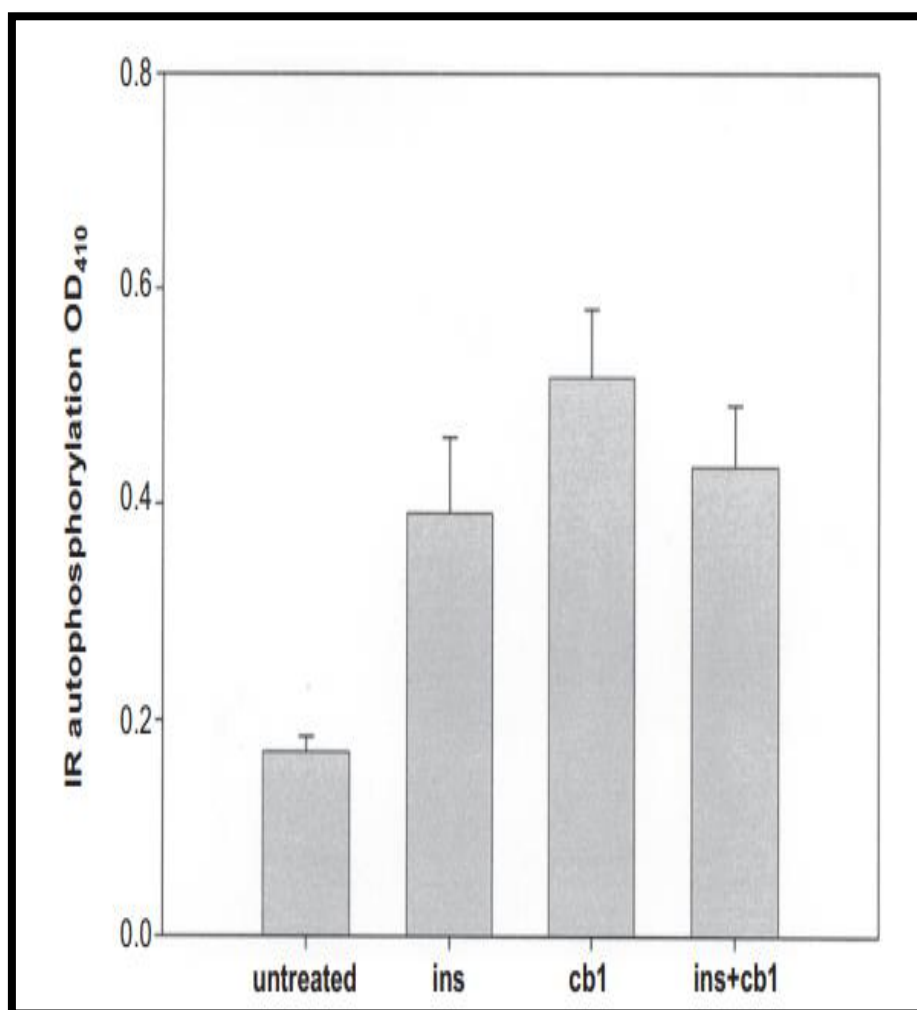


Fig.2 ELISA for insulin receptor phosphorylation in intact cells

4. DISCUSSIONS

Individuals with type 2 diabetes who are not insulin-dependent may have uncontrolled diabetes mellitus due to insulin resistance. Promising results have been seen in treating type 2 diabetes with drugs that promote autophosphorylation of insulin receptors (IR) and consequently activate IR signaling in the body. The glucose transport mechanism may require a series of events that start with receptor activation, such as insulin binding, receptor autophosphorylation, and endogenous substrate phosphorylation mediated by a receptor. The amount of tyrosine phosphorylation at the insulin receptor was dramatically enhanced by cinnamontannin B1. The binding of insulin activates tyrosine kinase, which results in the autophosphorylation of numerous Tyr residues found within the cell. Tyrosine residues on the IRS protein are also phosphorylated in this process.

5. CONCLUSION

I discovered cinnamontannin B1, a bioactive substance, in *C. zeylanicum*. It seems to have an insulin-like action by attaching to a receptor tyrosine residue in the cell membrane and causing the tyrosine residues to self-phosphorylate. Insulin is a hormone that regulates blood sugar levels. Autophosphorylation activates PI3K, GLUT4 translocation, and glucose absorption. Studies have demonstrated that cinnamontannin B1 is involved in inhibiting insulin receptor-subunit phosphorylation, which is an important target for diabetes treatment.

REFERENCES

- Huda, N., "Effectiveness of Cinnamon (*Cinnamomum burmannii*) Ethanol Extract Against *Staphylococcus aureus* Growth". *Tropical Health and Medical Research*, Vol 1, issue (2), Page 39- 43,2019.
- Qureshi, A.S., "Effect of ethanolic preparations of cinnamon (*Cinnamomum zeylanicum*) extract on hematologic and histometric parameters of selected organs in Alloxan® induced diabetic female albino rats". *J Diabetes Metab Disord* Vol 18, issue (2), Page 505–512,2019
- Gupta Jain, S., "Effect of oral cinnamon intervention on metabolic profile and body composition of Asian Indians with metabolic syndrome: a randomised, double-blind control trial". *Lipids Health Dis*, Vol 16, issue (2), Page 113-119,2017.
- Muhammad D "Cinnamon and its derivatives as a potential ingredient in functional food—A review", *International Journal of Food Properties*, Vol 20, issue (2), Page 2237-2263,2017.
- Krishnan R, "Isolation and analysis of polymeric polyphenol from black tea". *Food Chemistry*, Vol 96, issue (2), Page 331- 340,2006
- Sivapriya T "Antimicrobial activity of *Cinnamomum Zeylanicum* aqueous extract against bacteria and fungi responsible for urinary tract infection". Vol 1, issue (2), Page 187-195,2020
- Hariri, M. "Cinnamon and chronic diseases. In *Drug Discovery from Mother Nature*", Vol 1, issue (2), Page 1-24,2016.
- Sivapriya T "Qualitative and quantitative and antioxidant analysis of phytochemicals present in *Cinnamomum zeylanicum* species", Vol 1, issue (2), Page 43-49,2020
- Rajadurai M., "Herb Used for the Treatment of Coronary Heart Disease in Malayali Tribes, Pachamalai Hills". *International Journal of Pharmacognosy and Phytochemical Research*, Vol 8, issue (7), Page 1218- 1222,2016
- Jayaprakasha., "Volatile Constituents from *Cinnamomum zeylanicum* Fruit". *Journal of Agricultural and Food Chemistry*, Vol 51, Issue (15), Page 4344-4348,2003.
- Kamiya, K., C. "Studies on Constituents of *Parameria laevigata* MOLDENKE". *Chemical and Pharmaceutical Bulletin*. Vol 49, Issue (5), Page 551-557,2003.
- Foo, L. Y., Y. "A-Type Proanthocyanidin Trimers from Cranberry that Inhibit Adherence of Uropathogenic P-Fimbriated *Escherichia coli*". *Journal of Natural Products*. Vol 63, issue (9), page 1225-1228,2000.
- Baek, N. I., E. J. "Flavonoids and a Proanthocyanidin from Rhizomes of *Selligie feei*". *Phytochemistry*. Vol 36, issue (2), page 513-518,1994.
- Bilia, A. R., I. Morelli, M. "Flavans and A-Type Proanthocyanidins from *Prunus prostate*". *Phytochemistry*. Vol 43, issue (4), page 887-892,1996.
- Sidhu, M.C.; "Medicinal Plants From Twelve Families Having Antidiabetic Activity: A Review". *Am. J. PharmTech Res*, Vol 3, Vol 1, issue (2), Page 36–52,2013.
- Muhammad, A., "Biologically active flavonoids from *Dodonaea viscosa* and their structure-activity relationships". *Ind. Crop. Prod.* Vol 78, issue (2), Page 66–72,2015.
- Muthukumran, P., "Antidiabetic activity of *Dodonaea viscosa* (L) leaf extracts". *Int. J. Pharm. Tech. Res.* Vol 3, issue (2), Page 136–139,2011.
- Nguyen, P. H., "Protein tyrosine phosphatase 1B (PTP1B) inhibitors from *Morinda citrifolia* (Noni) and their insulin mimetic activity". *J. Nat. Prod.* Vol 76, issue (2), Page 2080–2087,2013.
- Nieto-Vazquez, I., "Protein-tyrosine phosphatase 1Bdeficient myocytes show increased insulin sensitivity and protection against tumor necrosis factor-alpha-induced insulin resistance". *Diabetes*, Vol 56, issue (2), Page 404–413,2007.
- Uddin, Z., Li, Z., "Visconata: a rare flavonol having long chain fatty acid from *Dodonaea viscosa* which inhibits Human neutrophil elastase (HNE)". *Tetrahedron Lett*, Vol 58, issue (2), Page 2507–2511,2017