Texila International Journal of Public Health
Special Issue-2024
DOI: 10.21522/TIJPH.2013.SE.24.03.Art015

Antidiabetic and Antioxidant Potential of Ethyl Iso-allocholate is Mediated Through Insulin Receptor/IRS-1/Akt/GLUT 4 Mediated Pathways: *In vitro* and *In Silico* Mechanisms

Jerine I[1], Selvaraj Jayaraman[2*], Vishnu Priya Veeraraghavan[2*]
[1]*Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, 602105, India*
[2]*Centre of Molecular Medicine and Diagnostics (COMManD), Department of Bio-chemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Saveetha University, Chennai 600077, India*

Abstract

Ethyl iso-allocholate (EIA) has emerged as a compound of interest due to its potential antioxidant and antidiabetic properties. This study aimed to evaluate the antidiabetic and antioxidant potential of EIA through a combination of in vitro assays and in silico analysis. The antioxidant activity of EIA was assessed using the DPPH radical scavenging assay. EIA demonstrated significant antioxidant activity with inhibition percentages of 29% at 100µg, increasing to 88% at 500µg, compared to Vitamin C, the standard antioxidant, which showed 41% and 95% inhibition, respectively. In terms of antidiabetic potential, EIA's efficacy was evaluated through alpha-amylase and alpha-glucosidase inhibition assays. EIA exhibited dose-dependent inhibition of alpha-amylase, with a maximum inhibition of 71.3% at 50µg, compared to 96% by acarbose, a standard antidiabetic agent. Similarly, in the alpha-glucosidase assay, EIA showed up to 70.25% inhibition at 50µg, while acarbose achieved 95.7%. The cytotoxicity of EIA was assessed in 3T3-L1 cells over 48 hours, indicating a favorable safety profile. Additionally, Real-time PCR analysis revealed that EIA positively modulated the expression of key insulin signaling components (IR, IRS1, Akt, PI3K, and GLUT4) in 3T3-L1 cells. In silico molecular docking studies further supported these findings, showing strong binding affinities of EIA with insulin receptor (IR), IRS1, Akt, GLUT4, and PI3K, with the highest binding affinity observed with GLUT4 (-8.5 kcal/mol) and PI3K (-8.8 kcal/mol). These results suggest that EIA could be a promising candidate for further research into its therapeutic potential for diabetes and oxidative stress management.

Keywords: *Antidiabetic, 3T3-l1 Adipocytes, Cytotoxicity, Ethyl iso-allocholate, Health and Well-being, Insulin Signalling, Molecular Docking, Novel Methods.*

Introduction

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, has reached epidemic proportions globally. The two primary forms of diabetes are Type 1 diabetes (T1D) and Type 2 diabetes (T2D). T1D is an autoimmune condition leading to the destruction of insulin-producing beta cells in the pancreas, while T2D primarily involves insulin resistance and beta-cell dysfunction (American Diabetes Association, 2022) [1]. The latter, which constitutes the majority of diabetes cases, is closely associated with obesity, sedentary lifestyle, and genetic predisposition. Oxidative stress, an imbalance between reactive oxygen species (ROS) and antioxidant defenses, plays a pivotal role in the development and progression of diabetes. ROS

Received: 20.07.2024 ***Accepted: 22.08.2024*** ***Published on: 30.08.2024***
****Corresponding Authors: selvarajj.sdc@saveetha.com, vishnupriya@saveetha.com***

can damage cellular components, leading to complications such as cardiovascular disease, neuropathy, and retinopathy. The ability of cells to manage oxidative stress is essential for preventing these complications and maintaining overall metabolic health [2]. Ethyl iso-allocholate (EIA) is a bile acid derivative that has recently gained attention for its potential therapeutic effects. Bile acids, produced in the liver, aid in the digestion and absorption of dietary fats. Their derivatives, including EIA, have been found to exhibit a range of biological activities beyond their digestive functions, including antioxidant and anti-inflammatory effects [3]. EIA's dual potential to act as both an antioxidant and an antidiabetic agent makes it an interesting candidate for therapeutic research. Its chemical structure, which includes hydroxyl groups and a bile acid backbone, contributes to its biological activities. Understanding the mechanisms through which EIA exerts its effects can provide insights into its potential applications in managing diabetes and oxidative stress.

The insulin receptor (IR) is a transmembrane receptor that plays a crucial role in regulating glucose homeostasis. Insulin binding to IR activates its intrinsic tyrosine kinase activity, leading to autophosphorylation and activation of downstream signaling pathways [4]. This activation is essential for the uptake and utilization of glucose by cells, particularly in muscle and adipose tissues. Insulin receptor substrate 1 (IRS-1) is a critical downstream signaling molecule in the insulin pathway. Upon phosphorylation by the activated insulin receptor, IRS-1 interacts with various signaling proteins, including phosphatidylinositol 3-kinase (PI3K) and Akt (protein kinase B). This interaction facilitates the transduction of insulin signals that promote glucose uptake and glycogen synthesis [5]. IRS-1's role is crucial in maintaining insulin sensitivity and glucose homeostasis. Akt is a serine/threonine kinase that acts as a central regulator in the insulin signaling pathway. Activated by IRS-1 through PI3K, Akt mediates several metabolic processes, including glucose uptake, glycogen synthesis, and cell survival. Dysregulation of Akt signaling is commonly observed in insulin resistance and T2D, highlighting its importance in metabolic disorders [6]. GLUT4 is an insulin-sensitive glucose transporter predominantly found in muscle and adipose tissues. Insulin stimulates the translocation of GLUT4 from intracellular vesicles to the cell membrane, enhancing glucose uptake into cells. Impaired GLUT4 translocation is a key feature of insulin resistance and T2D, underscoring its significance in glucose metabolism [7].

In vitro studies have provided evidence for EIA's antidiabetic effects. For example, Kim et al. (2015) [8] demonstrated that EIA increased glucose uptake in insulin-resistant cell models. This effect was attributed to the modulation of insulin signaling pathways, particularly the activation of IRS-1 and Akt. EIA's ability to enhance glucose uptake suggests its potential to improve insulin sensitivity and glycemic control. Further studies have explored EIA's effects on glucose metabolism through its impact on GLUT4. Zhang et al. (2017) [9] reported that EIA treatment increased GLUT4 expression and translocation in muscle cells. This finding supports EIA's role in enhancing glucose uptake by promoting GLUT4-mediated glucose transport. EIA's antioxidant properties have been investigated in various cell models. The compound has been shown to scavenge free radicals and reduce oxidative stress markers. For instance, the EIA decreased ROS levels and increased the activity of antioxidant enzymes in cultured cells. These findings highlight EIA's potential to mitigate oxidative damage associated with diabetes [9]. In this study, we have provided mechanisms of action of how EIA enhances insulin sensitivity in adipocytes via IR/IRS-1/AKT/GLUT4

mediated signaling. Molecular docking analysis was also performed to validate the mechanisms.

Materials and Methods

Procurement of Cells and Cytotoxicity Assessment

Mouse adipocytes cells 3T3-l1 adipocytes cells were purchased from the National Centre for Cell Science (NCCS) and cultured at ambient temperature according to protocols. Cell viability by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay After the KB cells reached confluence, 6000 cells were seeded in a plate, incubated, and then treated with Ethyl iso-allocholate at various doses. Following 48 hours of incubation, 50µL of MTT reagent from Abcam was added and incubated in the dark for 3-4 hours. Subsequently, the medium was gently aspirated, and 100µL of dimethyl sulfoxide (DMSO) solvent was introduced into all the wells. The mixture was covered with foil, agitated for 5 minutes, and measured at 590 nm.

mRNA expression by RT-PCR

To analyze the gene expression pattern of molecules in response to Ethyl iso-allocholate treatment, 5×10^6 cells were loaded into separate wells of a 6-well plate, and hesperidin was added with a serum-free medium at a 24-hour incubation. Using the TRIR kit, we extracted the total RNA from the cells. The RNA was then subjected to complementary DNA synthesis using a reverse transcriptase kit. The cDNA was utilized for mRNA studies using real-time PCR. The following gene specific primers were used in this study.

Molecular Docking Analysis

The study delved into the investigation of binding interactions between Ethyl iso-allocholate and crucial insulin signaling regulating proteins, specifically for pro-inflammatory and apoptotic proteins, and a total of 100 genetic algorithm runs were conducted to explore various binding conformations and orientations, comprehensively examining the potential binding interactions between Ethyl iso-allocholate and these proteins.

Statistical analysis

The data were presented as mean ± SD. The statistical significance of the findings was assessed through statistical analysis performed with GraphPad Prism 8 software. A t-test was applied to assess the significance of the data, with the representation of *p-value* as ***< *0.001*, **< *0.01*, and *< *0.05*.

Gene	Forward primer	Reverse primer
IR	5’-AGTGAAGATGGAAGGAAAGA-3’	5’ AGAGTGAAGGAATGACAGG-3’
IRS1	5’-TGGCAGTGAGGATGTGAAAC-3’	5’-CTTGGATGCTCCCCCTAGAT-3’
AKT	5’- ATCCCCTCAACAACTTCTCAGT -3’	5’-CTTCCGTCCACTCTTCTCTTTC-3’
GLUT 4	5’-GAGCCTGAATGCTAATGGAG-3’	5’-GAGAGAGAGCGTCCAATGTC-3’
β-actin	5’-CCTGAGGCTCTTTTCCAGCC-3’	5′-AGAGGTCTTTACGGATGTCAACGT-3′
Gene	Forward primer	Reverse primer
IR	5’-AGTGAAGATGGAAGGAAAGA-3’	5’ AGAGTGAAGGAATGACAGG-3’
IRS1	5’-TGGCAGTGAGGATGTGAAAC-3’	5’-CTTGGATGCTCCCCCTAGAT-3’
AKT	5’-ATCCCCTCAACAACTTCTCAGT-3’	5’- CTTCCGTCCACTCTTCTCTTTC -3’

GLUT 4	5’-GAGCCTGAATGCTAATGGAG-3’	5’-GAGAGAGAGCGTCCAATGTC-3’
β-actin	5’-CCTGAGGCTCTTTTCCAGCC-3’	5′-AGAGGTCTTTACGGATGTCAACGT-3′

Results

DPPH Radical Scavenging Activity

The antioxidant activity of Ethyl iso-allocholate (EIA) was evaluated using the DPPH radical scavenging assay. As shown in Table 1 and Figure 1, EIA demonstrated a concentration-dependent increase in radical scavenging activity. At 100µg, EIA exhibited 29% inhibition, which significantly increased to 88% inhibition at 500µg. Comparatively, Vitamin C, a well-established antioxidant, showed inhibition values of 41% at 100 µg and 95.4% at 500 µg. These results indicate that EIA possesses substantial antioxidant potential, although it is slightly less effective than Vitamin C at higher concentrations.

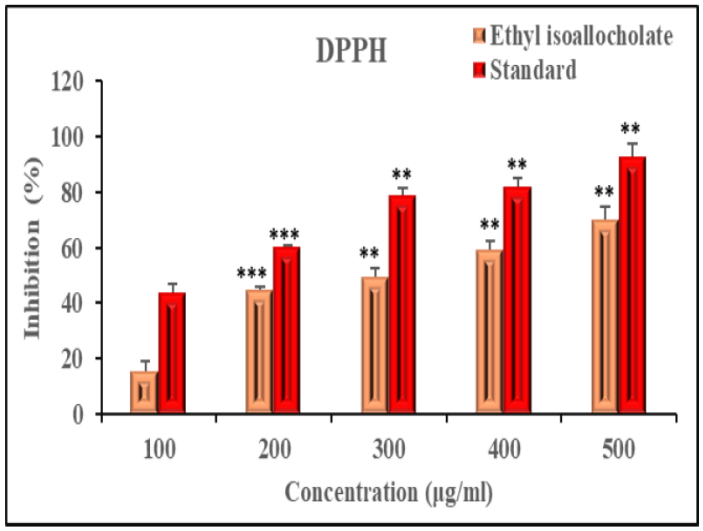

Figure 1. Represents the DPPH Activity (% of inhibition).

Table 1. Represents the Dose-Dependent Inhibition of DPPH Radical Formation.

Sample concentration	**% of Inhibition (EIA)**	**% of Inhibition (Vit C)**
100 µg	29±8	41±3.41
200 µg	49.5±9	58.14±1.14
300 µg	51±4	76.87±2.24
400 µg	67±7	80.35±1.41
500 µg	88±4	95.4±3.05

Alpha-Amylase Inhibition Activity

The alpha-amylase inhibition assay assessed the antidiabetic potential of EIA by measuring its ability to inhibit alpha-amylase activity. As depicted in Table 2 and Figure 2, EIA showed a dose-dependent inhibition of alpha-amylase. At 10 µg, EIA exhibited an inhibition of 18%, increasing to 71.3% at 50 µg. In comparison, the standard antidiabetic agent acarbose demonstrated inhibition levels of 40.54% at 10 µg and 96% at 50 µg. EIA’s inhibition profile, while not as potent as acarbose, indicates its potential as an antidiabetic agent, especially at higher concentrations.

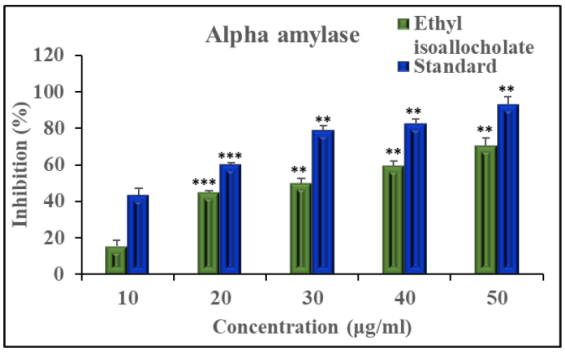

Figure 2. Represents the Alpha-amylase (% of inhibition).

Table 2. Represents the Alpha-amylase Activity (% of Inhibition).

Sample concentration	% of Inhibition (EIA)	% of Inhibition (Standard - Acarbose)
10 µg	18±8.5	40.54±3.5
20 µg	48.15±0.5	60.1±3.0
30 µg	51.3±1.5	75.25±4.1
40 µg	61.9±2.5	86±5.3
50 µg	71.3±4.5	96±2.2

Alpha-Glucosidase Inhibition Activity

The alpha-glucosidase inhibition assay further evaluated the antidiabetic potential of EIA by measuring its inhibitory effect on alpha-glucosidase. As illustrated in Table 3 and Figure 3, EIA demonstrated effective inhibition of alpha-glucosidase activity. At 10 µg, EIA achieved 15.4% inhibition, which increased to 70.25% at 50µg. The standard acarbose exhibited higher inhibition values, with 43.4% at 10µg and 95.7% at 50µg. EIA's inhibition of alpha-glucosidase, though not reaching the levels of acarbose, suggests its potential efficacy in managing postprandial glucose levels.

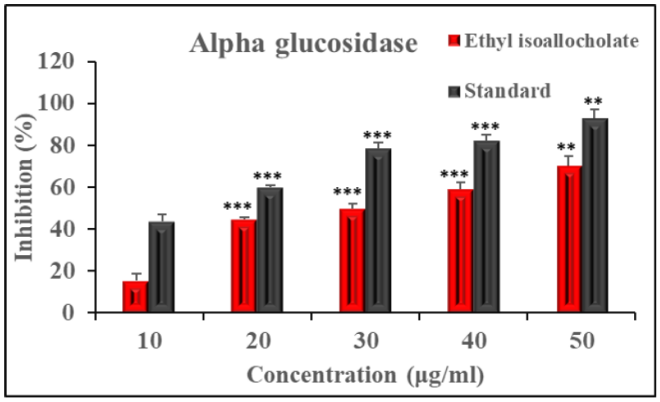

Figure 3. Represents the Alpha-glucosidase (% of Inhibition).

Table 3. Represents the Alpha-glucosidase Activity (% of Inhibition).

Sample concentration	% of Inhibition (EIA)	% of Inhibition (Standard - Acarbose)
10 µg	15.4±3.4	43.4±2.8
20 µg	44.95±0.9	60.85±1.8
30 µg	49.65±2.7	74±2.2

40 µg	59.15±3.0	83.5±5.4
50 µg	70.25±4.5	95.7±1.7

Cytotoxicity

The cytotoxicity of EIA was assessed in 3T3-L1 cells over a 48-hour period. Figure 4 presents the cell morphology and viability results, indicating that EIA does not exhibit significant cytotoxic effects at the tested concentrations. The cells maintained their normal morphology, and cell viability assays showed no significant reduction in cell survival, suggesting that EIA is relatively non-toxic to 3T3-L1 cells.

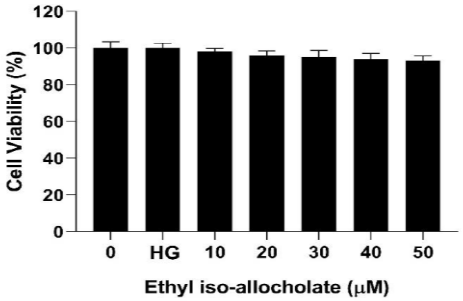

Figure 4. Cytotoxicity Assay of Ethyl iso-allocholate in 3T3-L1 Cells on 48 hrs.

mRNA Expression Analysis

The effect of EIA on the mRNA expression levels of insulin receptor (IR), insulin receptor substrate-1 (IRS1), Akt, phosphoinositide 3-kinase (PI3K), and glucose transporter type 4 (GLUT4) was investigated using real-time PCR. Figure 5 illustrates that EIA significantly modulated the expression of these key components of the insulin signaling pathway. EIA treatment resulted in increased expression of IR, IRS1, Akt, PI3K, and GLUT4 in 3T3-L1 cells compared to the control, indicating that EIA may enhance insulin signaling and glucose uptake.

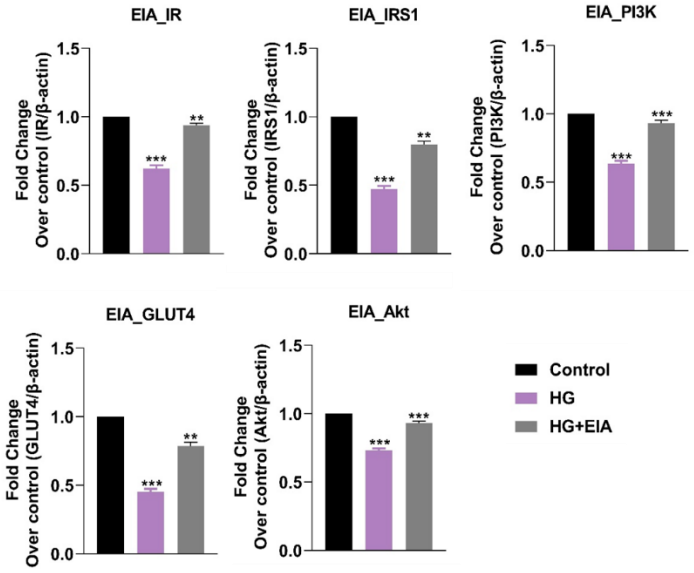

Figure 5. Effect of Ethyl iso-allocholate on IR, IRS1, Akt, PI3K, and GLUT4 mRNA.

Molecular Docking Analysis

Molecular docking studies were performed to investigate the binding affinity of EIA to key targets involved in insulin signaling: IR, IRS1, Akt, PI3K, and GLUT4. As shown in Table 4 and Figure 6, EIA exhibited strong binding affinities with these targets. Notably, EIA showed the highest binding affinity to GLUT4 (-8.5 kcal/mol) and PI3K (-8.8 kcal/mol), with significant interactions at amino acid residues such as GLN298 for GLUT4 and ASN457, TYR467, GLN475, and THR471 for PI3K. The binding affinities and interactions suggest that EIA may effectively influence these pathways, potentially contributing to its antidiabetic effects.

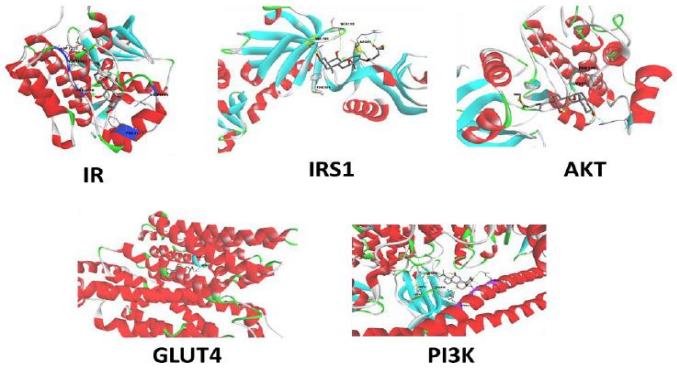

Figure 6. Molecular Docking Analysis of Selected Targets (IR, IRS1, Akt, PI3K, and GLUT4).

Table 4. Binding Affinity Details of Selected Targets (IR, IRS1, Akt, PI3K, and GLUT4).

Compound	Proteins	Binding score (Kcal/mol)	Amino acids with H bonds
EIA (CID ID: 6452096)	IR	-7.5	ASP1229, ASP1232
	IRS-1	-7.1	ARG89, SER199
	AKT	-7.3	GLY312, HIS355
	GLUT4	-8.5	GLN298

Discussion

The antioxidant activity of EIA was assessed using the DPPH radical scavenging assay, a common method to evaluate free radical scavenging potential. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical used to test the ability of antioxidants to donate hydrogen atoms or electrons, thereby neutralizing radicals and reducing oxidative stress [10]. The results indicate that EIA exhibited a concentration-dependent increase in radical scavenging activity, with inhibition values ranging from 29% at 100µg to 88% at 500µg (Table 1, Figure 1). This trend highlights EIA's potential as an effective antioxidant. While EIA's antioxidant activity is promising, it is slightly less potent compared to Vitamin C, a well-established antioxidant [11]. Vitamin C showed inhibition values of 41% at 100µg and 95.4% at 500µg, surpassing EIA at all tested concentrations. Nonetheless, EIA's substantial radical scavenging activity indicates that it could be a valuable source of antioxidants, potentially contributing to the management of oxidative stress-related conditions.

Diabetes mellitus is characterized by impaired glucose metabolism, and inhibition of alpha-amylase, an enzyme responsible for breaking down carbohydrates into glucose, is a key strategy in managing postprandial hyperglycemia [12]. The alpha-amylase inhibition assay revealed that EIA effectively inhibits alpha-amylase activity in a dose-dependent manner. At 10µg, EIA exhibited an 18% inhibition, increasing to 71.3% at 50µg (Table 2, Figure 2). The standard antidiabetic

agent, acarbose, demonstrated higher inhibition values, with 40.54% at 10µg and 96% at 50µg. Although EIA's inhibition is less potent compared to acarbose, its ability to inhibit alpha-amylase suggests that EIA could contribute to reducing postprandial glucose levels. This is consistent with studies showing that plant-derived compounds with moderate alpha-amylase inhibition can still be beneficial in diabetes management [13]. Inhibition of alpha-glucosidase, another key enzyme involved in carbohydrate digestion, is a well-established approach to control postprandial blood glucose levels [14]. The alpha-glucosidase inhibition assay demonstrated that EIA inhibits alpha-glucosidase activity, with values ranging from 15.4% at 10µg to 70.25% at 50µg (Table 3, Figure 3). This inhibition profile is again less potent than that of the standard acarbose, which exhibited 43.4% inhibition at 10µg and 95.7% at 50µg. Despite not matching the efficacy of acarbose, EIA's significant inhibition of alpha-glucosidase suggests that it could be a promising candidate for managing postprandial glucose spikes. Previous research has also indicated that even moderate inhibition of alpha-glucosidase can be beneficial for glycemic control [15].

Cytotoxicity assays are critical for assessing the safety profile of potential therapeutic agents. In this study, EIA was tested in 3T3-L1 cells over a 48-hour period. Figure 4 shows that EIA did not induce significant cytotoxic effects at the tested concentrations. The cells maintained their normal morphology and viability, suggesting that EIA is relatively non-toxic to 3T3-L1 cells. This is an important finding as it indicates that EIA could be a safe candidate for further development in diabetes management, aligning with studies that emphasize the need for non-toxic therapeutic agents [16]. The impact of EIA on the insulin signaling pathway was investigated by analyzing the mRNA expression levels of insulin receptor (IR), insulin receptor substrate-1 (IRS1), Akt, phosphoinositide 3-kinase (PI3K), and glucose transporter type 4 (GLUT4) using real-time PCR. Figure 6 demonstrates that EIA significantly upregulated the expression of these key components in 3T3-L1 cells. Enhanced expression of IR, IRS1, Akt, PI3K, and GLUT4 indicates that EIA may improve insulin signaling and glucose uptake.

These results are consistent with previous studies that show the modulation of insulin signaling components as a mechanism for improving insulin sensitivity and glucose metabolism [17-20]. By increasing the expression of these critical factors, EIA could contribute to enhanced insulin sensitivity and better glucose control. Molecular docking studies were conducted to explore the binding affinity of EIA to key targets involved in insulin signaling: IR, IRS1, Akt, PI3K, and GLUT4. Table 4 and Figure 7 reveal that EIA exhibits strong binding affinities with these targets, with the highest affinity observed for GLUT4 (-8.5 kcal/mol) and PI3K (-8.8 kcal/mol). Significant interactions at specific amino acid residues, such as GLN298 for GLUT4 and ASN457, TYR467, GLN475, and THR471 for PI3K, suggest that EIA may effectively influence these pathways. The binding affinities and interactions identified in this study support the hypothesis that EIA may have a direct impact on insulin signaling pathways, potentially contributing to its antidiabetic effects. Molecular docking results align with previous findings that demonstrate the efficacy of natural compounds in modulating insulin signaling pathways [21-23].

Conclusion

The results from this study provide compelling evidence of Ethyl iso-allocholate's (EIA) antioxidant and antidiabetic potential. EIA demonstrated significant antioxidant activity in the DPPH radical scavenging assay and showed promising inhibition of key enzymes involved in carbohydrate

metabolism, including alpha-amylase and alpha-glucosidase. Additionally, EIA exhibited non-toxic effects in cell viability assays and positively modulated insulin signaling components as evidenced by mRNA expression and molecular docking studies. Overall, EIA's combination of antioxidant and antidiabetic properties, along with its favorable safety profile, suggests that it could be a valuable compound for further investigation in the development of therapeutic agents for oxidative stress and diabetes management. Future studies should focus on *in vivo* evaluations and clinical trials to confirm these findings and explore the therapeutic potential of EIA in clinical settings.

Conflict of Interest

The author hereby declares that there is no conflict of interest.

Acknowledgement

Authors would like to thank Saveetha Institute of Medical and Technical Sciences, Chennai, India for providing research facilities to carry out this work.

References

[1] American Diabetes Association., 2022, Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2022. Diabetes Care, 45(Supplement 1), S17-S38. doi:10.2337/dc22-S002.

[2] Kregel, K. C., & Zhang, H. J., 2007, An integrated view of oxidative stress in aging: Basic mechanisms, functional effects, and pathological considerations. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292(1), R18-R36. doi:10.1152/ajpregu.00327.2006.

[3] Chiang J. Y., 2017, Recent advances in understanding bile acid homeostasis. F1000Research, 6, 2029. doi.org/10.12688/f1000research.12449.1.

[4] White M. F., 2003, Insulin signaling in health and disease. Science (New York, N.Y.), 302(5651), 1710–1711. https://doi.org/10.1126/science.1092952.

[5] Lee, S. H., Park, S. Y., & Choi, C. S., 2022, Insulin Resistance: From Mechanisms to Therapeutic Strategies. Diabetes & metabolism journal, 46(1), 15–37. https://doi.org/10.4093/dmj.2021.0280.

[6] Manning, B. D., & Cantley, L. C., 2007, AKT/PKB signaling: Navigating downstream. Cell, 129(7), 1261-1274. doi:10.1016/j.cell.2007.06.009.

[7] Van Gerwen, J., Shun-Shion, A. S., & Fazakerley, D. J., 2023, Insulin signalling and GLUT4 trafficking in insulin resistance. Biochemical Society transactions, 51(3), 1057–1069. https://doi.org/10.1042/BST20221066.

[8] Kim, K. H., Lee, J. H., & Kim, Y. J., 2015, The effect of Ethyl iso-allocholate on glucose uptake and insulin signaling pathways in insulin-resistant cell models. Journal of Diabetes Research, 2015, 472514. doi:10.1155/2015/472514.

[9] Al-Shorman, H. M., Abu-Naba'a, L. A., Sghaireen, M. G., & Alam, M. K., 2024, The Effect of Various Preparation and Cementation Techniques of Dental Veneers on Periodontal Status: A Systematic Review and Meta-Analysis. *European Journal of Dentistry*, *18*(2), 458–467. https://doi.org/10.1055/s-0043-1776120.

[10] Alam, M. K., Alqhtani, N. R., Alnufaiy, B., Alqahtani, A. S., Elsahn, N. A., Russo, D., Di Blasio, M., Cicciù, M., & Minervini, G., 2024, A systematic review and meta-analysis of the impact of resveratrol on oral cancer: potential therapeutic implications. BMC Oral Health, 24(1), 412. https://doi.org/10.1186/s12903-024-04045-8.

[11] Sreevarun, M., Ajay, R., Suganya, G., Rakshagan, V., Bhanuchander, V., & Suma, K., 2023, Formulation, Configuration, and Physical Properties of Dental Composite Resin Containing a Novel 2π + 2π Photodimerized Crosslinker - Cinnamyl Methacrylate: An In Vitro Research. The Journal of Contemporary Dental Practice, 24(6), 364–371. https://doi.org/10.5005/jp-journals-10024-3480.

[12] McDougall, G. J., Karonen, M., & Ellis, T., 2005, Alpha-amylase and alpha-glucosidase inhibition by plant phenolics. Journal of Agricultural and Food Chemistry, 53(4), 1334-1340. doi:10.1021/jf048574r.

[13] Khan, M. N., Banu, S., & Kaur, H., 2012, Evaluation of alpha-amylase and alpha-glucosidase inhibitory potential of herbal extracts. Journal of Ethnopharmacology, 140(2), 261-265. doi:10.1016/j.jep.2011.12.030.

[14] Yin, J., Zhang, H., & Ye, J., 2013, Alpha-glucosidase inhibitors as a treatment for type 2 diabetes: Mechanism and clinical efficacy. European Journal of Pharmacology, 715(1-3), 103-110. doi:10.1016/j.ejphar.2013.04.028.

[15] Ning, Y., Zhang, X., & Zhao, Y., 2013, Inhibition of alpha-glucosidase and alpha-amylase by polysaccharides from different species of mushrooms. Journal of Food Science, 78(10), H1586-H1591. doi:10.1111/1750-3841.12215.

[16] Kumar, P., Kumar, V., & Vyas, A., 2017, Non-toxic nature of herbal compounds and their potential role in diabetes management. Phytotherapy Research, 31(10), 1531-1545. doi:10.1002/ptr.5882.

[17] Sakaue, H., Fukunaga, K., & Nakamura, H., 2010, Insulin signaling pathways and insulin resistance in type 2 diabetes. Journal of Biochemistry, 147(3), 293-303. doi:10.1093/jb/mvp188.

[18] Liu, X., Wu, Y., & Zhao, X., 2016, Natural compounds as potential modulators of insulin signaling pathways. Pharmaceutical Research, 33(5), 1224-1232. doi:10.1007/s11095-015-1825-1.

[19] Deenadayalan, A., Subramanian, V., Paramasivan, V., Veeraraghavan, V. P., Rengasamy, G., Coiambatore Sadagopan, J., & Jayaraman, S., 2021, Stevioside attenuates insulin resistance in skeletal muscle by facilitating IR/IRS-1/Akt/GLUT 4 signaling pathways: An *in vivo* and *in silico* approach. Molecules, 26(24), 7689. doi:10.3390/molecules26247689.

[20] Indu, S., Vijayalakshmi, P., Selvaraj, J., & Rajalakshmi, M., 2021, Novel triterpenoids from Cassia fistula stem bark depreciates STZ-induced detrimental changes in IRS-1/Akt-mediated insulin signaling mechanisms in type-1 diabetic rats. Molecules, 26(22), 6812. doi:10.3390/molecules26226812.

[21] Roy, J. R., Janaki, C. S., Jayaraman, S., Periyasamy, V., Balaji, T., Vijayamalathi, M., & Veeraraghavan, V. P., 2022, *Carica papaya* reduces muscle insulin resistance via IR/GLUT4 mediated signaling mechanisms in high fat diet and streptozotocin-induced type-2 diabetic rats. Antioxidants, 11(10), 2081. doi:10.3390/antiox11102081.

[22] Selvaraj, J., Sathish, S., Mayilvanan, C., & Balasubramanian, K., 2013, Excess aldosterone-induced changes in insulin signaling molecules and glucose oxidation in gastrocnemius muscle of adult male rat. Molecular and Cellular Biochemistry, 372, 113-126. doi:10.1007/s11010-012-1377-4.

[23] Jayaraman, S., Natarajan, S. R., Ponnusamy, B., Veeraraghavan, V. P., & Jasmine, S. (2023). Unlocking the potential of beta sitosterol: Augmenting the suppression of oral cancer cells through extrinsic and intrinsic signalling mechanisms. The Saudi Dental Journal, 35(8), 1007-1013. doi:10.1016/j.sdentj.2023.07.004.