Journal of Namibian Studies, 33 (2023): 5951-5962 ISSN: 2197-5523 (online)

Effect Of Antioxidant Vitamins On Protein Kinase-C And Phosphotyrosine Phosphatase 1b Expression In The Liver Of Glyphosate - Induced Experimental Diabetic Rats

Preethi. K[1], Selvaraj Jayaraman[2]*, Kavitha.S[3]*, V. Vishnu Priya[4], R. Gayathri[5]

Abstract

INTRODUCTION: Antioxidant Activity is limitation of nutrient oxidation by restricting oxidative chain reaction. Studies showed that glyphosate and its formulated products are found to be carcinogenic. Supplementation with vitamin C and vitamin E influences the generation of free radicals and improves Antioxidant defenses.

AIM: Aim of the study is to analyze the effect of antioxidant vitamins on protein kinase C and Phosphotyrosine Phosphatase 1B in the liver of glyphosate induced diabetic rats.

METHODS: Adult male Albino rats were divided into 3 groups with 6 each. Group 1: Control rats. Group 2: Glyphosate induced rats. Group 3: Glyphosate induced rats treated with vitamin C and vitamin E for 30 days.

[1]Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

[2] *Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India Email: selvarajj.sdc@saveetha.com

[3] *Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. Email: kavithas.sdc@saveetha.com

[4] Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. Email: vishnupriya@saveetha.com

[5] Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. Email: gayathri.sdc@saveetha.com

***Corresponding author:** Selvaraj Jayaraman, Kavitha.S

*Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. Email: selvarajj.sdc@saveetha.com

*Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. Email: kavithas.sdc@saveetha.com

Fasting blood glucose, serum insulin, protein kinase C mRNA and phosphotyrosine phosphatase 1B mRNA were studied and data were statistically analyzed using one-way-ANOVA.

RESULTS: Vitamin C and vitamin E showed a significant decrease ($p<0.05$) in fasting blood glucose, reduced serum insulin concentration compared to control which was altered by glyphosate exposure. mRNA levels of protein kinase C compared to and phosphotyrosine phosphatase 1B were significantly raised in the glyphosate-exposed diabetic group compared to the healthy group. Vitamin supplementation significantly reduced the same effectively ($p<0.05$).

CONCLUSION: Vitamin C and vitamin E shows significant Anti-diabetic Activity by decreasing levels of fasting blood glucose and serum insulin and by altering gene expression of protein kinase C and phosphotyrosine phosphatase 1B.

Keywords: Innovative technique, Antioxidant vitamin, Diabetic rats, Glyphosate, PKC, PP1B, Vitamin C, Vitamin E, Novel method.

INTRODUCTION:

Glyphosate is a broad spectrum systemic herbicide and crop desiccant(1). It is an organophosphorus compound, specifically a phosphonate, which acts by inhibiting the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase. It is used to kill weeds,especially Annual Broadleaf weeds and grasses that compete with crops(2).

Antioxidants are compounds that inhibit oxidation reactions that can produce free radicals and chain reaction that may damage the cells of organisms(3,4). Antioxidants such as thiols or ascorbic acid (Vitamin C) may act to inhibit these reactions(5). To balance Oxidative stress, plants and animals maintain complex systems of overlapping Antioxidants, such as glutathione(6). The only dietary Antioxidants are A, C, E. While fruits and vegetables are rich sources of Antioxidant vitamins. According to some studies, supplements of vitamin A and vitamin E have no positive effect on mortality rate or cancer rate(7).

Diabetes Mellitus is a metabolic syndrome that affects millions of people world wide(8,9). Recent studies have demonstrated that protein kinase C Activation plays an important role in hyperglycemia induced atherosclerosis(10). Protein kinase C Activation is involved in several cellular responses such as the expression of various growth factors, activation of signaling pathway and enhancement of oxidative stress in hyperglycemia (11).

Protein phosphotyrosine phosphatase has been shown to be involved in the negative regulation of both insulin and leptin action at the in

vitro, ex vivo, in vivo levels(12). A growing body of human genetics data also support the hypothesis that phosphotyrosine phosphatase 1B has an important role in insulin signaling(13).
Oxidative stress is caused by an imbalance between production and accumulation of reactive oxygen species in cell (14,15). Supplementation with vitamin C and vitamin E influence the generation of free radicals and improves Antioxidant defenses (16,17). The objective of the study is to focus on the protective role of Antioxidant vitamins against glyphosate toxicity. Aim of the study is to analyze the effect of Antioxidant vitamins on protein kinase C and phosphotyrosine phosphatase 1B in the liver of glyphosate induced diabetic rats.

MATERIALS AND METHODS:

CHEMICALS:
All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from New England Biolabs (NEB), USA and the GoTaq Green master mix was purchased from Promega, USA. PKC, PTP-1B and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India. Antioxidant enzyme (GPX) and LPO ELISA kits were procured from Abbkine, (Bldg C17, Optics Valley International Biomedicine Park, Wuhan, China. 430223).

ANIMALS:
Adult male Albino Wistar rats weighing 150–180 g were used in our study. They were maintained as per the guidelines of the Indian National Law on Animal Care and Use at Biomedical Research unit and laboratory animal center (BRULAC), Saveetha dental college and hospitals, SIMATS, Chennai-77. The Institutional Animal Ethical Committee (IAEC) (Register Number: BRULAC/ SDCH/ SIMATS/IAEC/8-2021/086) approved all animal-related experimental methods. The animals were housed in a temperature (21 ± 2 °C)-controlled room with a standard 12 h light – 12 h dark cycle and were allowed free access to water and standard pellet diet at Biomedical Research unit and laboratory animal center(BRULAC), Saveetha dental college and hospitals, SIMATS, Chennai-77.

EXPERIMENTAL DESIGN:

Healthy male albino rats were divided into 3 groups consisting of 6 animals each.
Group I: Control rats (Control rate injected with corn oil intraperitoneally (ip) once daily as a vehicle).
Group II: Glyphosate treated [dissolved in water at a dose of 100g/kg/body wt/day at 8am] orally for 10 weeks.

Group III: Rats received simultaneous treatment of glyphosate + vitamin E (dissolved in olive oil at a dose of 50 mg/kg body weight) and vitamin C treated (100 mg/kg body weight dissolved in distilled water daily at 10 AM through gastric intubation for 30 days).

After the treatment period, animals were anesthetized with ether, blood was collected, sera separated and stored at −80 °C. Liver from control and treated animals were dissected out and subjected for the assay of various parameters.

FASTING BLOOD GLUCOSE:

Blood glucose was estimated using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA) after overnight fasting. Blood was collected by pricking the tip of the rat tail and results are expressed as mg/dl.

ELISA:

GPX and LPO were analyzed using Abbkine elisa kits as per the manufacturer's instruction.

RNA EXPRESSION BY RT-PCR:

Total RNA, 2 µg was used for reverse-transcriptase polymerase chain reaction (RT-PCR) analysis. RT-PCR was carried out using a two-step RT-PCR kit. In the first step, complementary DNA (cDNA) will be made from an mRNA template using OligodT, dNTPs, and reverse transcriptase. The components were combined with a DNA primer in a reverse transcriptase buffer for an hour at 37°C. After cDNA conversion, standard PCR was carried out using gene-specific oligonucleotide primers by the initial PCR activation at 95°C for 5 min. The three-step PCR cycles consisted of denaturation at 95°C for 2 min, annealing at 60°C 30 s, and extension at 73 °C for 30 s. The PCR amplification was carried out for 30 cycles and to ensure that the products are extended completely, a final extension at 73°C for 5

min was carried out. Gene-specific oligonucleotide primers for the house-keeping gene, β-actin, were added to the same PCR reaction vial and co-amplified.

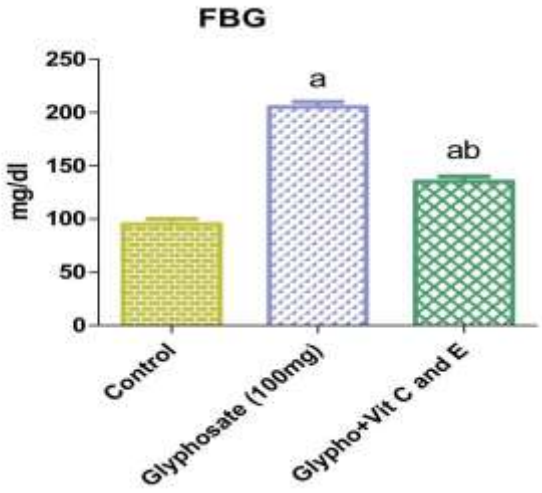
STATISTICAL ANALYSIS:

Data were expressed as the means ± SD of 3 individual experiments performed in triplicate. Statistical analysis was performed using the one-way ANOVA and $p<0.05$ was considered to indicate a statistically significant result.

RESULTS:

Impact of glyphosate on fasting blood glucose in glyphosate-induced rats

Compared to control, glyphosate induced rats showed a significant increase in the fasting blood glucose ($p<0.05$). Vitamin supplementation reduced hyperglycaemia near to that of the control level (Fig 1).

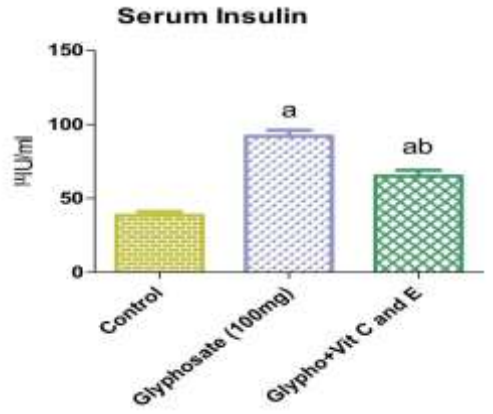


Mean	95	205	135
Std. Deviation	7.071	7.071	7.071
Std. Error	5	5	5

Fig. 1: Fasting blood glucose in glyphosate and vitamin treated rats. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to glyphosate induced rats. The mean of the control rats is 95 and the mean of glyphosate induced rats is 205 and the mean of rats treated with glyphosate, vitamin C and vitamin E is 135.

Impact of glyphosate on serum insulin in glyphosate-induced rats

Compared to control, glyphosate induced rats showed a significant increase in the serum insulin concentrations ($p<0.05$). Vitamin supplementation reduced hyperinsulinemia whose levels was found to be near to that of the control level (Fig 2).

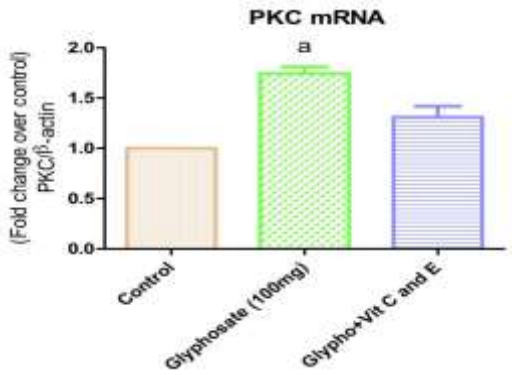


Mean	38.5	92	65
Std. Deviation	3.536	5.657	5.657
Std. Error	2.5	4	4

Fig. 2: Fasting serum insulin in glyphosate and vitamin treated rats. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to glyphosate induced rats. The mean of the control rats is 38.5 and the mean of glyphosate induced rats is 92 and the mean of rats treated with glyphosate, vitamin C and vitamin E is 65.

Impact of glyphosate on PKC mRNA expression in liver of glyphosate-induced rats

Compared to control, glyphosate induced rats showed a significant increase in the PKC mRNA expression ($p<0.05$). Vitamin supplementation reduced PKC mRNA whose level was found to be near to that of the control level (Fig 3).

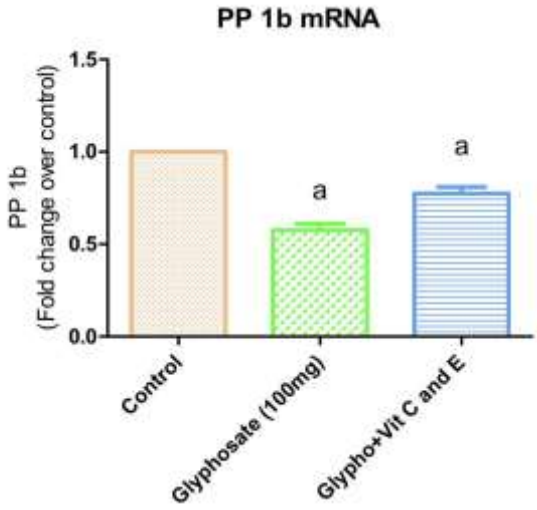


Mean	1	1.745	1.31
Std. Deviation	0	0.09192	0.1556
Std. Error	0	0.065	0.11

Fig. 3: PKC mRNA expression in the liver of glyphosate and vitamin treated rats. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to glyphosate induced rats. The mean of the control rats is 1 and the mean of glyphosate induced rats is 1.745 and the mean of rats treated with glyphosate, vitamin C and vitamin E is 1.31.

Impact of glyphosate on Phosphotyrosine phosphatases (PTPases) mRNA expression in liver of glyphosate-induced rats

Compared to control, glyphosate induced rats showed a significant increase in the PTP mRNA expression ($p<0.05$). Vitamin supplementation reduced PTP mRNA whose level was found to be near to that of the control level (Fig 4).



Mean	1	0.575	0.775
Std. Deviation	0	0.0495	0.0495
Std. Error	0	0.035	0.035

Fig. 4: PTP ase mRNA expression in the liver of glyphosate and vitamin treated rats. Each bar represents the mean ± S.E.M of 6 animals. a-compared to control; b-compared to glyphosate induced rats. The mean of the control rats is 1 and the mean of glyphosate induced rats is 0.575 and the mean of rats treated with glyphosate, vitamin C and vitamin E is 0.775.

DISCUSSION:

Recently,the protein phosphotyrosine phosphatase has been shown to be a negative regulator of the insulin signaling pathway,suggesting that inhibitors of this enzyme may be beneficial in the treatment of type 2 diabetes mice lacking phosphotyrosine phosphatase are resistant to both diabetes and obesity(18). Studies have identified that the activation of protein kinase C and increased diacylglycerol levels initiated by hyperglycemia are associated with many vascular abnormalities in retinal, renal and cardiovascular tissues(19). Transgenic Mice overexpressing PKC-beta isoform in the myocardium developed cardiac hypertrophy and failure,further supporting the hypothesis that PKC- beta isoform Activation can cause vascular dysfunction(20).

Insulin binds with the insulin receptor to form an insulin receptor substrate, which activates the PI3K enzyme. PI3K enzyme activates PKB enzyme(21,22). This stimulates GLUT 4, glucose transportation into the cell.phosphorylation of selected tyrosine sites on the receptor substrate is known to activate different pathways leading to increased

glucose uptake,lipogenesis which in turn activates the enzyme PP1B(23). PP1B reduces the levels of diabetes.

In Current research, The fasting blood glucose level in control rats is around 100 which is normal value; The fasting blood glucose level in glyphosate induced rats is increased around 200; When the Rats received simultaneous treatment of glyphosate,vitamin C and vitamin E the fasting blood glucose level is decreased from 200 to 150. In control rats the serum insulin level is around 50 which is normal value;The serum insulin level in glyphosate induced rats is increased around 100;When the Rats received simultaneous treatment of glyphosate,vitamin C and vitamin E the serum insulin level is decreased from 100 to 60.In control rats the PKC mRNA is around 1.0 which is normal value;The PKC mRNA in glyphosate induced rats is increased around 2.0;When the Rats received simultaneous treatment of glyphosate,vitamin C and vitamin E the PKC mRNAis decreased from 2.0 to 1.5.In control rats the PP1B mRNA is around 1 which is normal value;The PP1B mRNA in glyphosate induced rats is decreased around 0.5; When the Rats received simultaneous treatment of glyphosate,vitamin C and vitamin E the serum insulin level is increased from 0.5 to 0.8.

Vitamin C and Vitamin E treatment showed significant reduction in fasting blood glucose levels and serum insulin levels compared to diabetic rats. Vitamin C and Vitamin E decreased the expression of protein kinase C compared to the diabetic rats,which on activation induces inflammation.Vitamin C and Vitamin E caused upregulation in the expression of phosphotyrosine phosphatase 1B and mRNA compared to diabetes rats,whose expression will help to reduce the hyperlipidemia and hyperglycemia.

CONCLUSION:

Our study for the first time concludes that vitamin C and vitamin E play a significant role in reducing glyphosate induced hyperglycemia by modulating the expression of PKC and PP1B in the liver. Hence, antioxidant vitamins could be considered as therapeutic drug candidates for the management of type-2 diabetes. Further studies on clinical trials have to be done to develop vitamin c and vitamin E as an anti diabetic drug.

Conflict of Interest:

The authors hereby declare that there is no conflict of interest in this study.

Acknowledgement:

The authors express their gratitude to Saveetha Dental College & Hospitals for supporting and for successful completion of this project.

FUNDING AGENCY:

The present project is funded by
Saveetha Institute of medical and technical sciences
Saveetha dental college and hospital
Saveetha university
Loganathan Dental care Hospital.

Author Contribution:

A) Preethi K - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
B) Dr. Selvaraj - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
C) Dr. V. Vishnupriya - contributed in study design, guiding the research work, manuscript correction.
D) Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.
E) Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

REFERENCES:

Arcuri A. Glyphosate [Internet]. International Law's Objects. 2018. p. 234–46. Available from: http://dx.doi.org/10.1093/oso/9780198798200.003.0020

Green JM. The rise and future of glyphosate and glyphosate-resistant crops [Internet]. Vol. 74, Pest Management Science. 2018. p. 1035–9. Available from: http://dx.doi.org/10.1002/ps.4462

Bendich A. Antioxidant Vitamins and Human Immune Responses [Internet]. Vitamins & Hormones. 1996. p. 35–62. Available from: http://dx.doi.org/10.1016/s0083-6729(08)60406-9

[No title] [Internet]. [cited 2023 Sep 4]. Available from: https://www.researchgate.net/profile/Jaiganesh-Ramamurthy/publication/353723507_Evaluation_Of_Antioxidant_And_Anti_Inflammatory_Activity_Of_Grape_Seed_Oil_Infused_With_Silver_Nanoparticles_An_In_Vitro_Study/links/616032d5e7993f536ca55fa0/Evaluation-Of-Antioxidant-And-Anti-Inflammatory-Activity-Of-Grape-Seed-Oil-Infused-With-Silver-Nanoparticles-An-In-Vitro-Study.pdf

Dasgupta A, Klein K. Antioxidant Vitamins and Minerals [Internet]. Antioxidants in Food, Vitamins and Supplements. 2014. p. 277–94. Available from: http://dx.doi.org/10.1016/b978-0-12-405872-9.00015-x

Hennekens CH. Antioxidant Vitamins and Cardiovascular Disease [Internet]. Antioxidant Status, Diet, Nutrition, and Health. 2019. p. 463–77. Available from: http://dx.doi.org/10.1201/9780367811099-21

Bassuk S. Antioxidant Vitamins [Internet]. Clinical Trials in Heart Disease. 2004. p. 364–76. Available from: http://dx.doi.org/10.1016/b978-0-7216-0408-4.50033-0

Garg DS, Garg S, Solapur V. Prevalence of Complications in Diabetes Mellitus Type 2 [Internet]. Vol. 6, Journal of Medical Science And clinical Research. 2018. Available from: http://dx.doi.org/10.18535/jmscr/v6i1.57

Association Between Right Upper Molar Involvement And Diabetes Mellitus In Subjects With Chronic Periodon-titis [Internet]. [cited 2023 Sep 4]. Available from: https://www.academia.edu/download/73119179/IJDOS_2377_8075_08_6061.pdf

King G. Activation of protein kinase C pathway, a common pathway for hyperglycemia to cause vascular pathologies [Internet]. Vol. 13, American Journal of Hypertension. 2000. p. S316. Available from: http://dx.doi.org/10.1016/s0895-7061 (00)00785-8

Aakruti K. Thymol protects diabetic kidney by attenuating hyperglycemia induced oxidative stress [Internet]. Available from: http://dx.doi.org/10.26226/morressier.59d51849d462b80296ca304f

Begum N, Sussman KE, Draznin B. Differential effects of diabetes on adipocyte and liver phosphotyrosine and phosphoserine phosphatase activities [Internet]. Vol. 40, Diabetes. 1991. p. 1620–9. Available from: http://dx.doi.org/10.2337/diabetes.40.12.1620

Posner BI, Yale JF, Shaver A. S-1: The Insulin Receptor Kinase (IRK)-associated Phosphotyrosine phosphatase (PTP): A key regulator of IRK function and a therapeutic target [Internet]. Vol. 104, Experimental and Clinical Endocrinology & Diabetes. 2009. p. 1–2. Available from: http://dx.doi.org/10.1055/s-0029-1211487

Oxidative Stress [Internet]. Oxidative Stress and Disease. 2012. p. 129–30. Available from: http://dx.doi.org/10.1201/b13714-7

Sivakumar N, Geetha RV, Priya V. Gayathri R, Dhanraj Ganapathy. Targeted Phytotherapy For Reactive Oxygen Species Linked Oral Cancer. Int J Dentistry Oral Sci. 2021;8(1):1425–9.

Ali MA, El-Tahan RA, Kamel MA, Matar NA, Mahmoud SA. The diabetogenic effects of chronic supplementation of vitamin C or E in rats: Interplay between liver and adipose tissues transcriptional machinery of lipid metabolism. Life Sci. 2022 Jul 18;306:120812.

Pandiyan I, Sri SD, Indiran MA, Rathinavelu PK, Prabakar J, Rajeshkumar S. Antioxidant, anti-inflammatory activity of Thymus vulgaris-mediated selenium nanoparticles: An in vitro study. J Conserv Dent. 2022 Jun 13;25(3):241–5.

Namratha M, Lakshman M, Jeevanalatha M, Kumar A. Testicular Toxicity Induced by Glyphosate (GLP) and Ameliorative Effect of Vitamin C in Wistar Rats [Internet]. International Journal of Livestock Research. 2020. p. 1. Available from:

http://dx.doi.org/10.5455/ijlr.20191012074803

Tizhe EV, Ibrahim NDG, Fatihu MY, Igbokwe IO, George BDJ, Ambali SF, et al. Haematogical changes induced by subchronic glyphosate exposure: Ameliorative effect of zinc in Wistar rats [Internet]. Vol. 11, Sokoto Journal of Veterinary Sciences. 2013. Available from: http://dx.doi.org/10.4314/sokjvs.v11i2.5

Macfarlane DE, Manzel L. Activation of beta-isozyme of protein kinase C (PKC beta) is necessary and sufficient for phorbol ester-induced differentiation of HL-60 promyelocytes. Studies with PKC beta-defective PET mutant [Internet]. Vol. 269, Journal of Biological Chemistry. 1994. p. 4327–31. Available from: http://dx.doi.org/10.1016/s0021-9258(17)41782-0

Xu P, Jacobs AR, Taylor SI. Interaction of Insulin Receptor Substrate 3 with Insulin Receptor, Insulin Receptor-related Receptor, Insulin-like Growth Factor-1 Receptor, and Downstream Signaling Proteins [Internet]. Vol. 274, Journal of Biological Chemistry. 1999. p. 15262–70. Available from: http://dx.doi.org/10.1074/jbc.274.21.15262

Anti Proliferative Effect of Endogenous Dopamine Replica in Human Lung Cancer Cells (A549) Via Pi3k and Akt Signalling Molecules. Available from: https://pnrjournal.com/index.php/home/article/download/1228/1030

Gibbins JM. Study of Tyrosine Kinases and Protein Tyrosine Phosphorylation [Internet]. Platelets and Megakaryocytes. p. 153–68. Available from: http://dx.doi.org/10.1385/1-59259-783-1:153