Role of Antioxidant Vitamins on the Expression on the Sparc, Munc. 18 and Syntaxin Mrna in the Gastrocnemius Muscle of Glyphosate Induced Experimental Rats

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Abstract

Background:

Glyphosate is a herbicide that poses a significant threat to human and animal life. Hepatotoxicity induced by the ingestion of herbicide (glyphosate) in diabetic rats can be reduced/lessened by the action of antioxidant vitamins (vitamin A & vitamin C).

Aim:

The purpose of this study is to determine the effect of antioxidant vitamins on the expression of SPARC, MUNC.18, and syntaxin mRNA in the gastrocnemius muscle of glyphosate-induced rats.

Materials:

Adult male albino rats were divided into 3 groups. Group I: served as vehicle control (corn oil alone); Group II: Control rats were injected intraperitoneally daily with glyphosate; Group III: glyphosate induced rats treated with vitamins E (50 mg/kg body weight) and C (100 mg/kg body weight), once daily through gastric incubation for 30 days. On completion of treatment, animals were anesthetized, blood was collected, sera were separated, gastrocnemius muscle was dissected out and subjected to assessment of gene expression analysis. Fasting blood glucose, serum insulin levels were measured in the serum whereas, HNF-1 alpha mRNA and SREBP-1c proteins were measured in the skeletal muscle using Real Time-PCR analysis using gene specific primers and ELISA methods. The data were statistically analyzed using One-Way-ANOVA followed by Duncan's multiple range test were used to check the statistical significance and considered at the levels of $p<0.05$.

Results:

Fasting Blood Glucose (FBG) test and serum insulin levels were estimated for diabetes in this study. Results of this study showed that glyphosate exposure

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significantly raised the fasting blood glucose and insulin levels ($p<0.05$). However, treatment with the antioxidant (Vitmim C and E) improved the glycemic control and insulin levels near to that of the control levels ($p<0.05$). SPARC protein levels in comparison between three groups provides data that implies the efficacy of antioxidant vitamins on glyphosate induced rats. Also, MUNC. 18, Syntaxin protein expression was also determined at their mRNA level using real time/qPCR analysis. Those data provide a clear conclusion of the efficacy of antioxidant vitamins on glyphosate induced diabetes mellitus.

Conclusion:

Our current findings show that antioxidant vitamins E and C have a greater impact on the expression of SPARC, MUNC18, and syntaxin protein. The efficacy of naturally abundant antioxidants in tissue homeostasis is elaborated in link with Syntaxin and Munc 18 protein at its mRNA level expression. Also these findings pave the way for future research to gain a knowledge of antioxidants mechanism of action in glucose homeostasis.

Keywords: Innovative technique, Antioxidant vitamins (Vitamin C and E), SPARC, MUNC8, glyphosate, gastrocnemius muscle, novel method.

1. INTRODUCTION

Antioxidants are substances which prevent free radical stimulated oxidative stress damage. A chemical reaction can produce free radicals, which can damage the organism's cells in a chain reaction. Antioxidants like thiols and ascorbic acid may inhibit the reactions(1). Plants and animals maintain a complex system of overlapping antioxidants glutathione, superoxide dismutase by balancing oxidative stress. Vitamin C (ascorbic acid) is an essential cofactor for α-ketoglutarate-dependent dioxygenases. Vitamin C plays a major role in the biosynthesis of collagen and in down-regulation of hypoxia-inducible factor (HIF)-1 molecule (transcription factor that regulates many genes responsible for tumor growth, energy metabolism, and neutrophil function and apoptosis). Vitamin C-dependent inhibition of the HIF pathway may provide alternative or additional approaches for controlling tumor progression, infections and inflammation (2). Vitamin E (α-tocopherol) functions as an essential lipid soluble antioxidant, scavenging hydroperoxyl radicals in lipid milieu. Human symptoms of vitamin E deficiency suggest that its antioxidant properties play a major role in protecting erythrocyte membranes and nervous tissues (3). As an antioxidant, vitamin C provides protection against oxidative stress-induced cellular damage by scavenging of reactive oxygen species, vitamin E-dependent neutralization of lipid hydroperoxyl radicals, and by protecting proteins from alkylation by electrophilic lipid peroxidation products. These bioactivities bear relevance to inflammatory disorders. Vitamin C also plays a role in the function of endothelial nitric oxide synthase (eNOS) by recycling the eNOS cofactor, tetrahydrobiopterin, which is relevant to arterial elasticity and blood pressure regulation (4). Evidence from plants supports a role for vitamin C in the formation of covalent adducts with electrophilic secondary metabolites. Vitamins A, C, and E are examples of dietary antioxidants. It's also used in industrial chemicals to keep synthetic rubber, plastics, and other materials from oxidizing (5). Antioxidant vitamins are found in fruits and vegetables and are used as preservatives, food additives, and cosmetics. One of the natural antioxidants abundant in animals in the form of

ascorbic acid is vitamin C. It is an animal and plant-derived monosaccharide oxidation-reduction catalyst. It is also present in all parts of plants, some of the animals are able to produce this compound in their bodies and do not require it in their diet (6). Vitamin E is a fat-soluble vitamin that has antioxidant properties . It refers to a group of eight tocopherols that are related. One of the most important lipid-soluble antioxidants is alpha tocopherol (7). The Gastrocnemius muscle is a powerful bulk muscle found in the back of the human lower leg. The lateral head develops from the femur's lateral condyle. It is used for basic activities such as walking, jumping, posture etc. (8)**.** Our team has extensive knowledge and research experience that has translate into high quality publications (9–18))((19–28).

2. 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. Insulin receptor (IR), glucose transporter-4 (GLUT4) and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India and.

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 centre (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 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: Rats received ip injection of glyphosate with a dose limit (100mg).
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). In the present study, Glyphosate dose was selected based on our previous report of Anne et al. (2013) and Vit C and E doses were selected based on the report of our previous study. After the treatment period, animals were anesthetized with ether, blood was collected, sera separated and stored at −80 °C. Gastrocnemius muscle from control and treated animals were dissected out and subjected for the assay of various parameters.

Determination of fasting blood glucose (FBG) and serum insulin

The animals were starved overnight the day before sacrifice after receiving treatment for 30 days.The next day, blood was drawn from the rat tail tip to estimate glucose levels using On-Call Plus blood

glucose test strips. The data were displayed in milligrammes per decilitre on the meter display window.

INS GENLISA ELISA kit from Krishgen Biosystem, Mumbai-400018, India, was used to measure insulin levels in rat serum. According to the manufacturer's guidelines, the detection range and coefficient of variation were set. The serum insulin concentration was measured in pg/ml

Total RNA, cDNA Synthesis and Real-Time PCR

RNA was taken out from the livers of the rats in the present study. The reverse transcriptase RT kit was used to reverse transcript 2µg of RNA (Seraing, Belgium). The primers utilized in this study are listed in Table 1. Using housekeeping gene (β-Actin) as a reference gene, 40 cycles of 95°C, 59–60°C, and 72°C for 30s each were amplified in a Stratagene MX 3000P qRT-PCR system under the subsequent reaction conditions: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C, 59–60°C and 72°C for 30s each. The melt and amplification curves analyses were used to calculate relative quantification.

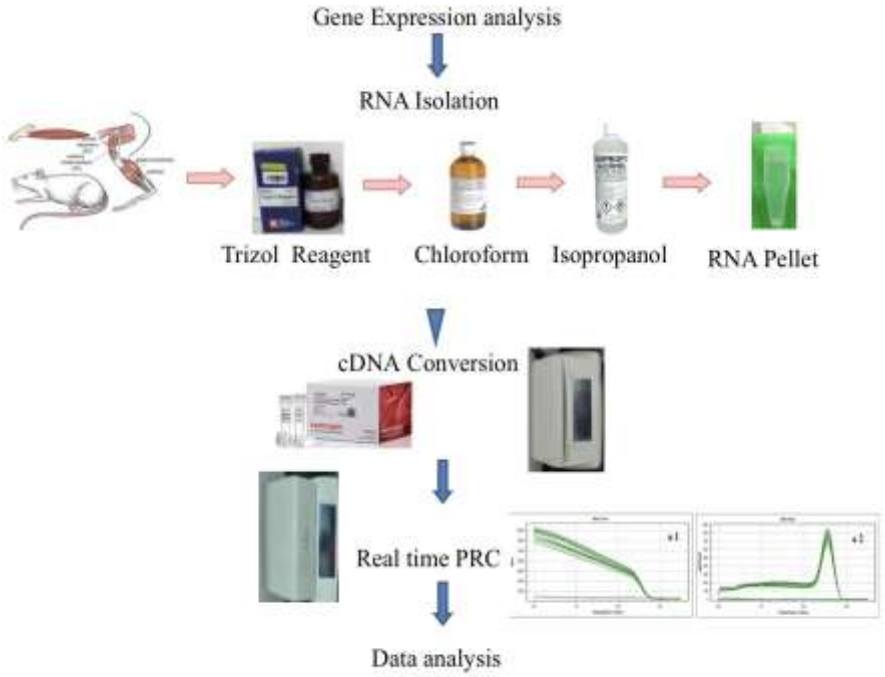


Figure 1 Summarizes the brief outline of overall experimentation.

3. RESULTS

Effect of antioxidant vitamins (Vitamin C and E) on fasting blood glucose and fasting serum insulin in glyphosate experimental rats

Glyphosate exposure significantly raised fasting blood sugar levels and serum insulin concentration ($p<0.05$) when compared with control suggesting that glyphosate administration causes hyperglycemia and hyperinsulinemia in experimental rats (Figure 1 & 2). However, Vitamin C and E administration for a period of 30 days, reduced hyperglycemia and hyperinsulinemia significantly and this study clearly indicates that antioxidant vitamins play a significant role in diabetes mellitus.

Figure 1

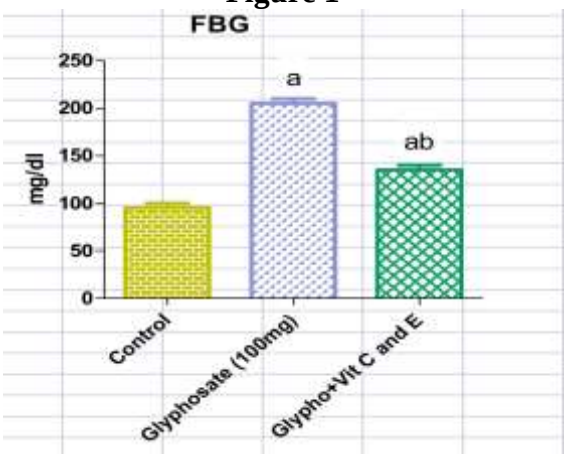


Figure 2

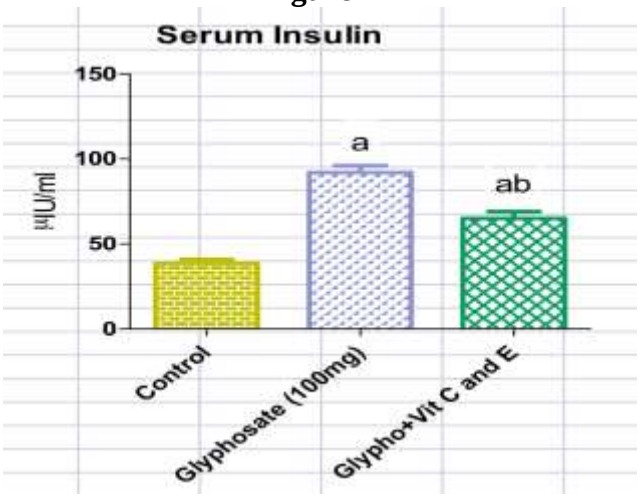


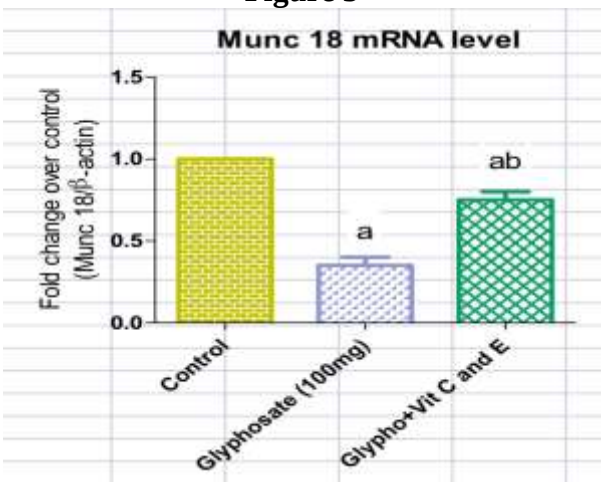
Figure 1 & 2 represents the significance of antioxidant vitamins when compared to other two groups. Effect of glyphosate and vitamins (C and E) supplementation on FBG and serum insulin in experimental diabetic rats. Each bar represents the mean±SEM of six animals (n=6). Significance at p<0.05, a—compared with control, b—compared with glyphosate induced type-2 diabetic rats. X-axis indicates group while Y-axis represents concentration of glucose given as mg/dl concentration while serum insulin has been expressed as micro international unit per ml.

Effect of antioxidant vitamins (Vitamin C and E) on Munc 18 mRNA level in gastrocnemius muscle of glyphosate induced rats

Munc 18 protein is involved in the neurotransmission process which has both synaptic excitatory and inhibitory action. Here in this graph. The expression of Munc 18 mRNA has been represented in terms of pg/ml. In this study, Munc18 mRNA levels were found to be significantly ($p<0.05$) lower in glyphosate induced rats compared to control rats (Figure 3). However, administration of vitamin C & E in combination increases the expression of protein (Munc18mRNA) whose effects were found to be

significantly lesser to that of the healthy control rats.

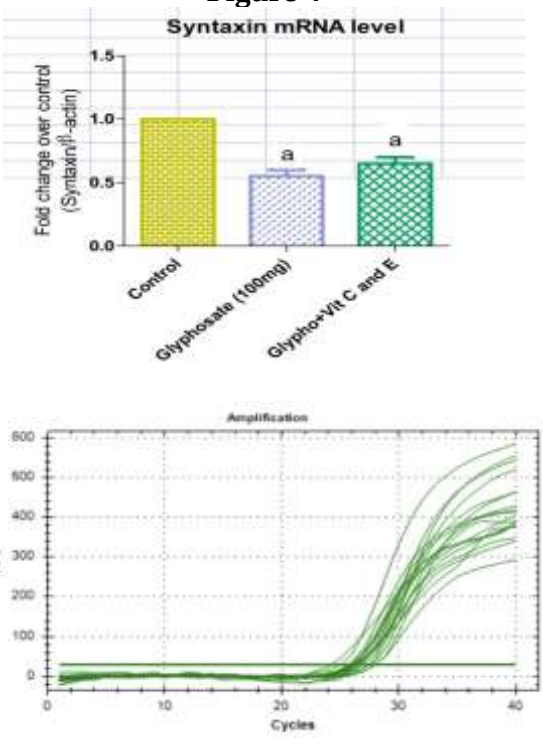
Figure 3

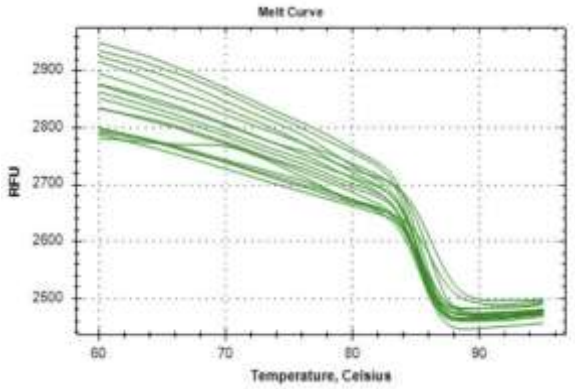


Effect of antioxidant vitamins (Vitamin C and E) on Syntaxin mRNA levels in the gastrocnemius muscle of glyphosate induced rats

Syntaxin is a group of integrated Q-SNARE proteins which plays a major role in exocytosis. It mediates vesicular fusion in a diverse vesicular transport process. Here in this graph. The expression of Syntaxin mRNA has been represented in terms of pg/ml.. In this study, Syntaxin mRNA levels were found to be significantly ($p<0.05$) lower in glyphosate induced rats compared to control rats (Figure 4). However, administration of vitamin C & E in combination increases the expression of protein (Syntaxin mRNA) whose effects were found to be significantly lesser to that of the healthy control rats.

Figure 4

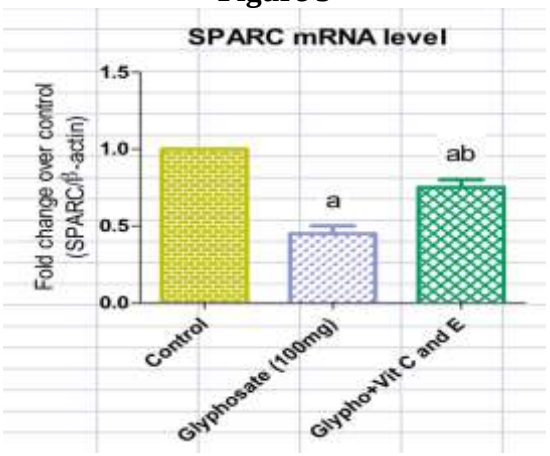




Effect of antioxidant vitamins (Vitamin C and E) on SPARC protein levels on the gastrocnemius muscle of glyphosate induced rats

SPARC is a matricellular protein which plays a crucial role of regulation between matrices and the cells. In this graph, the expression of SPARC protein has been represented in terms of pg/ml.. In this study, SPARC levels were found to be significantly ($p<0.05$) lower in glyphosate induced rats compared to control rats (Figure 5). However, administration of vitamin C & E in combination increases the expression of protein (SPARC) whose effects were found to be significantly lesser to that of the healthy control rats.

Figure 5



4. DISCUSSION

According to the findings, the role of SPARC in hematology and other malignancies is complex and it also varies depending on the types of malignancy. The link between MUNC.18 and syntaxin 4 are clearly explained by the mechanism of insulin granule exocytosis and Glut 4 vesicle translocation (29). In insulin secreting or insulin responsive cells, MUNC. 18 or syntaxin isoforms have demonstrated that both proteins can undergo tyrosine phosphorylation in a stimulus dependent manner. As a result, syntaxin 4 is the only syntaxin isoform known to be required for insulin-stimulated Glut 4 vesicle translocation. Recently, Glut 4 was discovered to be expressed in the hypothalamus, implying that syntaxin 4 also plays a role in the brain (30). As previously stated, glyphosate is a widely used herbicide that is shown to increase the risk of cancer. The mechanical phenotype plays an important role in malignant transformation. In other

study using a rat exposed to glyphosate displayed a pronounced important in liver function which was confirmed by histological and ultra structural alternation. Our team has extensive knowledge and research experience that has translated into high quality publications (31), (32), (33), (34), (35), (36,37), (38), (39), (40), (41).

5. CONCLUSION

The cocrystallization of the MUNC18 isoform with the N-terminal19 residue peptide of syntaxin 4 demonstrates the importance of this specific site for protein-protein interaction. According to the findings, antioxidant vitamins E and C have a greater impact on the expression of SPARC, MUNC18, and syntaxin protein. The efficacy of naturally abundant antioxidants in tissue homeostasis is elaborated in link with Syntaxin and Munc 18 protein at its mRNA level expression. Also these findings pave the way for future research to gain a knowledge of antioxidants mechanism of action in glucose homeostasis.

Future Scope:
Future research aimed at gaining the ability to control syntaxin 4 expression may be beneficial in improving whole-body glucose homeostasis.

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

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Author Contribution:
A) Venkateshwaran V - 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.

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