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Impact of Glyphosate on SREBP-1c and PPAR-Γ Expression in Adipose Tissue of Male Albino Wistar Rats

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Abstract

Glyphosate is used as an herbicide in agriculture. At sub-agriculture concentrations, glyphosate-based herbicide inhibits cell proliferation. Glyphosate is a chelating agent that interferes with the metabolic activities in plants thereby adversely affecting its metabolism. The study aimed to determine the glyphosate-induced detrimental changes in SREBP-1c and PPAR-γ mRNA expression in adipose tissue of adult male rats. Adult male Wistar albino rats were divided into 4 groups, each consisting of 6 animals. Group I served as normal control rats; Group II-IV consisted of rats exposed to glyphosate at different concentrations (50, 100, and 250 mg/kg body weight respectively) orally for 16 weeks. After 16 weeks of treatment, the animals were sacrificed, and adipose tissue was dissected out for the assessment of SREBP-1c and PPAR-γ mRNA by real-time PCR using gene-specific down-regulated primers. The results with the p<0.05 level were considered to be statistically significant. The results showed a significant dose-dependent increase (P <0.05) in the expression of SREBP-1c in all the glyphosate-exposed rats compared to control rats and PPAR-γ mRNA expression was found to be significantly reduced in a concentration-dependent manner (P<0.05) compared to normal control animals. The current findings for the first time report that glyphosate had detrimental changes in the expression of transcription factors such as SREBP-1c and PPAR-γ mRNA in adipose tissue and thereby glyphosate may lead to the development of type-2 diabetes or insulin resistance.

Keywords: *Adipose Tissue, Glyphosate, Innovative Technology, Novel Method, PPAR-Γ, SREBP - 1c, Type-2 Diabetes.*

Introduction

Glyphosate can be found widely in groundwater, surface water, and in sediments globally [1-3]. The maximum level of glyphosate and its metabolites is 700 micrograms/ litre [2]. Glyphosate is a commercial herbicide that is commonly used worldwide. Glyphosate is a chelating agent that interferes with the metabolic activities in plant thereby adversely affecting its metabolism. Glyphosate inhibits the enzyme 5-enol pyruvylshikimate -3-phosphate synthase involved in shikimic acid pathway. This pathway is essential in synthesis of aromatic amino acids in plants but is absent in animals [4, 5]. When glyphosate is ingested through food and water in small amounts, it is considered to be safe for people. Glyphosate-related herbicides are widely used in countries like Brazil, Argentina and USA. In Argentina, the Buenos Aires region has genetically

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modified soybean plants where glyphosate is found in large amounts in water and soil [6]. Taking this into consideration, the herbicide can spread across the food chain to reach animals and plants. In addition, by agricultural practices, herbicide residues may be exposed to humans [7]. The glyphosate-related herbicide also contains polyoxyethyleneamine, which acts as a surfactant. These compounds may also be toxic. Glyphosate formulations are shown to be toxic and potent endocrine disruptors affecting the hypothalamic-pituitary axis [8-10]. They trigger the key transcription factor expression in adipogenesis: PPAR-γ is involved in protein induction and its expression is increased in adipocytes.

SREBP- 1c (Sterol regulatory element-binding protein-1c) is a transcription factor involved in fatty acid and cholesterol metabolism. In cultured rat hepatocytes, SREBP-1c expression is stimulated by insulin and repressed by glucagon [11]. SREBP - 1c activates the genes which are required for the synthesis of fatty acids. Recently, SREBP - 1c is suggested to be a transcription factor that mediates the insulin action on glycerol kinase transcription in the liver [12, 13].

The ligand of PPAR- γ receptors are potent insulin sensitizers which are used as targets in type 2 diabetes treatment. PPAR-γ promotes the storage of fatty acid in fat depots and regulates the adipocyte secretome which subsequently impacts glucose homeostasis. PPAR and SREBP together alter hepatic fatty acid biosynthesis and insulin signalling leading to diabetes mellitus and liver diseases in response to glyphosate exposure. No previous literature was found in this study. The findings of the current study demonstrate that glyphosate causes lipid accumulation by up-regulation of lipogenesis-related genes and downregulation of lipolysis-related genes which is associated with the pathogenesis of diabetes. Glyphosate raises the environmental threat and public health concerns. Our team has extensive knowledge and research experience that has translated into high-quality publications [14-33]. Hence, we investigated the effect of glyphosate on the expression of Sterol regulatory element-binding protein-1c and PPAR-γ (Peroxisome proliferator-activated receptor -gamma) in adipose tissue of male albino Wistar 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. glyphosate was procured from Sigma Chemical Company St. Louis, MO, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from Genet Bio, South Korea purchased from Promega, USA. Sterol regulatory element-binding protein -1c receptor and peroxisome proliferator-activated receptor-gamma receptor were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India.

Animals

The present experimental study was approved by the institutional animal ethics committee (IAEC no.: BRULAC/SDCH/SIMATS/IAEC/02-2019/015). Adult male Wistar albino rats, weighing 180–200g, were obtained and maintained in clean propylene cages at the Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha Dental College and Hospitals, Saveetha University, India) in an air-conditioned animal house, fed with standard rat pelleted diet (Lipton India Ltd., Mumbai, India), and clean drinking water was made available ad libitum. Rats were divided into 3 groups, each consisting of 6 animals.

Experimental Design

An adult male albino rat of the Wistar strain (R*attus norvegicus) was divided into four groups each consisting of 6 animals.* Group I: Normal control rats fed with normal diet and drinking water; Group II: Glyphosate treated (dissolved in water at a dose of 50 mg/kg body weight/day at 8 to AM) orally for 16 weeks; Group III: Glyphosate treated (dissolved in water at a dose of 100 mg/kg body weight/day at 8 to AM) orally for 16 weeks; Group IV: Glyphosate treated (dissolved in water at a dose of250 mg/kg body weight/day at 8 to AM) orally for 16 weeks.

At the end of the treatment, animals were anaesthetized with sodium thiopentone (40 mg/kg b. wt), blood was collected through the cardiac puncture, sera were separated and stored at −80°C, and 20 ml of isotonic sodium chloride solution was perfused through the left ventricle to clear blood from the organs. Adipose tissue from control and experimental animals was immediately dissected out and used for assessing the various parameters.

Isolation of Total RNA

Total RNA was isolated from control and experimental samples using a TRIR (total RNA isolation reagent) kit. Briefly, 100 mg fresh tissue was homogenized with 1 ml TRIR, and the homogenate was transferred immediately to a microfuge tube and kept at -80°C for 60 min to permit the complete dissociation of nucleoprotein complexes. Then, 0.2 ml of chloroform was added, vortexed for 1 min, and placed on ice at 4°C for 5 min. The homogenates were centrifuged at 12,000 x g for 15 min at 4°C. The aqueous phase was carefully transferred to a fresh microfuge tube and an equal volume of isopropanol was added, vortexed for 15 sec, and placed on ice at 4°C for 10 min. The samples were centrifuged at 12,000 x g for 10 min at 4°C. The supernatant was discarded, and the RNA pellet was washed with 1 ml of 75% ethanol by vortexing and subsequent centrifugation for 5min at 7,500 x g (4°C). The supernatant was removed, and RNA pellets were mixed with 50 µl of autoclaved Milli-Q water and dissolved by heating in a water bath for 10 min at 60°C.

Quantification of RNA

Diluted RNA samples were quantified spectrophotometrically by measuring the absorbance (A) at 260/280 nm. 40 µg of RNA in 1 ml gives one absorbance at 260 nm. Therefore, the concentration of RNA in the given sample can be determined by multiplying its A260 by 40 and the dilution factor. The purity of RNA preparation can be calculated using the ratio between its absorbance at 260 and 280 nm. A ratio of absorbance at 260/280 nm> 1.8 is generally considered as good quality RNA (Fourney et al., 1988). The purity of RNA obtained was 1.8.

Reverse Transcriptase - Polymerase Chain Reaction (RT - PCR)

RT-PCR is an approach for converting and amplifying a single-stranded RNA template to yield abundant double-stranded DNA products. 1. First-strand reaction: Complementary DNA (cDNA) is made from the mRNA template using Oligo dT, dNTPs & reverse transcriptase. 2. Second strand reaction: After the reverse transcriptase reaction is complete, standard PCR (called the “second strand reaction”) is initiated. Principle RT-PCR is a method used to amplify cDNA copies of RNA. It is the enzymatic conversion of mRNA into a single cDNA template. A specific oligodeoxynucleotide primer hybridizes to the mRNA and is then extended by an RNA-dependent DNA polymerase to create a cDNA copy. First-strand DNA synthesis The RT kit was purchased from Eurogentec (Seraing, Belgium). Reagents 1. 10X RT buffer: One vial containing 1.4 ml of 10X RT buffer. 2. EuroScript reverse transcriptase: One tube containing 75 µl of Moloney Murine leukemia virus reverse transcriptase (3750 U at 50 U/µl).

cDNA Conversion and PCR Amplification

Briefly, 2µg of RNA was reverse transcribed using the reverse transcriptase RT kit from Eurogentec (Seraing, Belgium). Genes were amplified using SYBR green master mix in a real-time PCR system (Bio-Rad C1000 Touch, thermal cycler, Bio-Rad laboratories ltd. Bio-Rad House, 13 Maxted Road, Hemel Hempstead, Herts. HP2 7DX, United Kingdom), under the following reaction conditions: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 s, 59-60°C for 30 s, and 72°C for to calculate relative quantification, the melt and amplification curves analysis was employed. Details of primers used in the present study:

RAT PPAR-γ- FW: 5'-GGACGCTGAAGAAGA-3'; RW: 5'-GACCTG CCGGGTCCTGTCT GAGTATG-3'; SREBP-1c -forward, 5′-CGCTACCGTTCCTCTATCAATGAC-3; reverse, 5′-AGTTTCTGGTTGCTGTGCTGTAAG-3'; β-actin- FW: 5'-GACCTCTATGCCAACACAGT-3'; RW 5'-CACCAATCCACACAGAGTAC-3'.

Statistical Analysis

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ±standard deviation. Results were analyzed statistically by one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Duncan's multiple range test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were statistically significant.

Results

Impact of Glyphosate on the mRNA Expression of SREBP-1c and PPAR- γ in the Adipose Tissue of Experimental Rats

mRNA expression of SREBP-1c and PPAR-γ were assessed by Real-Time -PCR. There is a significant dose-dependent increase ($p<0.05$) in the expression of SREBP-1c in the glyphosate-treated rats compared to control rats (Figure 1) conversely PPAR- γ mRNA expression was also found to be significantly ($p<0.05$) down-regulated in glyphosate exposed rats compared to control in a dose-dependent manner (Figure 2) indicating that glyphosate has detrimental changes adipose tissue leading to the development of diabetes.

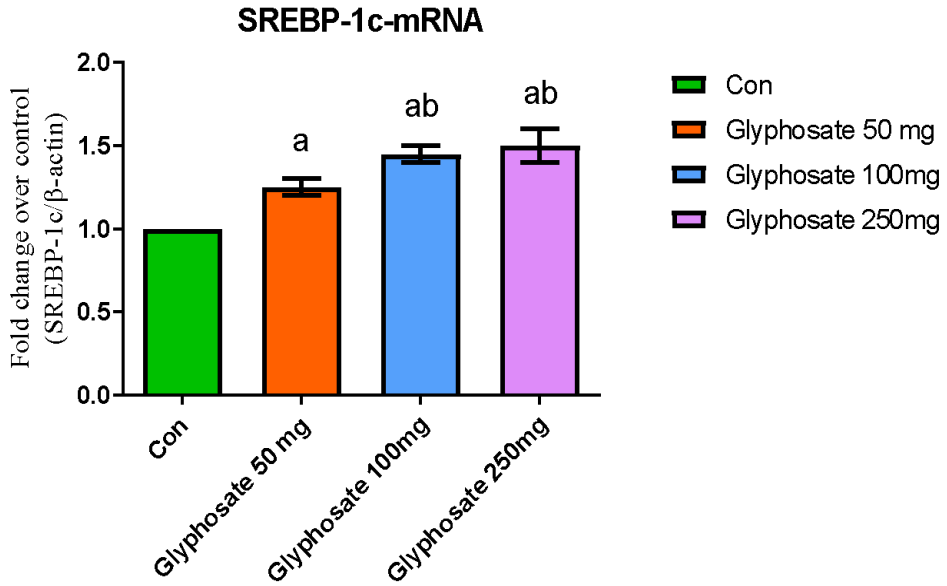

Figure 1. Impact of Glyphosate on the mRNA Expression of SREBP-1c in Adult Male Rats

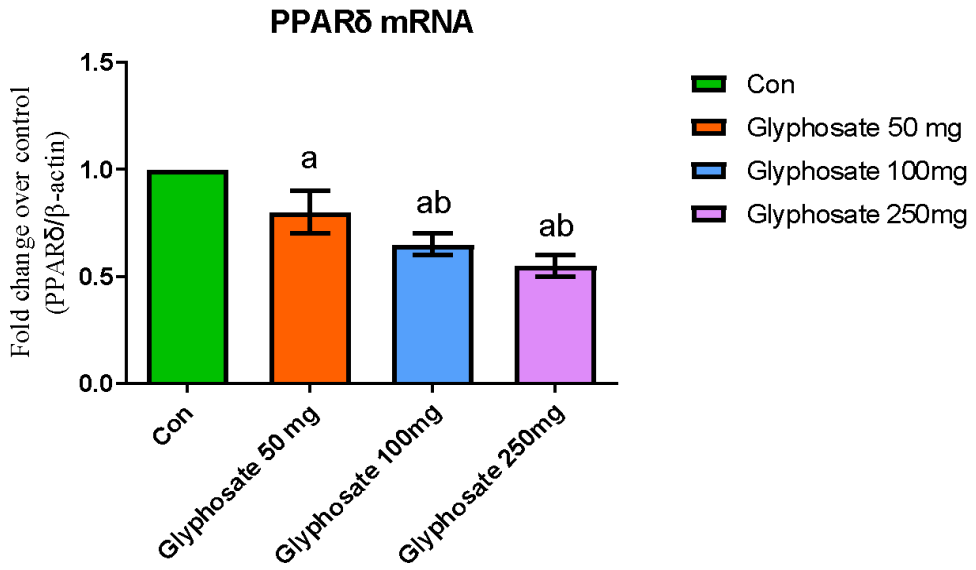

Figure 2. Impact of Glyphosate on the mRNA Expression of PPAR- **γ** in Adult Male Rats

Discussion

Glyphosate is used as an herbicide which raises concern about public health. Oxidative stress is considered the pathological mechanism associated with the toxicity of herbicides (34) [34]. Acute glyphosate exposure causes changes in the antioxidative defence mechanisms [35]. The studies also showed that glyphosate significantly decreases the level of gene expression and antioxidative activity enzymes at low-dose exposure. Apart from oxidative damage, inflammation is considered a secondary response to glyphosate [36]. Inflammatory mediators are associated with abnormal lipid metabolism involving lipid accumulation. There is a significant dose-dependent increase in the expression of SREBP-1c in the glyphosate-treated rats compared to control rats. SREBP - 1c upregulates gene expression enzymes involved in fatty acid and triglyceride synthesis such as acetyl - CoA carboxylase and Fatty acid synthase [37]. SREBP - 1c regulates lipogenesis by regulating genes coding for enzymes namely HMG CoA synthase, HMG CoA reductase, farnesyl diphosphate synthase, squalene synthase, and also low-density lipoprotein (LDL) receptor involved in uptake of cholesterol [38]. Glyphosate exposure might be able to increase lipogenesis as it increases the expression of SREBP - 1c.

SREBP has a basic helix–loop–helix leucine zipper motif. Different isoforms of SREBP arise due to alternative splicing of transcripts. SREBP-2 isoform is involved in the cholestrogenic pathway. SREBP-1 isoforms are involved in the synthesis of fatty acids. Particularly SREBP-1c is involved in insulin-dependent regulation of lipogenic genes and triglyceride synthesis. Exposure to glyphosate reduces the expression of this isoform SREBP-1c. Insulin can also activate the transcription of genes involved in fatty acid synthesis including SREBP-1c. PPAR-γ is also essential in regulating the secretion of leptin hormone in adipose tissue. Glyphosate can produce hepatic steatosis by modification of PPAR-γ. The modification of these genes can lead to the development of insulin resistance [38].

PPAR-**γ** belongs to the nuclear hormone receptor family of transcription factors. It is found in liver, heart, skeletal muscle and brown adipose tissue and is involved in regulation of fatty acid oxidation occuring mitochondria and peroxisomes. There is a significant dose-dependent decrease in the expression of PPAR-γ which is in a concentration-dependent and dose-dependent manner. A previous study by

Martini et al investigated the glyphosate effect on PPAR- γ induction in fibroblasts where they found that glyphosate exposure decreases the expression of PPAR-γ. This study supports our finding [39] where the impact of glyphosate on the mRNA expression of PPAR-γ in adult male rats leads to downregulation of lipolysis-related genes, leading to diabetes and PPAR-γ also participates in lipogenesis and lipolysis [40-47].

Even though the results were promising, only two parameters were used. Furthermore, the serum levels of glyphosate, diabetic profiles, blood glucose level parameters, and other signalling molecules were not analyzed. Current findings demonstrate that glyphosate causes lipid accumulation by up-regulation of lipogenesis-related genes and downregulation of lipolysis-related genes which can lead to diabetes [48-52].Further studies on downstream signalling molecules of proinflammatory signalling mechanisms are warranted to better understand glyphosate towards the development of new drugs.

Conclusion

The current findings for the first time report that glyphosate had detrimental changes in the expression of transcription factors such as SREBP-1c and PPAR-γ mRNA in adipose tissue and thereby glyphosate may lead to the development of type-2 diabetes or insulin resistance.

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Conflict of Interest

The author declares that there is no conflict of interest in the present study.

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