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Antioxidant Vitamins (Vit C and E) Protect the Kidney from Glyphosate-Exposed Renal Damage in Experimental Rats - A Molecular Study

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Abstract

Glyphosate is a widely used herbicide and the active ingredient in Monsanto's Roundup, among other herbicide formulations. Vitamin C (ascorbic acid) and vitamin E (tocopherol) are both antioxidants that play important roles in reducing oxidative DNA damage and protecting cells from the harmful effects of free radicals. The study aimed to analyse the effect of antioxidant vitamins (vitamins C and E) on the expression of IGF-1 and KIM-1 molecules in the kidneys of glyphosate-exposed rats. Adult male Albino Wistar rats were grouped into 3. Group I: Control; Group II: Glyphosate treated (100mg); Group III: Glyphosate+ vitamin E & C treated. Serum, insulin, and mRNA expression of insulin-like growth factor (IGF-1) and kidney injury molecules (KIM-1) mRNA expression by RT-PCR. Serum insulin was measured by the ELISA method. Glyphosate-exposed rats developed hyperglycaemia and hyper compared to control but antioxidant vitamins (Vitamin C and E) supplementation, reduced the glyphosate mediate increased fasting blood glucose and insulin near to that of the control level. Similarly, mRNA expression of both IGF-1 and KIM-1 were also significantly altered due to glyphosate induction whose effects were found to be reduced when vitamins C and E were treated. It is concluded that glyphosate exposure causes renal damage and develops diabetic nephropathy by modulating the expression of IGF-1 and KIM-1 molecules in the kidney. Vitamin C and E potentially were able to reduce the detrimental changes caused by glyphosate. Hence, vitamin C and E supplements could be considered therapeutic drugs for diabetic nephropathy.

Keywords: *Diabetes Nephropathy, Glyphosate, Kim-1 and Igf-1 Health and Well-Being, Vitamin C, Vitamin E.*

Introduction

Glyphosate, a widely used organophosphate herbicide found in products like Roundup, Rodeo, and Pond Master, poses economic significance in agriculture and environmental management but also carries risks to human and animal health. Its broad applicability and high solubility make it a popular choice among farmers for weed control. Glyphosate has a variable half-life, ranging from 0.8 to 150 hours, contributing to its versatility and effectiveness in various agricultural settings. From a chemical standpoint, glyphosate is a relatively straightforward molecule classified as an organophosphorus compound, specifically, a phosphonic acid. This compound results from the formal oxidative combination of the methyl group in methyl phosphonic acid with the amino group of glycine. It shares similarities with the natural amino acid glycine, featuring a basic amino group and a highly ionized 126-phosphate group. Consequently, glyphosate becomes a highly polar and

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amphoteric molecule. Currently, this herbicide is extensively employed by both developed and developing nations. The widespread use of glyphosate raises concerns about its potential accumulation due to its prolonged life, and it proves challenging to purify its effluents from environmental sources. The associated risk factors and concerns regarding glyphosate usage have sparked controversies, prompting debates on whether it should be banned, restricted, or promoted [1, 2].

Type-2 diabetes mellitus (T2DM) is associated with the dysfunction of signalling mechanisms, leading to either overexpression or reduced expression of multiple genes involved in glucose utilization. This, in turn, accelerates hyperglycemia. Free radicals, released by cells during oxidative stress in various biological processes, contribute to the development of several diseases, including T2DM [3]. Furthermore, these free radicals can be countered by natural antioxidants or synthetic antioxidants. The human body possesses natural antioxidants such as antioxidant enzymes (superoxide dismutase, catalase, peroxidase, and reductase) and vitamin C. Oxidative damage plays a significant role in the progression of various pathogenic conditions, including diabetes. An enhanced free radical scavenging system in the host can mitigate oxidative damage and potentially impede the progression of pathogenesis [4]. Well-known naturally available free radical scavengers include carotenoids, vitamin C, and vitamin E.

Additionally, enzymes like Cytochrome P450, glutathione transferases (GST), and glucose-6-phosphate dehydrogenase (G6PD) play crucial roles in detoxification mechanisms, which may be negatively influenced by the herbicide Roundup. An in vitro study explored the adverse effects of glyphosate on various hepatic enzymes, revealing alterations in the levels of these enzymes upon exposure to glyphosate [5-7].

Alpha-tocopherol is the only form of the fat-soluble vitamin E that is used by the human body. As an antioxidant, its primary function is to scavenge loose electrons, or "free radicals," which can harm cells [8-10]. Additionally, it improves immunological performance and stops blood clots from developing in heart arteries. When scientists realized that free radical damage was involved in the early stages of artery-clogging atherosclerosis and might also be a factor in cancer, vision loss, and several other chronic disorders [11], antioxidant vitamins, particularly vitamin E, came to the public's notice. In some circumstances, vitamin E can both stop the generation of free radicals and shield cells from their damage. The potential of utilizing high doses of vitamin E to prevent chronic diseases, meanwhile, has been somewhat dampened by inconsistent study findings.

Retinol, also known as retinoic acid, is a vitamin crucial for immunity, cell division, growth, and vision. Antioxidant properties are also present in vitamin A. When your body breaks down food or is exposed to radiation and tobacco smoke, free radicals are created. Antioxidants are compounds that may shield your cells from these impacts. Heart disease, cancer, and other illnesses could be caused by free radicals. Many foods, including spinach, dairy products, and liver, contain vitamin A. Beta-carotene-rich foods including green leafy vegetables, carrots, and melons are additional sources [12]. Beta-carotene is converted into vitamin A by your body.

Vitamins C and E are both antioxidants, which means they can help protect cells in the body from damage caused by substances called free radicals. Free radicals are unstable molecules that can cause damage to cells, leading to inflammation and a range of health problems. Antioxidants like vitamins C and E can help to neutralize free radicals and protect cells from their damaging effects [13-15].

Vitamin C is a water-soluble vitamin that is important for maintaining healthy skin, blood

vessels, and bones. It is also an antioxidant that can help protect cells from damage caused by free radicals. Vitamin C is found in a variety of foods, including citrus fruits, strawberries, and leafy green vegetables. Vitamin E is a fat-soluble vitamin that plays a role in the maintenance of healthy skin and eyes. It is also an antioxidant that can help protect cells from damage caused by free radicals. Vitamin E is found in a variety of foods, including nuts, seeds, and vegetable oils.

It is important to get enough of these vitamins as part of a balanced diet, but it is also possible to get too much of them. Taking high doses of vitamin C or E supplements can be harmful, so it is important to talk to a healthcare provider before starting any new supplement regimen.

By neutralizing free radicals, Antioxidants, which are included in some foods, may help to reduce some of the harm that they may do. Antioxidants, vitamins A, C, and E, as well as the minerals copper, zinc, and selenium, are some of these. Antioxidant-rich diets may lower the risk of several illnesses (including heart disease and certain cancers) [16-19]. Antioxidants remove free radicals from the body's cells and stop or lessen oxidation-related damage. Antioxidants' protective effects are still being researched globally. For instance, men who consume a lot of the antioxidant lycopene, which is present in red fruits and vegetables including tomatoes, apricots, pink grapefruit, and watermelon, may have a lower prostate cancer risk than other men.

Good sources of vitamin A are liver, sweet potatoes, carrots, milk, and egg yolks. Good sources of vitamin E are vegetable oils (like wheatgerm oil), avocados, nuts, seeds and whole grains. According to research, some vitamin supplements [20, 21] may make us more likely to get cancer. Vitamin A (beta-carotene), for instance, has been linked to a decreased risk of some cancers but an increase in others, such as lung cancer [22] in smokers (if vitamin A is purified from foodstuffs).

When taken as a supplement, vitamin E did not provide the same advantages, according to research looking at its effects [23]. Vitamin E is the primary lipid-soluble element of the cell's antioxidant defense mechanism, vitamin E can only be found in food. Because of its antioxidant action, it plays a variety of significant roles in the body. Vitamin E has been demonstrated to be beneficial against these since oxidation has been connected to a wide range of potential ailments and diseases, including cancer, ageing, arthritis, and cataracts. Vitamin E can also assist in inhibiting the formation of prostaglandins like thromboxane, which promote platelet clumping, as well as platelet hyperaggregation, which can result in atherosclerosis.

The current overview of the literature examines vitamin E's roles and functions in human health, various disorders, and the effects of vitamin E deficiency. The word "vitamin E" refers to a collection of fat-soluble chemicals with specific antioxidant properties that Evans and Bishop initially identified in 1922 and which are vital to human health [24]. Vitamin E may be found in foods that contain fat [25], and since it can be stored in the fatty tissues of both humans and animals due to its fat-soluble nature, it is not necessary to take it daily. When fat is subjected to oxidation and during the spread of free radical reactions, vitamin E, a powerful chain-breaking antioxidant, prevents the synthesis of reactive oxygen species molecules. Even though its concentration ratio may only be one molecule for every 2,000 phospholipid molecules, it is mostly found in the membranes of cells and organelles where it may exert its best protective impact. It protects the cell membranes from free radical damage and serves as the initial line of defence against lipid peroxidation [26]. This study aims to analyze the protective effect of antioxidant vitamins (vitamin C and E) on the kidney from glyphosate-exposed renal damage in experimental rats.

Materials and Methods

The Animals used were the Adult male Albino Wistar rats which weighed between 150-180g and were used in our study the ethical number is BRULAC/SDCH/SIMATS/IAEC/8-2021/086.

Experimental Design

18 Healthy male albino rats were taken. They were then divided into 3 groups consisting of 6 animals each. Group I contained the Control rats (The Control rats were injected with corn oil intraperitoneally (ip) once daily as a vehicle). Group II: Glyphosate-treated rats (Glyphosate was dissolved in water at a dose of 100g/kg/body wt/day at 8 am) and given to the rats orally for 10 weeks Group III: Rats received simultaneous treatment of vitamin E (dissolved in olive oil at a dose of 50mg/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).

Assessment of Fasting Blood Glucose (FBG)

Blood glucose was estimated using on-call blood glucose test strips (ACON) after overnight fasting. Blood was collected by pricking the tip of the rat tail and results are expressed as mg/dl.

Gene Expression Analysis

mRNA Expression Analysis of IGF-1 and KIM-1 by RT-PCR Analysis

Total RNA was isolated as per the standard methods using total RNA Isolation Reagent (TRIR) and quantified at 260/280 nm. Then a total of 2µg of RNA 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 Oligod T, dNTP and reverse transcriptase. The components were transcriptase buffers 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 mins. The three-step PCR cycles consisted of denaturation at 95°C for 2 min, annealing at 60°C 30s and extension at 73°C for 30s. 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, B actin were added to the same PCR reaction vial and co-amplified. The following primers were used for gene expression analysis:

Rat-β-actin: FW- ‘5 -AGC CAT GTA CGT AGC CAT CC-3’; Rat β-actin: R W – 5’-CTC TCA GCT GTG GTG GTG AA-3’; Rat- IGF1: F 5/ - CTG GGT GTC CAA ATG TAA CT-3’; Rat IGF1R: 5’ -GTA TCT TTA TTG GAG GTG CG-3’. Rat KIM-1; FW: 5′-GAGTTCATTAGAGCCATTTCCACTCC-3′; Rat KIM-1 Rw: 5′-GAAAGCCTGTGTCCTGCTCTCTCT-3′.

Statistical Investigation

GraphPad Prism 8.0 was used for statistical calculations. Mean values with SEM were presented. One-way ANOVA was used in statistical studies, and then the Newman-Keuls assessment for numerous comparisons. Statistics were deemed significant at $p < 0.05$.

Results

Effect of Vitamin C &E on the Fasting Blood Glucose in Glyphosate-Induced Type-2 Diabetic Rats

Fasting blood glucose (FBG) is a crucial parameter in the diagnosis, management, and monitoring of type 2 diabetes mellitus. It serves as a fundamental tool for assessing glycemic control and has several important implications in the context of diabetes care. Regular monitoring of FBG levels helps healthcare providers and individuals evaluate the effectiveness of treatment regimens, including lifestyle modifications and medication therapy. In the present study, glyphosate treatment

showed a significant increase ($p<0.05$) in the levels of fasting blood glucose compared to control (Fig. 1). However, T2DM rats treated with vitamins improved glycemic control.

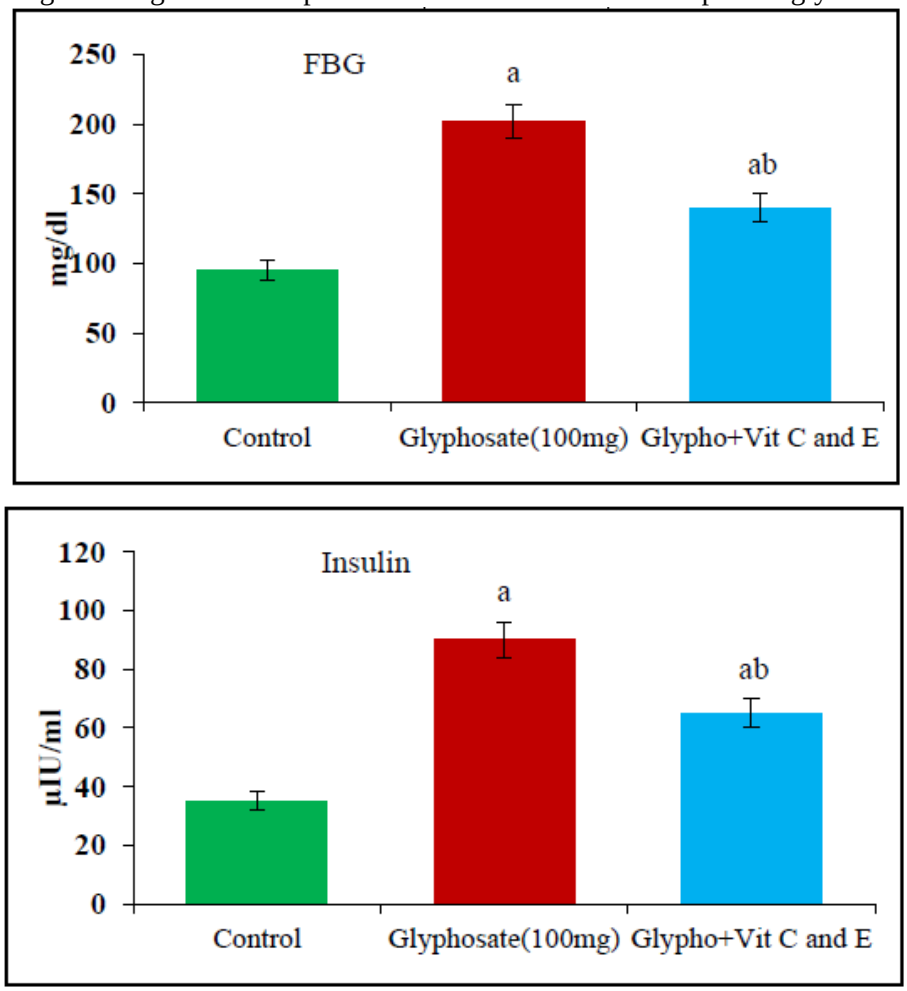

Figure 1 &2. The Effect of Vitamin C & E on FBG and Serum Insulin in Control and Glyphosate-Exposed Rats

Effect of Vitamin C &E on Serum Insulin Level in Glyphosate-Induced Type-2 Diabetic Rats

Serum insulin levels are an important parameter to assess in the context of diabetes and related metabolic conditions. Elevated serum insulin levels, when not accompanied by normal glucose levels, can be indicative of insulin resistance, a condition where the body's cells do not respond well to insulin. In this study, insulin concentration in glyphosate-exposed rats was significantly increased ($p<0.05$) compared to control rats. Fascinatingly, antioxidant vitamins (vitamins C & E) reduced hyperinsulinemia near to of the normal level suggesting that it control diabetic nephropathy (Fig.2).

Effect of Vitamin C & E on IGF-1 mRNA Expression in the Kidney of Glyphosate-Induced Type-2 Diabetic Rats

IGF-1 is a hormone that plays a crucial role in growth and development. It is also involved in tissue repair and regeneration, including in the kidneys. The expression of IGF-1 can be relevant to nephropathy, which refers to kidney disease or damage. Hence, in the present study, we measured gene level expression of IGF-1 mRNA in the control and treated rats. Glyphosate exposure caused a severe increase in the expression of IGF-1 compared to control whereas antioxidant vitamins reduced the drastic changes caused by glyphosate (Fig .3).

Effect of Vitamin C &E on KIM-1 mRNA Expression in the Kidney of Glyphosate-Induced Type-2 Diabetic Rats

Kidney Injury Molecule-1 (KIM-1) is a protein biomarker that plays a crucial role in the context of renal damage and various kidney diseases, including diabetic nephropathy. Here's how KIM-1 is related to renal damage and diabetic nephropathy. Hence, in the present study, we measured gene level expression of KIM-1 mRNA in the control and treated rats. Glyphosate exposure caused a severe increase in the expression of KIM-1 compared to control whereas antioxidant vitamins reduced the drastic changes caused by glyphosate (Fig. 4).

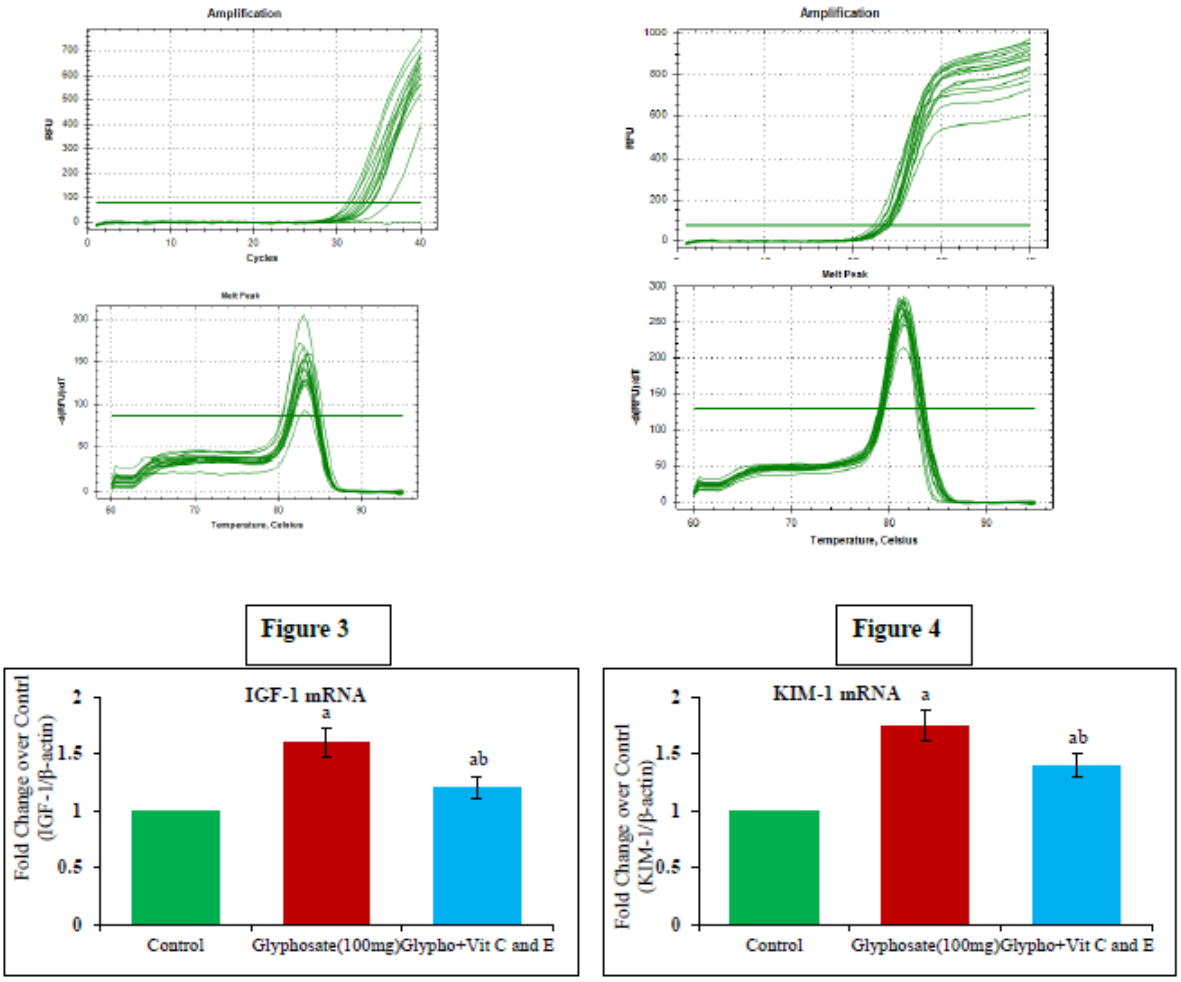

Figure 3

Figure 4

Figure 3 & 4. Effect of Vitamin C and E on mRNA Expression of IGF-1 and KIM-1 mRNA in Kidney Tissue of Glyphosate Exposed Rats

Discussion

Diabetic nephropathy is a serious complication of diabetes that affects the kidneys. It occurs when high blood sugar levels over an extended period damage the small blood vessels in the kidneys, leading to kidney dysfunction and, in severe cases, kidney failure. Fasting blood glucose levels are closely linked to the development and progression of diabetic nephropathy. elevated blood glucose levels, especially when poorly controlled over time, are a major risk factor for the development of diabetic nephropathy. Consistently high blood sugar levels can damage the delicate filtering units in the kidneys known as glomeruli. Some animal studies, particularly those conducted on rodents, have suggested a potential link between glyphosate exposure and kidney damage [27-29]. These studies have reported findings such as increased markers of kidney

injury and dysfunction in animals exposed to glyphosate. In this aspect, we measured fasting blood glucose in glyphosate (100mg) rats, and it showed a significant increase in the FBG, and vitamins showed reduced levels of hyperglycemia suggesting that vitamins C and E have a protective role over diabetic nephropathy. In addition, a previous study from our laboratory has also shown that glyphosate caused a significant increase in fasting blood glucose concentration in a dose-dependent manner to due the development of an increase in the reactive oxygen species [29] suggesting the diabetogenic potential of glyphosate. Antioxidant vitamins (Vit E & C) supplementation reduced hyperglycemia via the activation of insulin signalling in the gastrocnemius muscle of Diethyl hexyl phthalate-induced (DEHP) rats [30].

Further to check the molecular mechanisms, we measured IGF-1 mRNA expression in the control and glyphosate-treated rats. IGF-1 is involved in the growth and development of renal (kidney) cells. Abnormalities in IGF-1 expression during development can lead to structural and functional kidney issues that may predispose an individual to nephropathy later in life [31, 32]. In response to injury or damage, IGF-1 plays a role in the repair and regeneration of kidney tissue. Adequate IGF-1 expression is important for the kidneys to recover from various insults, including those associated with nephropathy. In our study, glyphosate exposure caused a severe increased in the expression of IGF-1 compared to control whereas antioxidant vitamins reduced the drastic changes caused by glyphosate and this study clearly demonstrate that vitamin C and E definitely regulated IGF-1 signaling in the renal tissues and control tissue damage caused by glyphosate exposure. In this regard, it has been shown that antioxidants like vitamin C can help combat oxidative stress and protect against cellular damage, potentially affecting IGF-1 signaling [33].

KIM-1 is expressed at very low levels in healthy kidneys but is significantly upregulated in response to renal injury, including diabetic nephropathy. It serves as a marker of tubular injury and is often used to detect and monitor kidney damage in diabetes.

A study published in "Diabetes Care" in 2009 investigated the utility of urinary KIM-1 levels as a biomarker for early diabetic nephropathy in patients with type 1 diabetes. The study found that elevated urinary KIM-1 levels were associated with the presence of kidney lesions, suggesting its potential as an early marker of renal damage in diabetes [34]. It has been studied as a predictive marker for the progression of diabetic nephropathy. High levels of KIM-1 in the urine have been associated with an increased risk of progression to advanced stages of kidney disease. In our study, we found that vitamins C and E lowered the expression of KIM-1 mRNA in rats exposed to glyphosate. These findings demonstrate that vitamins play a central role in KIM-1-mediated tissue damage in the renal tissue. Studies have shown that antioxidant vitamins, such as vitamin C (ascorbic acid) and vitamin E (tocopherol), have been studied for their potential protective effects on kidney health. These vitamins are known for their ability to combat oxidative stress and reduce cellular damage caused by free radicals, which may play a role in various kidney conditions [35, 36]. Taken together our findings clearly demonstrate that antioxidant vitamins control diabetic nephropathy via the modulation of IGF-1/KIM-mediated mechanisms thereby enhancing insulin signaling in the renal tissues. Further studies on the effect of antioxidant vitamins on insulin signaling molecules in the kidney are warranted to find out potential mechanisms of action. [37-39].

Conclusion

Our Study Concludes That

Glyphosate is a widely used herbicide, and its potential impact on various aspects of health, including its association with diabetic nephropathy and insulin signaling. In our study we are providing experimental evidence on protective effects of antioxidant vitamins in controlling diabetic nephropathy by controlling hyperglycemia and hyperinsulinemia in the kidney through IGF-1/KIM-1 signaling mechanisms. Hence, vitamin C and E can be considered as natural antioxidants to combat diabetic nephropathy.

Conflict of Interest

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

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Author Contribution

1. Keshav Rajesh - contributed to designing the study, execution of the project, statistical analysis, manuscript drafting.
2. Dr. Selvaraj - contributed to designing the study, execution of the project, statistical analysis, manuscript drafting.
3. Dr. V. Vishnupriya - contributed to study design, guiding the research work, manuscript correction.
4. Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.
5. Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

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