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Phytol Reduces Oxidative Stress and Cyclooxygenase-2 Expression in Kidney of Diabetic Wistar Rats

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Phytol Reduces Oxidative Stress and Cyclooxygenase-2 Expression in Kidney of Diabetic Wistar Rats

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Abstract
Diabetes mellitus is characterized by hyperglycemia, which induces oxidative stress and inflammation. The role of Phytol in oxidative stress and inflammation was investigated in diabetic rats. Fifteen Wistar rats were divided into five groups ($n = 5$). Groups 1, 2, and 3 served as normal control, diabetic untreated, and diabetic treated with 250 mg/kg Phytol, respectively. Rats were treated for 28 days with Phytol, and then blood samples were collected under sodium thiopental (30 mg/kg *i.p*) anesthesia for assay. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined using commercially available Randox kits. Cyclooxygenase-2 (COX-2) expressions in kidney samples were determined using immunostaining procedure. Statistical analysis was done using one-way analysis of variance and level of statistical significance taken at $p < 0.05$. Results showed a significant increase ($p < 0.05$) in CAT and GPx activities in diabetic treated with 250 mg/kg Phytol when compared with diabetic untreated with Phytol. SOD activity significantly decreased in diabetic untreated and diabetic treated with 250 mg/kg Phytol when compared with normal control. COX-2 was significantly expressed in diabetic untreated when compared with normal control and diabetic treated with 250 mg/kg Phytol. Oral administration of Phytol reduces oxidative stress damage and inflammation of kidney tissue caused by hyperglycemia in diabetes mellitus.

Keywords: Phytol; Hyperglycemia; Oxidative stress; Inflammation; Diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus is a chronic disease characterized by sustained hyperglycemia [1]. It occurs when the pancreas does not produce sufficient insulin essential for glucose uptake or the body tissues cannot efficiently utilize the insulin produced [2]. This disease affects more than 400 million people worldwide, and the prevalence is said to increase in the nearest future [3]. Ethnicity, family history of diabetes, age, overweight and obesity, unhealthy diet, physical inactivity, and smoking are potential risk factors for development of diabetes.

Diabetes mellitus is often associated with increased production of reactive oxygen species (ROS) and inflammation. ROS, which are by-products of oxygen metabolism, plays an important role in cell signaling and maintenance of homeostasis [4]. ROS production increases in prolonged hyperglycemia and could lead to oxidative stress in many tissues, if the level rises beyond the antioxidant defense capacity [5]. This oxidative stress damages several macromolecules leading to total destruction of cells [6].

The body tissues respond to hyperglycemia by stimulating immune cells and molecular mediators for defense against damage. This response may eliminate the cell injury, clear out necrotic cells and tissues damaged from the diabetes, and initiate tissue repair [7]. If this inflammatory response is not terminated, it may lead to chronic inflammation and cellular destruction [8].

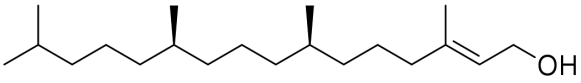
Diabetes mellitus could lead to serious damage of internal organs such as the heart, kidneys, eyes, blood vessels, and nerves if left untreated [9]. Although, diet and lifestyle changes are the main focus of treatments but, oral hypoglycemic agents are readily available and also useful for treatments. Phytol is an acyclic diterpene alcohol and a precursor for the synthesis of vitamin E. Once ingested, it is converted in the liver to phytanic acid before storage in adipocytes [10]. Phytol has been shown to reduce insulin resistance in diabetes mellitus and also has the potential of lowering circulating blood glucose level [11]. However, its role in oxidative stress damage and inflammation has not been fully elucidated. This study was, therefore, designed to investigate the effect of Phytol on oxidative stress and inflammatory markers of diabetic Wistar rats.

2. MATERIALS AND METHODS

2.1. Experimental Animals and Drug Purchase

Fifteen (15) Wistar rats were purchased from the animal house of College of Medicine, University of Ibadan, Nigeria. They had access to food and water ad libitum. The research was conducted following the guidelines from the National Institute of Health [12] and approved by animal research committee of the University of Ibadan, Nigeria. Phytol was purchased from Santa-Cruz Biotechnology, Inc., Germany with Catalogue number: sc-250719.

Figure 1: Structure of Phytol [11].



2.2. Experimental Design and Diabetic Induction

Rats were divided into three groups of five rats per group. Group 1 served as normal control, which received 0.3 ml of distilled water. Group 2 served as diabetic untreated and received 0.3 ml of distilled water, whereas Group 3 served as diabetic treated with 250 mg/kg Phytol. Diabetes was induced in Groups 2 and 3 using 120 mg/kg alloxan monohydrate as described by Kumar *et al*. [13]. Only rats with fasting blood glucose of 250 mg/kg were considered diabetic and selected for this study.

2.3. Blood Collection and Determination of Oxidative Stress Markers

Rats in Group 3 were treated with 250 mg/kg Phytol for 28 days. Blood samples were thereafter collected through retro-orbital sinus under mild anesthesia (30 mg/kg *i.p*, sodium thiopental) into plain tubes. The blood samples were centrifuged at 3000 rpm to obtain serum, and the serum was carefully aspirated into another plain tube using Pasteur pipette. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were quantified from each group using commercially available Randox kits, and their absorbance was measured using spectrophotometry procedure, as described by Christine *et al*. [14].

2.4. Kidney Immunohistochemical Staining

After 28 days posttreatment, kidneys were excised and stored in 10% formalin. Within 24 h, the kidney samples were processed and fixed into blocks. Then, the kidneys were cut, immunostained, and photomicrographed as described by Maity *et al*. [15] to show cyclooxygenase-2 (COX-2) expression.

2.5. Statistical Analysis

Results were analyzed using one-way analysis of variance followed by Neuman–Keuls post hoc test. Data were expressed as mean ± standard error of mean (SEM). The level of statistical significance was taken at $p < 0.05$.

3. RESULTS

Table 1: Effect of Phytol on superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities.

Experimental groups	SOD (U/L)	CAT (U/ml)	GPx (U/L)
Normal control	1.72 ± 0.27	0.67 ± 0.004	7.07 ± 0.67
Diabetic untreated	1.04 ± 0.44*	0.36 ± 0.014#	1.31 ± 0.54#
Diabetic + 250 mg/kg Phytol	1.08 ± 0.36*	0.71 ± 0.04	9.06 ± 0.48

Data were expressed as mean ± SEM; $p < 0.05$ * indicates value significantly different from normal control, while # indicates values significantly different from normal control and diabetic treated with 250 mg/kg Phytol.

Figure 2 (A–C): Immunostained sections showing COX-2 expression in the kidney: A (control), B (diabetic untreated), and C (diabetes treated with 250 mg/kg Phytol). A and C show no expression of COX-2, while B shows significant expression of COX-2 in the glomerulus and the surrounding basement membranes (blue arrows) X 400.
Cyclooxygenase-2 Expression

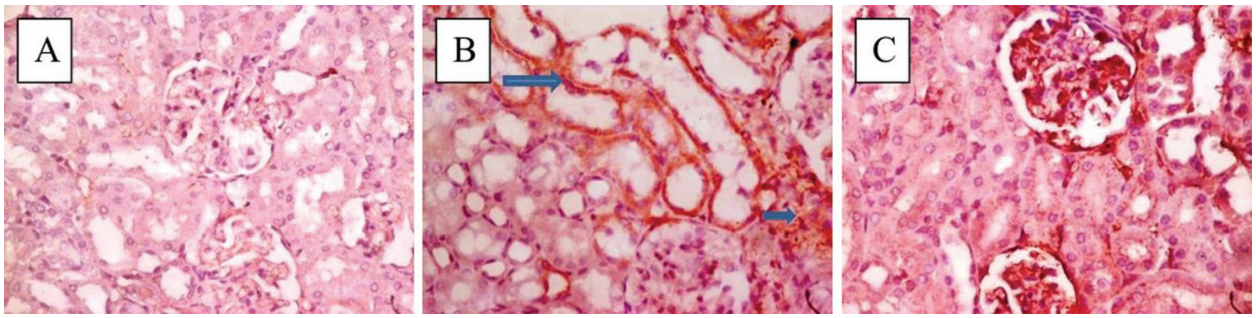


Table 1 shows changes in SOD, CAT, and GPx activities in normal control, diabetic untreated, and diabetic treated with Phytol.

There was no significant difference in SOD activity in diabetic treated with 250 mg/kg Phytol when compared with diabetic untreated with Phytol. However, there was a significant decrease ($p < 0.05$) in SOD activity in diabetic untreated and diabetic treated with 250 mg/kg Phytol when compared with normal control.

CAT activity significantly decreased ($p < 0.05$) in diabetic untreated when compared with normal control. However, CAT activity significantly increased ($p < 0.05$) in diabetic treated with 250 mg/kg Phytol when compared with diabetic untreated. The percentage increase in CAT activity was 49.29%, which was comparable to the normal control.

There was a significant decrease ($p < 0.05$) in GPx activity in diabetic untreated when compared with normal control. However, GPx activity significantly increased ($p < 0.05$) diabetic treated with 250 mg/kg Phytol when compared with diabetic untreated. The percentage increase in GPx was 85.54%.

4. DISCUSSION

This study investigated the role of Phytol in oxidative stress and inflammation in diabetic Wistar rats. Hyperglycemia is a common feature of diabetes mellitus, which induces mitochondria dysfunction and endoplasmic reticulum stress, leading to ROS production, inflammation, and cellular damage [16].

Enzymic antioxidants that are present in the body provide first line of defense by mopping up excess free radicals produced from oxidative stress. The major enzymic antioxidants are SOD, CAT, and GPx. CAT enzyme is a regulator of hydrogen peroxide metabolism [17] that could be toxic at high concentration especially in kidney tissues if allowed to accumulate as observed in diabetic untreated rats. From this study, Phytol may have potentiated CAT activity to breakdown hydrogen peroxide to water and oxygen that are less harmful to tissues. The GPx is a ubiquitous enzyme that acts as a cofactor in reducing glutathione (GSH) to glutathione disulfide (GS-SG) [18]. Treatment with Phytol may have potentiated the activity of GPx to catalyze the breakdown of hydrogen peroxide to water, thereby preventing oxidative damage [18].

Inflammation is an adaptive response of the body elicited as a principal component of tissue repair to deal with injuries and microbial infections [19]. It can be elevated in chronic conditions such as peripheral neuropathy, retinopathy, nephropathy, and fatty liver [20]. Diabetes mellitus is characterized with chronic inflammation, which causes the release of cytokines capable of activating different cyclooxygenase (COX) isoforms. COX is the rate-limiting step in the synthesis of prostanoids, a large family of arachidonic acid metabolites comprising prostaglandins, prostacyclin, and thromboxanes [21]. The elevated COX-2 expression in diabetes is consistent with our findings in diabetic untreated rats, and this suggests that COX-2 may have been upregulated and cause the formation of prostanoids that are involved in inflammatory reactions in tissues, especially the kidney and brain [21]. However, treatment with Phytol reduces COX-2 expression to a level comparable to the normal control. This effect of Phytol may be due to its ability to inhibit COX-2 expression in the kidney and prevent formation of prostanoid metabolites. Phytol may have acted through mechanisms similar to nonsteroidal anti-inflammatory drugs in reducing COX-2 expressions in the kidney [22].

In conclusion, Phytol caused decreased CAT and GPx activities. It also reduces COX-2 expressions in the kidney. Therefore, Phytol may be useful in reducing oxidative stress damage and inflammation in diabetes mellitus.

Author Contributions

Adeyomoye Olorunsola Israel 70%, Adewoye Elsie Olufunke 30%.

Conflict of Interest

The authors declare no conflict of interest.

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None.

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