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Effect of Piperine on Ir/IRS -1/AKT Signaling Molecules in High-Fat Diet and Sucrose-Fed Type 2 Diabetic Rat

Abisha[1], Gayathri R.[2*], V. Vishnu Priya[2], Selvaraj Jayaraman[2], Kavitha S.[2], Hema Shree K[3]
[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*
[3]*Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India*

Abstract

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Type 2 diabetics are insulin resistant. Insulin acts on AKT signaling molecules which then act on GLUT 4 which increases glucose uptake and glucose in the case of diabetic patients this pathway is absent to indicate that the pathway piperine is used. This study aims to evaluate the effect of piperine on Ir/ IRS-1/AKT signalling molecules in high-fat diet and sucrose-fed type 2 diabetic rats. Healthy adult male albino rats of Wister strain weighing 180 to 200 g were used for the evaluation of the effect of piperine. Fasting blood glucose and serum insulin levels are decreased with piperine administration. From this study, it is proved that piperine activates the insulin signaling pathway thus proving the potential anti-diabetic role.

Keywords: *Diabetic Rats, IR/IRS-1/AKT Signaling Molecules, Innovative Technique, Piperine.*

Introduction

Diabetes is a metabolic disease that causes the body to manufacture or utilize glucose inefficiently, which raises blood sugar levels. The main source of energy for cells in the human body is glucose, which is produced from food. Because the body does not produce enough insulin, glucose does not reach the cells in diabetes patients, causing it to accumulate in the bloodstream. The hormone insulin controls sugar levels in the blood and guards against hypo- or hyperglycemia [1]. There are two main forms of diabetes: type 1 & type 2. When a person has type 1 diabetes, their body is unable to manufacture insulin, making it difficult to control blood glucose levels. Conversely, type 2 diabetes is defined by the body's inability to use insulin as intended, which results in elevated blood sugar levels. It's crucial to remember that type 2 diabetes is more common in fat people.

Diabetes can lead to other health-related ailments, such as stroke, dental problems, and kidney-related issues [2]. Spices derived from medicinal plants have been used to cure deadly diseases. Traditional medicines have been used since ancient times. The plant, known as *Piperine nigrum*, belongs to the Piperaceae family. Black pepper is a commonly used spice worldwide, containing a percentage of 2–7.4% piperine [3]. Pepper contains a chemical compound known as piperine. It has a variety of pharmacological actions, including antioxidant, anti-obesity, and anti-diabetic qualities [4].

Piperine, a drug with various metabolic effects, has been subjected to numerous experiments, both *in-vivo* and *in-vitro* [5]. The

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****Corresponding Author: gayathri.sdc@saveetha.com***

purpose of these studies was to examine the drug's physiological effects.

In 1819, the Danish chemist Hans Christian Oersted extracted it from black pepper [6]. The properties of piperine, namely its anti-allergic, anti-inflammatory, and neuroprotective benefits, have been utilized for various purposes [7]. Previous research has examined the impact of piperine on multiple aspects of metabolic disorders [8]. However, none of these studies has specifically targeted the insulin signalling pathway. We aim to shed light on piperine's potential applications as a natural substance to control insulin signalling and enhance glucose metabolism in type 2 diabetics. To achieve this, we'll look into the consequences for type 2 diabetic mice given a diet heavy in fat and sugar.

Materials and Methods

Chemicals and Reagents

RNA isolation reagents, reverse-transcriptase enzymes (e.g., SuperScript™ III Reverse Transcriptase), and Go Taq Green master mix (e.g., Promega GoTaq® Green Master Mix) were acquired from different suppliers. Primers for IR, IRS-1, AKT, and β-actin and ELISA kits for glutathione peroxidase (e.g., Abcam Glutathione Peroxidase ELISA Kit) and LPO (e.g., Thermo Fisher Scientific Lipid Peroxidation Assay Kit) were procured.

Wistar Strain Male Albino Rats

Wistar strain male albino rats in good health weighing 180–200 g were used in this investigation [9]. The Lab Animal Center and Biomedical Research Unit housed these rats in polypropylene cages. The cages were kept at a consistent temperature and humidity level with a 12-hourly dark and light cycle [10]. The rats had a standard rat-pelleted diet and had unrestricted access to clean drinking water.

Male adult Wistar strain albino rats weighing 180 and 200 grams at birth were randomly assigned to five groups, each with six animals. The following were the groups: Group I was healthy rats; Group II involved high-fat diet-induced Type-2 diabetic rats; Group III involved oral piperine administration (40 mg/kg, b.wt/day for 30 days) for 30 days; Group IV involved oral metformin treatment (50 mg/kg, b.wt/day for 30 days); and Group V involved piperine administration (40 mg/kg, b.wt/day for 30 days) for Type-2 diabetic rats.

Biochemical Analysis

Blood glucose was calculated following an overnight fast. The rat tail tip was punctured to get blood, and then the results were reported in milligrams per deciliter [11]. Abbkine ELISA kits measured LPO and glutathione peroxidase levels [12]. Gene-specific oligonucleotide primers were used for standard PCR, with the first PCR activation being conducted for five minutes at 95ºC. β-actin was amplified with gene-specific oligonucleotide primers put into the same vial as the PCR reaction [13]. In our study, we performed the Real-Time q-RT-PCR method to analyze mRNA levels of IR, IRS-1 and Akt. Since the fluorescent dye SyBr is added in the PCR vial during the reaction itself, CT values of respective samples are being given as real-time data and that is calculated in terms of Fold change expression considering control as 1-fold.

Hence, in q-PCR analysis, the amplified products are not run on agarose gel using ethidium bromide staining

Statistics

The means ± SD of three distinct experiments conducted in triplicate were used to express the data, and a one-way ANOVA was used for statistical analysis [14]. A p-value of less than 0.05 showed a statistically significant outcome.

Result

The result is depicted in Figure 1 and Figure 2, which shows the levels of FBG, serum insulin, IR mRNA, IRS1 mRNA, and AKT among various study groups. Piperine treatment in diabetic rats decreased fasting blood glucose

and serum insulin levels. The findings suggest that piperine may positively impact insulin sensitivity and signalling pathways. However, metformin treatment in diabetes patients is still slightly more effective than piperine.

The outcomes showed significant variations between groups for the categories of diabetes with piperine ($p < 0.001$). The high F-statistics (563986.829 and 2202435.800, respectively) confirmed this. The minimal variance within each group further confirms the strength of these findings (Mean Squares: Diabetes = 0.023, Diabetes + Piperine = 0.003). (Table 1) The analysis of Diabetes with Metformin also identified notable differences between groups, although the specific values for the F-statistic and significance level were not provided. However, the extremely low within-group variance (Mean Square = 0.000) indicates considerable effects that necessitate further investigation.

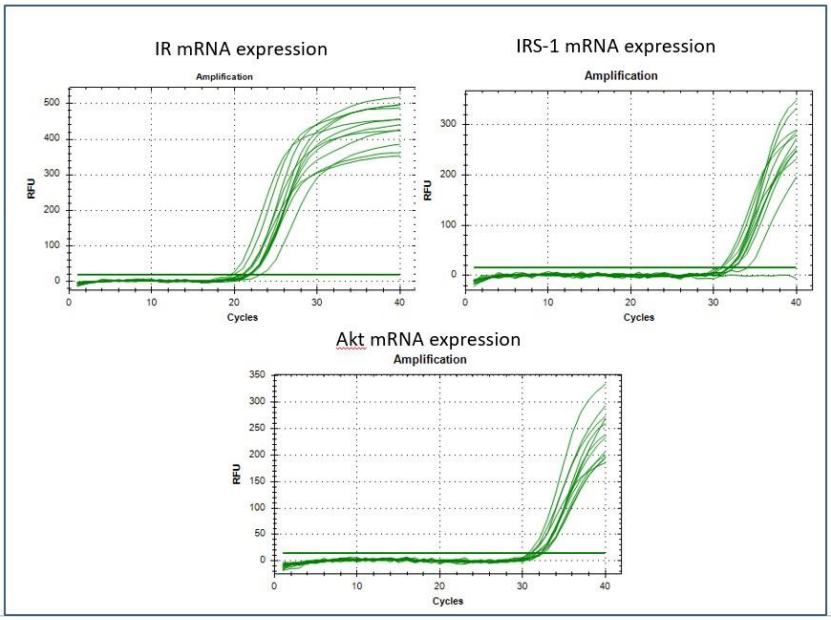

Figure 1. Amplification Plot Shows IR mRNA, IRS-1 mRNA, and AKT mRNA Expression. X-axis Represents No of Cycles and the Y-axis Denotes the Fluorescence of Amplification

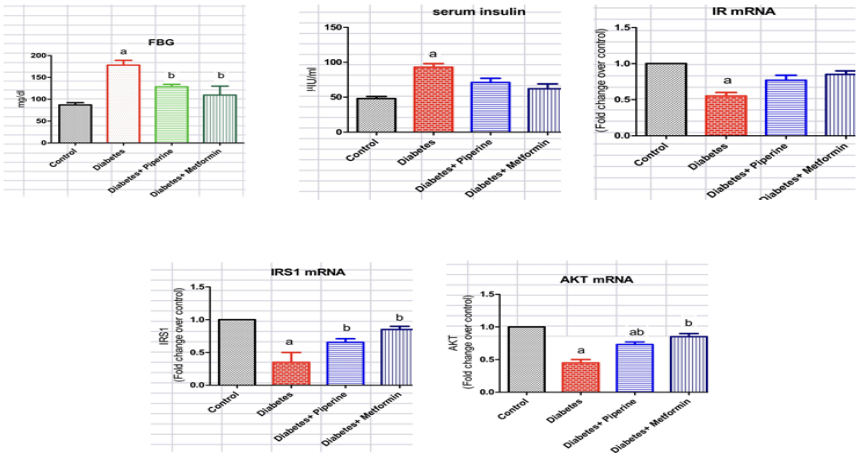

Figure 2. FBG, Serum Insulin, IR mRNA, IRS 1, mRNA, and AKT mRNA of Various Groups in mg/dl and Fold Change per Control

Table 1. ANOVA Showing Comparison of Diabetes, Diabetes + Piperine, and Diabetes + Metformin with Control Group

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Diabetes	Between Groups	26319.385	2	13159.693	563986.829	.000
	Within Groups	.047	2	.023		
	Total	26319.432	4			
Diabetes+piperine	Between Groups	14682.905	2	7341.453	2202435.800	.000
	Within Groups	.007	2	.003		
	Total	14682.912	4			
Diabetes+Metformin	Between Groups	11326.572	2	5663.286		
	Within Groups	.000	2	.000		
	Total	11326.572	4			

Discussion

Black pepper contains a natural substance called piperine, which has been shown to offer several health advantages. It has been shown to reduce obesity associated with a high-fat diet by modulating the gut microbiota. Piperine also protects cardiomyocytes (heart muscle cells) by reducing apoptosis caused by hypoxia/reoxygenation (H/R)-induced stress and activating the signalling pathway PI3K/AKT [15]. It also prevents myocardial ischemia-reperfusion damage by blocking the miR-383/RP105/AKT pathway, which inhibits pyroptosis [16]. Additionally, by blocking the signalling pathway involving PI3K and Akt and triggering apoptosis, piperine can stop the growth in human gastrointestinal cancer cells [17]. However, the effects of piperine on IR/IRS-1/AKT messenger proteins in diabetes type 2 diabetic rats given a high-fat and sugar-filled diet are not mainly discussed in the articles.

In this study, a group of diabetic rats was divided into five sub-groups. The first group comprised healthy control rats. The second group consisted of rats that were made diabetic by being fed a high-fat diet. Rats with diabetes who received a piperine treatment (a substance present in black pepper) made up the third group. Rats with type 2 diabetes were given metformin, a standard medicine, and made up the fourth group of rats. Finally, the fifth group served as a control group for the diabetic rats and received no treatment. Rats given diabetes have higher serum insulin levels and fasting blood glucose, a finding supported by earlier rat research.

Treatment with piperine has been demonstrated to have a considerable effect on serum insulin levels and fasting blood glucose levels. This suggests that Piperine has the potential to positively affect insulin sensitivity and signalling pathways, which could prove beneficial for individuals with diabetes. Additionally, the study suggests that piperine treatment may benefit diabetics by improving

blood sugar levels, sensitivity to insulin, and signalling pathways. In addition, previous research has shown that Piperine is linked with AKT signalling pathways, thus supporting our results and providing evidence of connective action on insulin sensitivity [18]. Further investigation is necessary to authenticate these discoveries and gain a comprehensive understanding of how Piperine impacts the regulation of diabetes.

The results were consistent with IRS1 mRNA and IRmNA, and upregulation was observed after piperine treatment [3, 19, 20]. However, diabetes patients treated with metformin have a slight overedge than piperine. However, considering the side effects of metformin, piperine would be the better alternative, or it could aid in reducing the metformin dosage [15, 21].

After comparing the impact of Piperine with metformin, we observed that both treatments led to improvements. On the other hand, piperine markedly affected serum insulin and fasting blood glucose levels, indicating that it may be a helpful treatment choice. It is critical to recognize the well-known side effects associated with metformin use despite the slight advantage it showed in our study. Piperine could offer a preferable alternative or potentially assist in reducing the required metformin dosage, thereby minimizing adverse effects [22].

Our findings have clinical implications that underscore the potential of Piperine as a promising intervention for individuals with diabetes. Translating these results from animal studies to human applications requires careful consideration[23,24,25]. Future research should focus on conducting clinical trials to validate the efficacy of Piperine in human subjects, exploring optimal dosage regimens, treatment durations, and potential combination therapies [26, 27, 28].

Even while our work offers insightful information, it is important to recognize its limitations, especially regarding the utilization of animal models. Further research is warranted to understand how Piperine impacts the regulation of diabetes in humans, recognizing the complexity of diabetes.

Conclusion

Our research indicates that piperine may help type 2 diabetic rats' glucose homeostasis by stimulating the insulin signalling system. For the treatment of diabetes, piperine shows a promising therapeutic approach.

Conflict of Interest

At this time, the authors affirm that there isn’t a conflict of interest in this research.

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