Effect of Piperine on Adipokine Signaling Molecules in High Fat Diet Fed Type 2 Diabetic Experimental Rats

Varsha Mugundhan[1], V. Vishnu Priya[2*], Selvaraj Jayaraman[3], R. Gayathri[4], Kavitha.S[5]

[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 Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[4]Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[5]Department of Biochemistry, Saveetha Dental college and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Abstract: Introduction: Diabetes mellitus is a disease of inadequate control of blood glucose level. Diabetes mellitus is one of the most common disease and about 10% of the whole of united states population is diagnosed with it. They are mainly classified into 3 types , type 1, type 2 and gestational diabetes out of which , type 2 diabetes is proved to be the most commonly occurring one. Treatment of type 2 diabetes is commercially done through metformin which works by lowering glucose production in liver and increases body sensitivity towards insulin. Piperine is an alkaloid used in black pepper , The distinctive biting quality of black pepper is due to piperine. Piperine offers a wide range of pharmacological actions and health advantages, especially when used to treat chronic conditions. Some of these advantages include the treatment of hepatic steatosis and the lowering of insulin resistance. Albino male wistar rats have used in our study , it has been the first and the most popular choice for leading pharmaceutical , chemical and contact research due to its smaller size and high rate of survival

Aim and Objective:

To analyze the effect of piperine on adipokine signaling molecules in high fat diet fed type 2 experimental rats

Materials and Methods:

A healthy adult male albino rats of wistar strain weighing about 180-200g following the national guidelines and protocols has been selected to perform this study. They were given an intraperitoneal injection of streptozotocin after feeding them with high fat and later were divided into groups and treated with the prepared extract and later were subjected to undergo tests including RT-PCR and ELISA.

Result:

Graphs were prepared from the results obtained with the adiponectin and leptin genes and were compared with the different groups of rats hence classified.

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Conclusion:
Piperine holding the potent antioxidant property controls the high fat diet induced diabetes by regulating the leptin and adiponectin gene expression.

Keywords:
Novel method, Adipokine, albino male wistar rats , leptin ,adiponectin , type 2 diabetes , piperine, innovative technique.

1. INTRODUCTION

An absolute or relative shortfall in insulin synthesis or action results in hyperglycemia,The majority of the food you consume is converted by your body into sugar (glucose), which is then released into your circulation. Your pancreas releases insulin when your blood sugar levels rise. In order for blood sugar to enter your body's cells and be used as energy, insulin functions like a key. When you have diabetes, your body either produces insufficient insulin or uses it improperly. Too much blood sugar remains in your circulation when there is insufficient insulin or when cells cease reacting to insulin. This is the hallmark of the varied group of illnesses known as diabetes mellitus.

Diabetes mellitus is majorly divided into three types which includes , type 1 , type 2 and gestational diabetes type 2 being the most common one and about over 90% of the population with diabetes is subjected to have type 2. Diabetes mellitus type 2 is a complex illness caused by both hereditary and environmental causes. Only a small percentage of type 2 diabetes cases are caused by a single gene abnormality, such as juvenile diabetes. People with type 2 diabetes are more than likely to be subjected to cardiovascular disease which may further cause conditions like heart attack or stroke. Short term complications of type 2 diabetes includes hypoglycemia, hyperosmolar hyperglycemic nonketotic syndrome. Long term complications includes diabetic retinopathy, diabetic neuropathy and macrovascular problems. (1)

People with type 2 diabetes are more than likely to develop various medical issues including damage to their eyes and nerves, as well as heart attacks and strokes (1,2). The main goal of taking medication is to prevent this. The most common drug used to treat type 2 diabetes is metformin. Metformin has been around for a while and helps in lowering the body's production of glucose. It is one of the best tolerated diabetic medications and the one with the most research behind it. For this reason, medical professionals often advise taking metformin first. If metformin isn't taken with a meal, the potential adverse effects include diarrhea and feeling ill (3)
However, recent observational studies have shown that long-term metformin medication raises the risk of vitamin B12 and folate deficiencies and may hasten the development of diabetic peripheral neuropathy.(4). Various herbal remedies have been demonstrated to influence blood sugar levels through a variety of pathways, although they are typically constrained by toxicity or a relative lack of efficacy when compared to conventional medicines. Another issue is the lack of consistency in the components and methods of preparation.(5).

A member of the Piperaceae family, Piper nigrum is one of the most often used spices worldwide.In multiple in vitro and in vivo experimental studies, piperine has a wide range of pharmacological actions, including antiproliferative, anticancer, antiangiogenesis, antioxidant, antidiabetic, anti-obesity, cardioprotective,

antibacterial, antiaging, and immunomodulatory properties.(6). Piper nigrum is a crucial species in biology. Different tests on the biological function of this species have shown that piperine and its secondary metabolites have anti-apoptotic, anti-bacterial, anti-colon toxin, antidepressant, anti-fungal, anti-diarrheal, anti-inflammatory, anti-mutagenic, and anti-metastatic activity. Antithyroid, Antitumor, Antispermatogenic, Antioxidant, Antipyretic, potentiator of cipro, diseases of the stomach, hepatoprotective, and cold extremities(6,7).

Leptin (ob), resistin (rstn), and fasting-induced adipose factor (fiaf) are examples of adipokine genes that have been linked to chronic low-grade inflammation and have been involved in the regulation of body weight and energy metabolism.(6–8) Because it can agglutinate both human and human erythrocytes, lectin was formerly known as hemagglutinin or agglutinin. They are a diverse collection of nonimmune proteins or glycoproteins.(9). Our team has extensive knowledge and research experience that has translate into high quality publications (10–19))(20–29)).

Invivo mode of research has been equipped in our study. It's a growing field for research and has been opted as its eco friendly and cost effective. In this research wistar rats were induced with type 2 diabetes and were tested for the same with piperine formulated drug.

2. MATERIALS AND METHODS

Chemicals:
Total RNA isolation reagent (TRIR) was acquired from Invitrogen in the United States, along with other supplies from Sigma Chemical Company in St. Louis, MO, Eurofins Genomics India Pvt Ltd in Bangalore, India, New England Biolabs (NEB), Promega, and Invitrogen. Purchases of the GoTaq Green master mix and the reverse-transcriptase enzyme (MMuLv) were made from Promega and New England Biolabs, respectively, in the United States. Primer sets for IL-6, TNF-alpha, and -actin were bought from Bangalore, India's Eurofins Genomics India Pvt Ltd. Abbkine provided ELISA kits for leptin and IL-6 (Bldg C17, Optics Valley International Biomedicine Park, Wuhan, China. 430223).

Animals:
In the current study, healthy adult male albino Wistar strain rats weighing 180 to 200 g (100 days old) were used. They were maintained in accordance with the National Guidelines and Protocols approved by the Institutional Animal Ethical Committee (IAEC No: BRULAC/SDCH/SIMATS/IAEC/07-2019/028 dated 13.07.2019). Animals were housed in polypropylene cages at the Biomedical Research Unit and Lab Animal Center (BRULAC), Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Chennai - 600 077, under specific humidity (65%5%) and temperature (21 °C2 °C) conditions with a constant 12 h light and 12 h dark schedule. They were given clean drinking water at will along with a regular rat pelted meal from Lipton India, Mumbai, India.

Experimental Design:
Male albino adult rats of Wistar strain (150–180 days old) weighing 180–200g were divided into five groups at random. Each group had six animals.

Group I-control rats;
Group II- High fat diet induced Type-2 diabetic rats;
Group III-Type-2 diabetic rats administered orally with piperine (40 mg/kg, b.wt/day for 30 days), for 30 days;

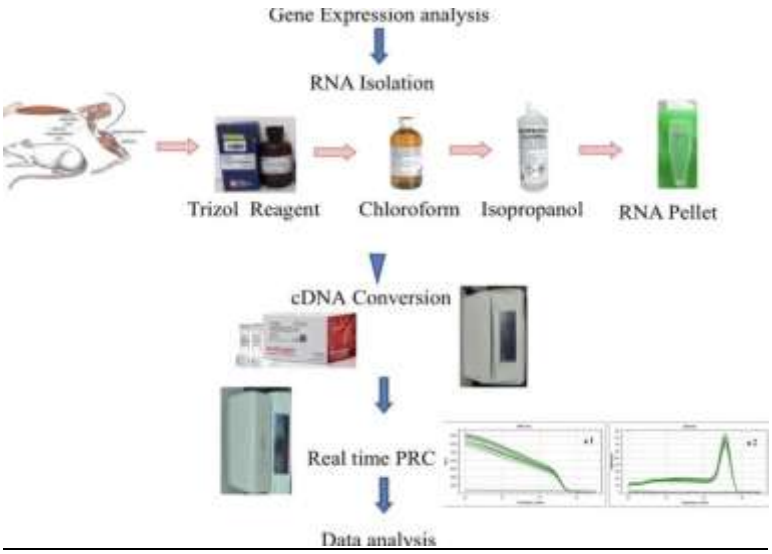
Group IV-Type-2 Diabetic rats treated orally with metformin (50 mg/kg, b.wt/day for 30 days. Group V- Control with piperine (40 mg/kg, b.wt/day for 30 days).

Experimental rats' fasting blood glucose (FBG) levels were examined. Experimental rats were given 40 mg of sodium thiopentone per kg of body weight as anesthetic at the conclusion of the investigation. Blood was drawn using a heart puncture, the sera were separated, and the blood was stored at -80°C. 20 ml of isotonic sodium chloride solution was perfused into the left ventricle to remove the blood from various organs. Adipose tissues were promptly removed and used for the subsequent investigation.

Fasting Blood Glucose:
After an overnight fast, blood glucose levels were measured using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA). Results were obtained by pricking the rat tail tip, and they are presented as mg/dl.

Homar and Quicki:
According to the methodology of Matthews et al. (1985), HOMA-IR was calculated using the formula (fasting blood glucose X fasting serum insulin/405) and QUICKI was computed using the formula 1/(log fasting serum insulin + log fasting blood glucose), according to the methodology of Katz et al (2000).

Elisa:
According to the manufacturer's instructions, Abbkine ELISA kits were used to analyse leptin and IL-6.



3. RESULTS AND DISCUSSION:

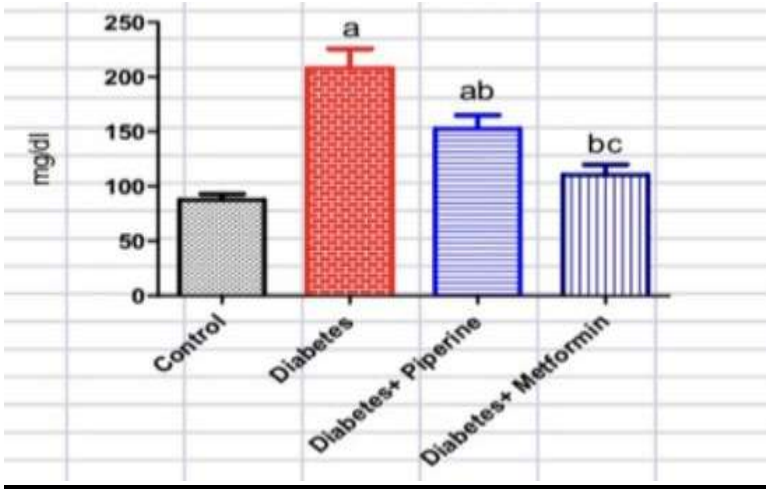
mRNA expression analysis of IL-16 , TNF - alpha and beta actin genes by RT-PCR
Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis was performed using 2 g of total RNA. A two-step RT-PCR kit was used to perform the RT-PCR. Using OligodT, dNTPs, and reverse transcriptase, complementary DNA (cDNA) will be produced from an mRNA template in the initial phase. The elements were mixed with a DNA primer for an hour at 37 °C in a reverse transcriptase buffer. Using gene-specific oligonucleotide primers and standard PCR after cDNA conversion, the first step was activated at 95 °C for 5 min. Denaturation at 95 oC for 2 min, annealing at 60 oC for 30 s, and extension at 73 oC for 30 s made

up the three-step PCR cycles. An additional 30 cycles of PCR amplification were performed to guarantee that the products are fully expanded. Our team has extensive knowledge and research experience that has translate into high quality publications (30), (31), (32), (33), (34), (35,36), (37), (38), (39), (40).

Data were presented as the means SD of three separate studies that were each carried out in triplicate. One-way ANOVA was used for the statistical analysis, and a result was regarded statistically significant if p 0.05 or above.

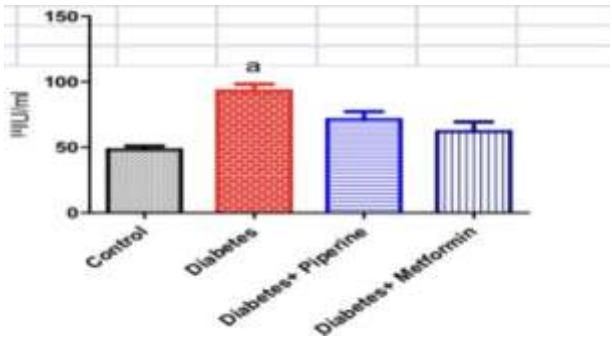
Gene name	Primer sequence	Reference
TNF-α	Sense primer: 5´ -GTCGTAGCAAACCACCAAGC-3´ Anti-sense primer: 5´ -CTCCTGGTATGAA ATGGCAAA-3´	Ziamajidi 2017
IL-6	Sense primer: 5´ -GTGAGAAGTATGAGAAGTGTGA-3´ Anti-sense primer: 5´ -GCAGGATGAGAATGATCTTTG-3´	Lu 2007
Rat β-actin	Sense primer: 5′ - GAC GTT GAC ATC CGT AAA GAC C-3′ Anti-sense primer: 5′ - TGC TAG GAG CCA GGG CAG TA-3′	Prasad 2022

Figure 1:
Total Cholesterol:



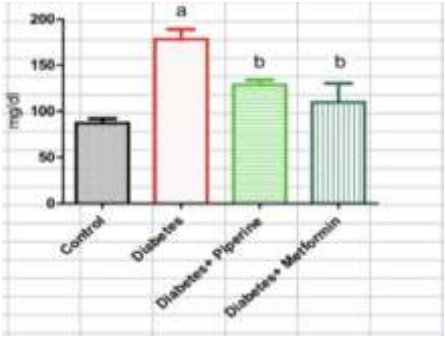
A measurement of your blood's overall cholesterol level. Both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol are included in it. The major cause of cholesterol accumulation and artery obstruction is LDL (bad) cholesterol. Total cholesterol of the four groups are compared with the control / commercially available metformin and it shows a much better result than the metformin (Figure 1).

Figure 2:
SERUM INSULIN:



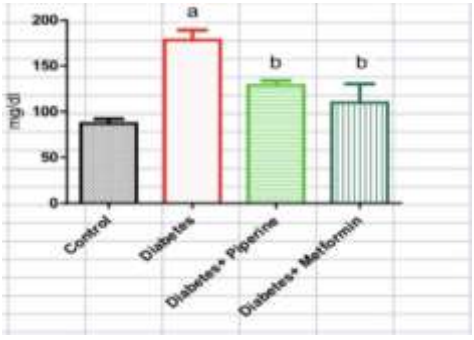
The pancreas beta cells create the hormone insulin. In addition to controlling glucose absorption and utilization, it also controls protein synthesis and triglyceride accumulation.
The high fat diet fed rat shows an increase in serum insulin while in piperine treated one the blood insulin level was restored back to normal (Figure 2).

Figure 3:
FBG:



Fasting blood glucose (FBG), random blood sugar, glucose challenge test, oral glucose tolerance test (OGTT). The expected values for normal fasting blood glucose concentration are between 70 mg/dL (3.9 mmol/L) and 100 mg/dL (5.6 mmol/L). The piperine-treated one in the fasting blood glucose was observed to reach the normal concentration while the metformin-treated one was still not observed to be as effective (Figure 3).

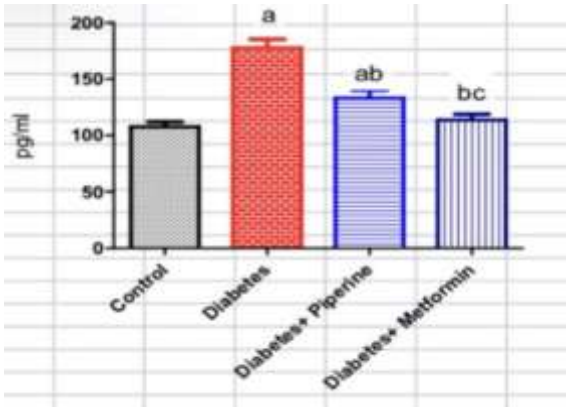
Figure 4:
ADIPONECTIN:



Adipose (fat) tissue secretes a hormone called adiponectin that reduces inflammation and improves insulin sensitivity. Obesity, Type 2 diabetes, and

atherosclerosis are just a few of the illnesses that have been linked to low levels of adiponectin (Figure 4).

Fig 5:
LEPTIN:



The central nervous system is instructed to alter food intake and energy expenditure in accordance with the level of circulating leptin, which acts as a marker for energy reserves. Leptin acts on the brain to control hunger, with immediate effects. The leptin and adiponectin were altered in diabetic conditions to show the effect of a high fat diet in piperine treatment; these molecules were altered to show the anti-diabetic activity of piperine (Figure 5).

4. CONCLUSION

Piperine holding potent antioxidant property controls high fat diet induced diabetes by regulating the leptin and adiponectin gene expression. Our study has proved to be effective in terms of analyzing the anti diabetic property of piperine in a non toxic herbal way. Therefore the antidiabetic property of piperine can find its application in various medicinal practices.

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