

## Piperine Attenuates Insulin Resistance in Adipocytes by Modulating the Expression of Proinflammatory Signaling Molecules in Experimental Diabetic Wistar Rats

Anuska Balaji<sup>1</sup>, Gayathri.R<sup>1\*</sup>, V. Vishnu priya<sup>1</sup>, J. Selvaraj<sup>1</sup>, Kavitha.S<sup>1</sup>, Hema Shree K<sup>2</sup>  
<sup>1</sup>Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, India  
<sup>2</sup>Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600 077, India

### Abstract

*The development of insulin resistance and diabetes in obese people is caused by metabolic inflammation. In adipose tissues in the abdomen, macrophages infiltrate and cause insulin resistance. As a result of the macrophages adopting an M1 polarization, pro-inflammatory cytokines are released. Piperine, known for its potent anti-inflammatory properties, is being studied as a potential treatment for inflammatory diseases. Our findings suggest that piperine, an immunomodulator, can effectively treat obesity-related diabetes.*

**Keywords:** Insulin Resistance, Piperine, Pro-inflammatory, Serum Insulin, Wistar Rats.

### Introduction

The primary location in the body where excess energy is stored is white adipose tissue (WAT). Apart from providing insulation and protection, WAT also secretes a range of adipokines crucial for regulating the body's metabolism. These adipokines maintain the overall balance of the body's metabolic processes [1, 2].

Worldwide, diabetes type 2 mellitus (T2DM) and obesity are growing major health concerns. According to the 9th version of the Global Diabetes Atlas published by the International Diabetes Federation, 463 million people worldwide—or 9.3% of the world's population—had diabetes in 2019. In 2045 this percentage will increase to 700 million people and 10.9%. Obesity is a common cause of metabolic illnesses, including steatohepatitis, Type 2 diabetes, fatty liver disease, and various cardiovascular conditions [3]. Type 2 Diabetes mellitus (T2DM) and Obesity share a common trait of chronic, low-grade inflammation. It is

mainly ascribed to the important role that immune cells called macrophages play in these circumstances. In both rodents and humans, the adipose tissue of obese individuals shows a considerable increase in macrophage infiltration [3].

Fat-cell hypertrophy and fat mass tend to increase due to heightened energy consumption, leading to the pathophysiology commonly observed in cases of obesity. One of the most important health concerns of our day is the rising prevalence of obesity [4–6]. The risk of metabolic disorders, which include type 2 diabetes, dyslipidemia, and coronary heart disease, increases with excess fat [7, 8]. The brain regulates food intake and energy expenditure to control fat storage (energy homeostasis) [9]. The body provides sensory information through vagal efferents from the gut and circulating hormones. This information combines environmental cues and the organism's emotional state [10].

Black pepper (*Piper nigrum* L.) is a popular spice in human diets and belongs to the Piperaceae family. It is native to South India, particularly to the tropical islands in Sri Lanka and the Malabar coast in India [12–15]. Indian Ayurveda doctors have long used black pepper as a spice with possible health benefits [11]. Piperine has many biological potentials, including modulating medication bioavailability, anticancer effects, antioxidant properties, and anti-inflammatory properties [16–18]. However, due to its lipophilic nature, piperine is difficult to dissolve and maintain bioavailability in the human body and thus can be administered with suitable carriers which can enhance its activity [19].

The primary alkaloid found in black pepper (*Piper nigrum*), long pepper (*Piper longum*), and other Piper genus species is piperine [8,20, 21]. Piperine showcases a range of biological characteristics, including modulation of the immune system, prevention of oxidation, regulation of lipid metabolism, and reduction of inflammation [11–14]. Its capacity to control immunological inflammation in a variety of disease models, such as ulcerative colitis, clone diseases, and arthritis, is of special interest to us. Its pharmacological activities in this regard are of great significance [7, 13–17]. Previous studies have confirmed that piperine has a beneficial effect on reducing dyslipidemia and body fat accumulation in mice fed a high-fat diet. Nevertheless, whether piperine helps prevent metabolic diseases by reducing metabolic inflammation in obese people is still unknown [11, 18]. This work aimed to examine the effects of piperine on pro-inflammatory chemicals in Wistar rats with diabetes. Specifically, it aimed to determine if piperine could improve adipocyte insulin resistance [17, 19].

## Materials and Methods

### Laboratory Supplies and Reagents

Chemicals and reagents were purchased from several suppliers, including Promega,

Invitrogen, Sigma Chemical Company, Eurofins Genomics India Pvt Ltd, and New England Biolabs (NEB). Invitrogen's Total RNA isolation reagent (TRIR), NEB's reverse-transcriptase enzyme (MMuLv) and Promega's Go Taq Green master mix were essential components used in the experiment. The TNF-alpha, the IKKB, IL-1,6 & beta-actin protein primers were supplied by Eurofins Genomic India Pvt Ltd. TNF-alpha and LPO ELISA kits were purchased through Abbkine of China's Wuhan city.

### Animal Model and Ethical Approval

The study involved the utilization of healthy mature albino Wistar rats, which were 100 days old and weighed between 180-200 grams. The Institutional Animal Ethical Committee approved the ethical guidelines for the study (IAECNo:BRULAC/SDCH/SIMATS/IAEC/07-2019/028 dated 13.07.2019). The rats were housed in the BRULAC and kept under specific conditions as per the guidelines.

### Induction of Type 2 Diabetes

The experiment involved feeding rats a high-fat diet for a month to induce type-2 diabetes. Following that, one infusion of streptozotocin (about 35 mg/kg body weight) was administered to the rats. After that, the rats were fed a diet consisting of 25% sucrose through drinking water, 30% coconut oil, 1% cholic acid, 66% regular rat feed, and 3% cholesterol for a 30-day duration. The streptozotocin was administered in a low dose to cause mild damage to the pancreatic beta cells, miming a chronic hypoinsulinemic insulin-resistant state.

### Study Design

Five groups of adult male albino rats were selected randomly based on the Wistar variety. The rats were between 150-180 days old and weighed 180 and 200 grams. Each group consisted of six animals. One group served as the control, while the second group was induced with type 2 diabetes through a high-fat diet. For 30 days, the third group—which had

type 2 diabetes—was given an oral dose of piperine (40 mg/kg b.wt/day). For 30 days, the fourth group received oral metformin at a dose of 50 mg/kg b.wt/day. The fifth group was given piperine as a control for 30 days at a dose of 40 mg/kg b.wt/day. The blood glucose levels of the rats were measured after they had fasted during the experiment. The laboratory rats were put to sleep after the study using 40 milligrams per kilogram of sodium thiopentone. A cardiac puncture was used to extract blood, and sera were separated and kept at -80°C. Twenty millilitres of isotonic sodium chloride solution were perfused via the left ventricle into various organs to eliminate blood. The fat tissues were quickly removed and utilized in the next studies.

### ELISA and RT-PCR Analysis

ELISA kits were employed to analyze TNF-alpha and LPO levels. The total RNA sample (2 µg) was used for RT-PCR, and a dual-step RT-PCR kit was used. For the amplification procedure, gene-specific primers for TNF-alpha, IL-1β, IL-6, IKKB, and β-actin were employed.

### Primers

Rat TNF-α, Rat IL-6, Rat IKKB, and Rat β-actin.

### Statistical Analysis

Software like Orange and SPSS were used to perform statistical analysis on the data. The aim was to identify any notable variations between the groups.

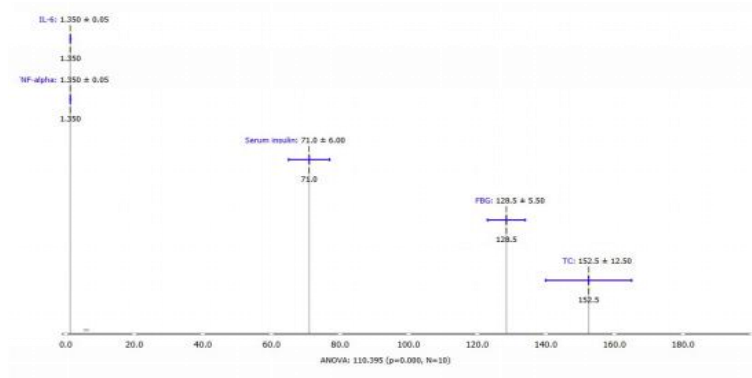
### Results

Table 1 data illustrates variations in critical parameters among different groups: Control, Diabetic, Diabetes + Piperine, and Diabetes + Metformin. Piperine and Metformin show potential in mitigating diabetic conditions, as evidenced by their positive impact on FBG, serum insulin, total cholesterol, and inflammatory markers. These findings suggest a therapeutic role for Piperine in managing diabetes-associated complications. It is also depicted in Figure 1(statistics) and Figure.2

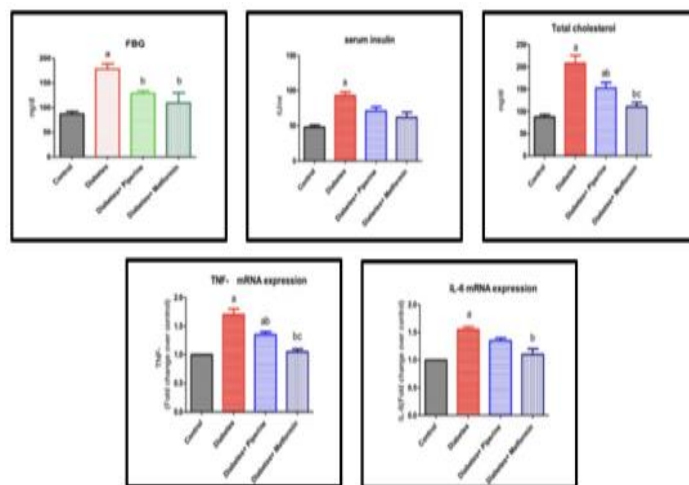
### Top of Form

**Table 1.** FBG, Serum Insulin, TC, TNF-alpha, IL-6 Levels Among Various Groups

	<b>Control</b>	<b>Diabetic</b>	<b>Diabetes + Piperine</b>	<b>Diabetes + Metformin</b>
<b>FBG</b>	82	167	134	89
	92	189	123	130
<b>Serum insulin</b>	45	98	77	55
	51	88	65	69
<b>TC</b>	82	190	140	101
	93	226	165	120
<b>TNF-alpha</b>	1	1.6	1.3	1
	1	1.6	1.4	1.1
<b>IL-6</b>	1	1.6	1.3	1
	1	1.5	1.4	1.2



**Figure 1.** Multiple ANOVA Showing Means of IL-6, TNF-alpha, Serum Insulin, FBG, and TC in Diabetes + Piperine Group



**Figure 2.** Distribution of Levels of FBG, Serum Insulin, Cholesterol, TNF-alpha, and IL-6 Among Various Groups

In MSG-obese insulin-resistant mice, the effects of piperine on established obesity were investigated by measuring body weight, Lee's index, mesenteric fat deposition, and insulin sensitivity glycolipid metabolic rate, following piperine therapy. According to the results, rats given diabetes had higher FBG, serum insulin, and Cholesterol levels. Animals given piperine showed a considerable reduction in serum insulin, FBG, and cholesterol levels.

## Discussion

The degree of metabolic inflammation and the severity of insulin resistance are tightly related in diabetic individuals [3, 18]. Directly inhibiting inflammatory reactions can potentially treat or reduce insulin resistance and T2DM. In this study, we primarily looked at how piperine affected metabolic inflammation

and insulin resistance brought on by obesity [20]. Mice that had become obese due to MSG were given piperine treatment. This treatment was effective in reducing their obesity and decreasing inflammation in their adipose tissue macrophages (ATMs). As a result, these mice were shielded from developing insulin resistance [21, 22].

When compared with mice on a high-fat diet (HFD), it has been proposed that a piperine-supplemented diet can result in a significant decrease in body and visceral fat, by 12% and 38%, respectively [23]. In obese HFD mice, piperine supplementation dramatically cures hepatic steatosis and insulin resistance, according to research by Choi et al [18]. According to our research, piperine may lower blood lipid profiles, abdominal adipose index,

mesenteric fat production, and body weight [24]. According to the findings of the ITT study, piperine may aid in lowering insulin resistance and improving insulin sensitivity in mice that are obese with MSG. It has been found that piperine helps obese mice with glucose intolerance and increases islet cell sensitivity to glucose stimulation. It is clear that glucose consumption fully returned to normal throughout the 10 weeks is indicative of this condition [24, 25].

Our study findings suggest that MSG-induced obese mice may benefit from piperine in terms of enhancing insulin sensitivity and reducing inflammation in adipose tissue [17]. The immune-modulating and anti-inflammatory properties of additional alkaloids will be further investigated as a result of this investigation [26]. The present study does have certain restrictions, though. For instance, piperine was utilized in a single dose [24, 25, 27]. As a result, we could not investigate how piperine affected T2DM at various doses. We only looked at piperine's suppressive effect on LPS-induced polarization of macrophages in vitro tests; we didn't investigate the signaling pathways that are involved. More research is needed [28, 29, 30] to clarify piperine's influence on macrophage polarization and its role in the pathological development of adipocytes and macrophages in rats.

## References

- [1] Matafome P, Seiça R (2017) Function and Dysfunction of Adipose Tissue. *Obesity and Brain Function* 3–31.
- [2] Gray SL, Vidal-Puig AJ (2007) Adipose Tissue Expandability in the Maintenance of Metabolic Homeostasis. *Nutr Rev* 65:S7–S12.
- [3] Roy PK, Islam J, Lahlhenmawia H (2023) Prospects of potential adipokines as therapeutic agents in obesity-linked atherogenic dyslipidemia and insulin resistance. *Egypt Heart J* 75:24.
- [4] Suwana J, Mahidon M, Mahāwitthayālai

## Conclusion

Our research indicates that administering piperine to obese mice that have developed insulin resistance and glucose intolerance may help to relieve these conditions. This treatment may also lead to some weight loss and correct abnormalities in glycolipid metabolism. The reason for piperine's effectiveness in treating obesity-related diabetes is likely due to its ability to strongly inhibit inflammation in both the body as a whole and in adipose tissues. Together, piperine's potential as a natural alkaloid offers enormous promise for creating and using anti-diabetic medications. It is necessary to conduct additional clinical studies to verify these effects.

## Funding

This work was made possible with the help of grants provided by Sri Amutha Dental Care in Thuraiyur and Trichy

## Acknowledgement

The authors would like to acknowledge the help and support rendered by the Department of Biochemistry and the Management of Saveetha Dental College and Hospitals for their constant assistance with the research.

## Conflict of Interest

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

Mahidon. Faculty of Science (2005) Effects of Piperine on Wistar Rats.

[5] BrahmaNaidu P, Nemani H, Meriga B, Mehar SK, Potana S, Ramgopalrao S (2014) Mitigating efficacy of piperine in the physiological derangements of high fat diet induced obesity in Sprague Dawley rats. *Chem Biol Interact* 221:42–51.

[6] Vm AE (2019) Epidemics and Pandemics in India throughout History: A Review Article. *Indian Journal of Public Health Research & Development*.

[7] Sivakumar N, Geetha RV, Priya V (2021) Gayathri R, Dhanraj Ganapathy. Targeted

- Phytotherapy For Reactive Oxygen Species Linked Oral Cancer. *Int J Dentistry Oral Sci* 8:1425–1429.
- [8] Nasim I, Rajesh Kumar S, Vishnupriya V, Jabin Z (2020) Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles. *Bioinformation* 16:831.
- [9] Tripathi AK, Ray AK, Mishra SK (2022) Molecular and pharmacological aspects of piperine as a potential molecule for disease prevention and management: evidence from clinical trials. *Beni Suef Univ J Basic Appl Sci* 11:16.
- [10] Sama V, Nadipelli M, Yenumula P, Bommineni MR, Mullangi R (2012) Effect of piperine on antihyperglycemic activity and pharmacokinetic profile of nateglinide. *Arzneimittelforschung* 62:384–388.
- [11] Pingale PL (2022) Drug Delivery Technology: Herbal Bioenhancers in Pharmaceuticals. Walter de Gruyter GmbH & Co KG.
- [12] Vishaka S, Sridevi G, Selvaraj J (2022) An in vitro analysis on the antioxidant and anti-diabetic properties of *Kaempferia galanga* rhizome using different solvent systems. *J Adv Pharm Technol Res* 13:S505.
- [13] Padmapriya A, Preetha S, Selvaraj J, Sridevi G (2022) Effect of *Carica papaya* seed extract on IL-6 and TNF- $\alpha$  in human lung cancer cell lines-an In vitro study. *Research Journal of Pharmacy and Technology* 15:5478–5482.
- [14] Pratheebha C, Devi RG, Priya AJ EFFECT OF PEPPERMINT AND ROSEMARY OIL ON CONSTIPATION. *Plant Cell Biotechnol. Mol. Biol.*
- [15] Knowledge about the effects of medicinal plants against COVID-19 among dental students-A questionnaire study.
- [16] Lakshmi T Medicinal value and oral health aspects of acacia catechu-an update. *Int. J. Dent. Oral Sci.*
- [17] Liu C, Yuan Y, Zhou J, Hu R, Ji L, Jiang G (2020) Piperine ameliorates insulin resistance via inhibiting metabolic inflammation in monosodium glutamate-treated obese mice. *BMC Endocr Disord* 20:152.
- [18] Wang X, Zhang Y, Zhang L, Wang W, Che H, Zhang Y (2022) Piperine attenuates hepatic steatosis and insulin resistance in high-fat diet-induced obesity in Sprague-Dawley rats. *Nutr Res* 108:9–21.
- [19] Woo H-M, Kang J-H, Kawada T, Yoo H, Sung M-K, Yu R (2007) Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. *Life Sci* 80:926–931.
- [20] Dinda B (2022) Natural Products in Obesity and Diabetes: Therapeutic Potential and Role in Prevention and Treatment. Springer Nature.
- [21] Koodathil J, Venkatachalam G, Bhaskaran K (2023) and antidiabetic activity of bitter honey in streptozotocin-nicotinamide-induced diabetic Wistar rats. *J Med Life* 16:91–100.
- [22] Abijeth B, Ezhilarasan D (2020) Syringic acid induces apoptosis in human oral squamous carcinoma cells through mitochondrial pathway. *J Oral Maxillofac Pathol* 24:40–45.
- [23] Heidari H, Bagherniya M, Majeed M, Sathyapalan T, Jamialahmadi T, Sahebkar A (2023) Curcumin-piperine co-supplementation and human health: A comprehensive review of preclinical and clinical studies. *Phytother Res.* <https://doi.org/10.1002/ptr.7737>.
- [24] Kumar Seetharaman P, Ramalingam P, Chandrika M, Rajan R, Chelliah J, Bo L (2023) Antidiabetic potential of Gymnemic acid mediated gold nanoparticles (Gym@AuNPs) on Streptozotocin-induced diabetic rats-An implication on in vivo approach. *Int J Pharm* 636:122843.
- [25] Muller CJF, de Beer D, Joubert E, Louw J, Gabuza K, Fey SJ (2011) Assessment of the Antidiabetic Potential of an Aqueous Extract of Honeybush (*Cyclopia Intermedia*) in Streptozotocin and Obese Insulin Resistant Wistar Rats.
- [26] Kishore SOG, Priya AJ, Narayanan L Controlling of oral pathogens using turmeric and tulsi herbal formulation mediated copper nanoparticles. *Plant Cell Biotechnol. Mol. Biol.*
- [27] Bhupatiraju L, Bethala K, Wen Goh K, et al (2023) Influence of extract on diabetes induced rat brain aging. *J Med Life* 16:307–316.
- [28] Karthik EVG, Priya V (2021) Gayathri. R, Dhanraj Ganapathy. Health Benefits Of *Annona Muricata*-A Review. *Int J Dentistry Oral Sci*

8:2965–2967

[29] Priya DV, (2020) Knowledge and awareness on HIV/AIDS among college students in A university hospital setting. *Int J Dent Oral Sci* 1182–1186

[30] Ganapathy D, (2021) Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. *Int J Dent Oral Sci* 2976–2980