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Piperine Modulates High Fat Diet - Induced Renal Damage by Regulating Kim-1 and Igf-1 Beta Signaling Molecules in Male Wistar Rats

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Abstract

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and other symptoms which ultimately cause various other complications like retinopathy , micro angioplasty and nephropathy . piperine shows antidiabetic activity by improving insulin level, signifying its usage in the management of hyperglycemia. IGF-1 is the insulin-like growth factor receptor . KIM-1 has proved to be an outstanding early indicator of kidney injury in the rats. Induction of type 2 diabetes to male wistar albino rats , they were divided into four groups , one group were treated with piperine , fasting blood glucose was analysed. Biochemical analysis and mRNA expression analysis of KIM-1 and IGF-1 by RT-PCR is done. Fasting blood glucose and the serum insulin was elevated in diabetes induced rats . piperine-treated animals exhibited a significant decrease in the level of FBG and serum insulin from the above study, it could show that piperine possesses antidiabetic activity.

Keywords: *Piperine, Innovative Technique, Wistar Male Rats, KIM-1, IGF -1, Nephropathy, High Fat Diet, Novel Method.*

1. Introduction

Diabetic mellitus is a chronic metabolic disorder. It is a fast-growing global problem with huge social health and economic consequences. It is a heterogeneous group of disorders characterized by hyperglycemia due to deficiency in insulin production[1]. Diabetic mellitus is divided into some divisions there are type 1 diabetics, type 2 diabetics, gestational diabetics. Type 2 diabetes mellitus is an expanding global health problem [2]. It is a leading cause of renal failure, ASCVD, non-traumatic lower limb amputation, blindness, and death worldwide. It is a serious chronic medical condition that necessitates the collaboration of a multidisciplinary team of healthcare professionals, dietitians, patient educators, patients, and their families[3].

Environmental factors such as unhealthy diet, obesity, physical inactivity and genetic factors contribute to the multiple Pathophysiological effects that are responsible for impaired glucose homeostasis in type 2 diabetes mellitus [4]. The only effective treatment for type one diabetes is insulin. Injected insulin acts just like naturally occurring insulin to lower the blood glucose. People with type 2 diabetics initially have insulin resistance - this is when the cells of the body do not respond to insulin in the same way as people without diabetes [5]. Oral antidiabetic agents work in various ways to reduce blood sugar levels in people with type 2 diabetes; some stimulate insulin secretion by the pancreas, others improve the responsiveness of cells to insulin or prevent glucose production by the liver. Others slow the absorption of glucose after meals [6].

Many people with type 2 diabetes eventually require insulin to manage their high blood sugar levels. some common antidiabetic agents are :

- alpha-glucosidase inhibitors (acarbose, miglitol)
- amylin analogs (pramlintide)
- dipeptidyl peptidase 4 inhibitors (alogliptin, linagliptin, saxagliptin, sitagliptin)
- incretin mimetics (albiglutide, dulaglutide, exenatide, liraglutide, lixisenatide)
- insulin
- meglitinides (nateglinide, repaglinide)
- non-sulfonylureas (metformin)
- SGLT-2 inhibitors (canagliflozin, dapagliflozin, empagliflozin)
- sulfonylureas (chlorpropamide, glimepiride, glipizide, glyburide, tolazamide, tolbutamide)
- and thiazolidinediones (rosiglitazone, pioglitazone)

The major classes of oral antidiabetic medications include biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, and α-glucosidase inhibitors. 3. There are currently several options for pharmacologic therapy to lower blood glucose levels, which have revolutionized long-term DM management.[7, 8].

New era antidiabetic drugs are characterized by cardiovascular safety. It has been postulated that the favorable effects of new antidiabetic agents are related both to better control of blood pressure (BP) levels and to activation of multiple anti-atherosclerotic properties.[9].To guarantee patient adherence to therapy and reduce major CV morbidity and mortality, it is important to thoroughly explain to patients the concerns with polypharmacy, diabetes management, hypertension, hyperlipidemia, and aspirin use.[8]

Every anti diabetic agents have side effects. Sulfonylureas is a type of anti-diabetic agent and it is associated with weight gain and hypoglycemia. Metformin which is proven to be an anti-diabetic drug and has major side effects in gastrointestinal tract, with nausea, cramps and diarrhea. A combination of herbal products or (isolated phytochemicals) are found to be beneficial in certain diseases when given along with conventional drugs [10 11 12]

Black pepper (piper nigrum L), belonging to the family piperaceae, is widely used in the human diet. It is a native South Indian spice , found at the Malabar coast Indian and islands of Sri Lanka . Anciently , the use of black pepper has been in practice by ayurvedic physicians in India with potential beneficial action[12,13]. Despite its wide biological potential, such as modulating the bioavailability of drugs, anti tumor effects, antioxidant and anti inflammatory, the lipophilic character of piperine makes it difficult to dissolve its access to site of action and its bioavailability in the body.

Piperine , the chief alkaloid of piper nigrum has been known for its antidepressant , antioxidant , endocrine , and several other activities. In pharmacodynamic studies , the combination of glimepiride with piperine proved to be significant by providing significant protection against the diabetes induced alterations in the biochemical parameters[14]. Piperine has the potential to be used in conjunction with metformin as a bio-enhancer, which could assist lower the dose of the drug and lessen any side effects.[15].

KIM -1 expression is predominantly found in the s3 segment of the proximal tubules', in human ischaemic and toxic acute kidney injury (AK1) it is found in the three segments of the proximal tubules. KIM 1 has proved to be an outstanding early indicator of kidney injury in rats . IGF-1 is the insulin-like growth factor in our blood.

IGF 1 is a hormone that manages the effects of growth hormone (GH) in our body together IGF1 and GH promote normal growth of bones and tissues. The aim of the current study is to analyze whether piperine modulates high fat diet - induced renal damage by regulating KIM 1 and IGF 1 beta signaling modules in male wistar rats.

2. Materials and Methods

Chemicals used

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis , MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from New England Biolabs (NEB), USA and the GoTaq Green master mix was purchased from Promega, USA. KIM 1, IGF 1 beta and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India. Renal Function test kits (Creatinine, Urea, Uric acid) were purchased from Grenier diagnostics, Germany.

Animals used

Healthy adult male albino rats of Wistar strain (*Rattus Norvegicus*) weighing 180 to 200 g (100 days old) maintained as per 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) were used in the present study. Animals were housed in polypropylene cages under specific humidity (65%±5%) and temperature (21 °C±2 °C) with constant 12 h light and 12 h dark schedule at Biomedical Research Unit and Lab Animal Center (BRULAC), Saveetha Dental College & Hospitals, Saveetha Institute of Medical & Technical Sciences, Chennai – 600 077. They were fed with standard rat pelleted diet (Lipton India, Mumbai, India), and clean drinking water *ad libitum*.

Induction of Type 2 Diabetes

Adult male rats were made diabetic (type-2) by a single intraperitoneal injection of streptozotocin (35 mg/kg body weight), after feeding the animals with high-fat diet containing 3% of cholesterol, 1%of cholic acid, 30% of coconut oil, 66% of standard rat feed and 30% of sucrose feeding through drinking water (25%) for 30 days. The low dose of streptozotocin is given to generate a slight trauma to beta cells of pancreas to mimic the chronic hyperinsulinemia insulin resistant condition (Balaji *et al*., 2012).

Experimental design

Wistar strains of adult male albino rats (150-180 days old) with 180-200g body weight were split into five groups randomly. Each group consisted of 6 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).

Fasting blood glucose levels (FBG) were analyzed in experimental rats. At the end of the study, experimental rats were anesthetized with 40 mg of sodium thiopentone per kg body weight. Through cardiac puncture, blood was collected and sera were separated and kept at -80°C. To clear the blood from various organs, 20 ml of isotonic sodium chloride solution was perfused by way of the left ventricle. Kidneys were immediately dissected and utilized for further study.

Fasting blood glucose (FBG)

Blood glucose was estimated using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA) after overnight fasting. Blood was collected by pricking the tip of the rat tail and results are expressed as mg/dl.

Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and Quantitative Insulin Sensitivity Check Index (QUICKI)

HOMA-IR was calculated using the formula (fasting blood glucose X fasting serum insulin/405) as per the method of Matthews *et al* (1985) and QUICKI was calculated using the formula 1/ (log fasting serum insulin + log fasting blood glucose) as per the method of Katz *et al* (2000).

Biochemical analysis

Renal Function test kits Creatinine, Urea, Uric acid were obtained from Grenier diagnostics and the tests were done according to the provided user manual.

mRNA expression analysis of KIM 1, IGF 1 and β-actin genes by RT-PCR:

Total RNA, 2 µg 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 OligodT, dNTPs, and reverse transcriptase. The components were combined with a DNA primer in a reverse transcriptase buffer for an hour at 37ºC. After cDNA conversion, standard PCR was carried out using gene-specific oligonucleotide primers by the initial PCR activation at 95ºC for 5 min. The three-step PCR cycles consisted of denaturation at 95ºC for 2 min, annealing at 60ºC 30 s, and extension at 73 ºC for 30 s. The PCR amplification was carried out for 30 cycles and to ensure that the products are extended completely, a final extension at 73ºC for 5 min was carried out. Gene-specific oligonucleotide primers for the house-keeping gene, β-actin, were added to the same PCR reaction vial and co amplified .

3. Results And Discussion

	control	diabetic	Diabetes+ Piperine	Diabetes+ Metformin
FBG	82	167	134	89
	92	189	123	130
serum insulin	89	117	114	149
	96	139	133	139
KIM1	1	1.206	1.202	0.82
	1	1.02	1.617	0.519
IGF-1β	1	0.706	1.251	0.71
	1	0.42	1.31	0.827

FASTING BLOOD GLUCOSE:

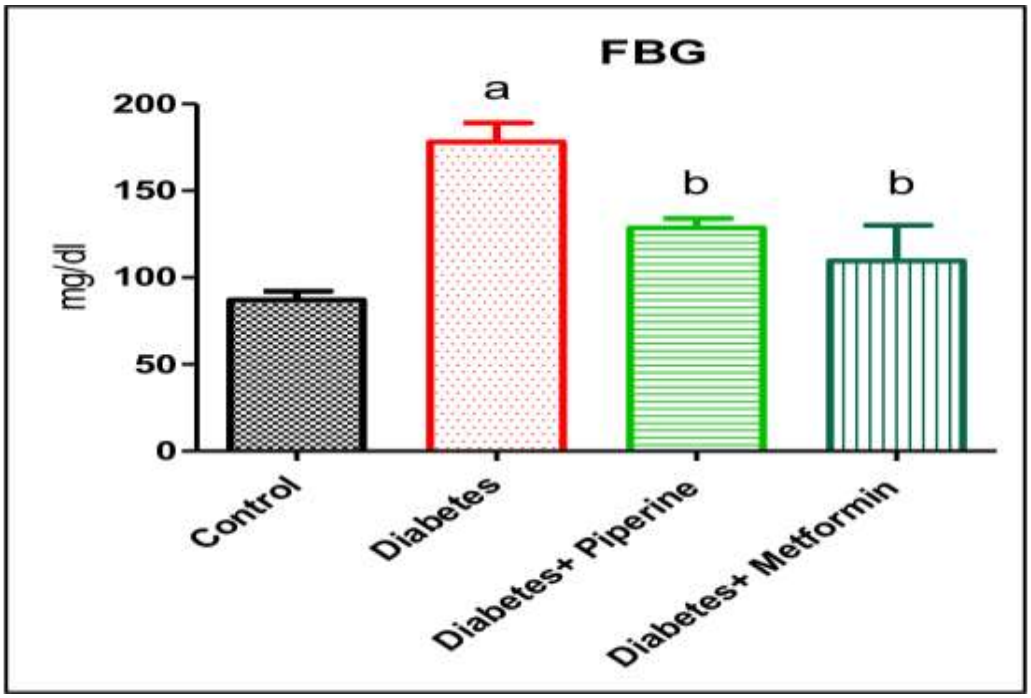

Figure 1. Fasting blood glucose level: As compared to control, fasting blood glucose was elevated in diabetes induced rats. In piperine treated animals there was a significant decrease in the level of fasting blood glucose

Serum Insulin:

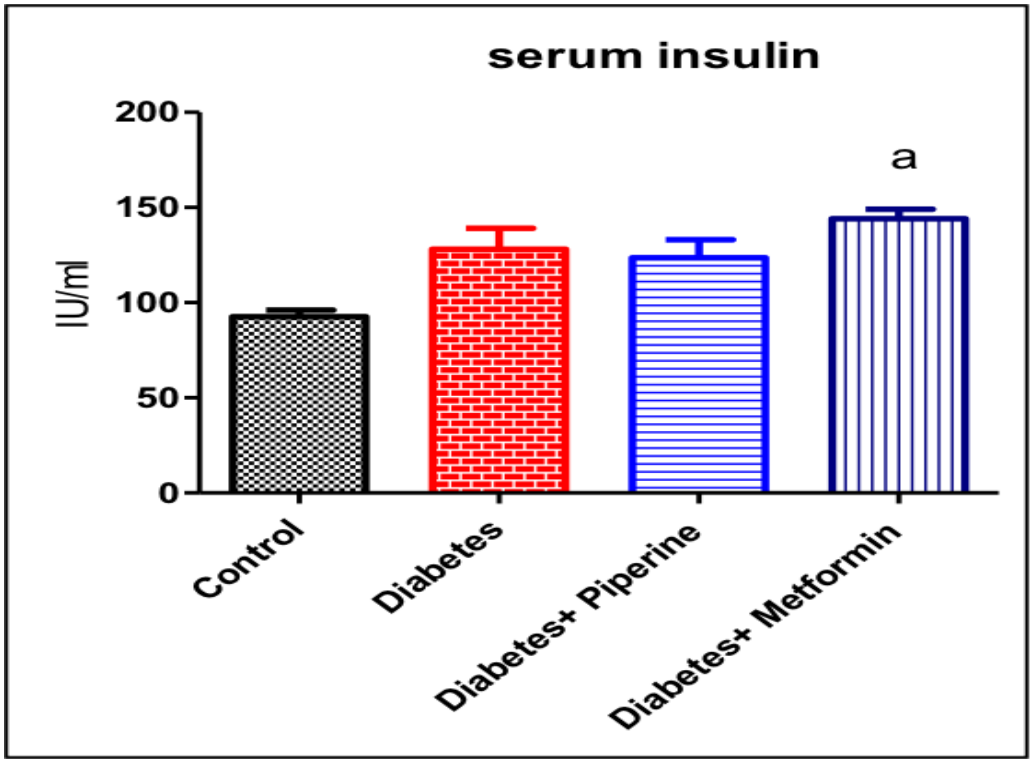

Figure 2. Serum insulin

As compared to control serum insulin was elevated in diabetes induced rats . piperine treated animals exhibited a significant decrease in the level of serum insulin.

KIM – 1

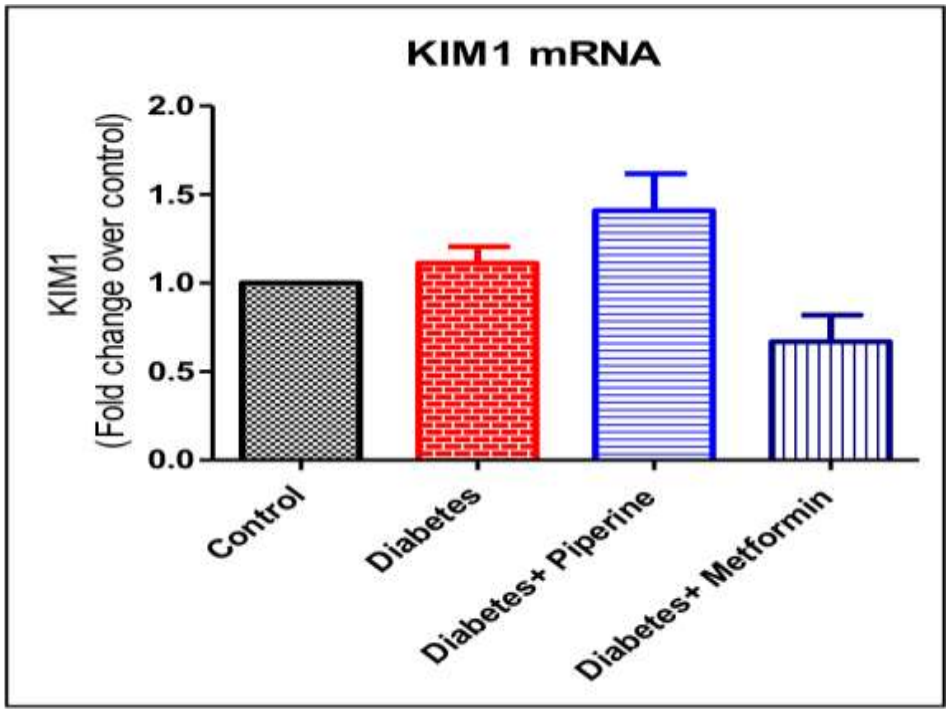

Figure 3. KIM - 1: mRNA expression for kim -1 was significantly increased in diabetic induced rats which is evident for renal damage. The group treated with piperine showed significant decrease in KIM-1 RNA.

IGF-1

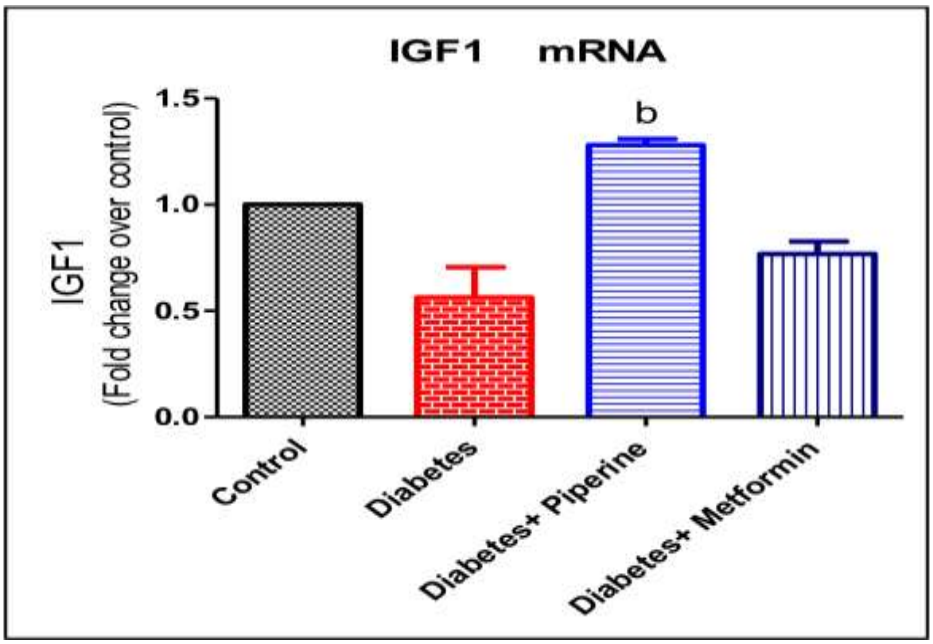

Figure 4. IGF-1: IGF -1 levels were increased in diabetic induced animals, contraindicated it is decreased in piperine treated animals.

Panda and kar's study on normal mice demonstrated that piperine had hypoglycemic impact . As a result, it might have application as an antidiabetic drug. In light of this, the current study was created to examine the impact of piperine on diabetic rats. It has been reported that piperine decreases retard oxidative phosphorylation and glucuronidation and improves absorption ,thereby improving bioavailability of drugs .[16].

It is suggested and strongly believed that piperine has antidiabetic properties in it and it also has great impact when it is used in combination with other drugs. The study conducted by[17] has concluded that cumerin - piperine combination reduced the degeneration of static nerve by lowering oxidative stress indicating neuroprotective effect in diabetic neuropathy, which is great contraindicating with conclusion of study [18], done with combination of curcumin and piperine. They provided evidence for the absence of any additional effects

in the antidiabetic and antioxidant activities of curcumin when administered with piperine. But it also stated a indicating conclusion that curcumin benefits if administered with higher dose of piperine

According to the study[19] , piperine is an antioxidant in streptozotocin - induced diabetic rats. The similar result has also been seen in the study [20], which concluded that small amounts of active ingredient piperine in black pepper have a significant effect against high - fat - induced oxidative stress to cells.

The study of [21], concluded that repeated use of piperine at appropriate dose over a period of time has potential to be used as an antidiabetic agent. which is similar to result of our study which is designed to evaluate the effects of piperine on blood glucose levels in diabetic induced male wistar rats.

Our study results depict that antidiabetic activity is evident at dose of 40mg/kg, we also compare the piperine treated wistar rats with metformin treated diabetic wistar rats, as metformin is proved to be an antidiabetic drug, which helped us to prove that piperine has nearly equal potential against diabetes as of metformin.

As compared to the control, FBG (fasting blood glucose) and serum insulin was elevated in diabetic induced rats. They showed significant reduction in their levels when treated with piperine which is comparatively equal to the results shown by diabetic induced rats treated with metformin (proven antidiabetic drug).

KIM-1 is an early marker for kidney damage. The diabetic induced rats showed significant increase in KIM-1 RNA providing evidence for renal damage. Thereby the group treated with piperine showed significant decrease in KIM-1 RNA proving that piperine has antidiabetic activity in it, protecting renal damage caused by diabetics . similarly, IGF-1 levels were increased in diabetic induced animals' contraindications it is decreased in piperine treated animals.

4. Conclusion

Chronic diabetes results in nephropathy, neuropathy and other associated complications. Hence a perfect antidiabetic drug is indeed to prevent diabetes and their consequent disorders. Every antidiabetic drug has side effects, their own pros and cons. so, it is strongly suggested to make use of ancient herbal medicines in place of allopathic medicine in order to reduce the side effects caused by them. Hereby we conclude with the above evidence that piperine has an effective antidiabetic property in it, which has to be used over a period of time in appropriate doses for effective results. From the study it is evident that intake of herbal extract rich in phytonutrients as a part of a regular diet, can ensure holistic health.

Conflict of Interest

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

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Author Contribution:

A) Kiruthigha T - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

B) Dr. Selvaraj - contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.

C) Dr.V.Vishnupriya - contributed in study design, guiding the research work, manuscript correction.

D) Dr. Gayathri R - study design, statistical analysis, manuscript proofreading and correction.

E) Dr. Kavitha S - study design, statistical analysis, manuscript proofreading and correction.

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