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ORIGINAL ARTICLE

Bio-enhancing Effect of Piperine with Metformin on Lowering Blood Glucose Level in Alloxan Induced Diabetic Mice

Shubham Atal, Sarjana Atal[1], Savita Vyas, Pradeep Phadnis[2]

Department of Pharmacology, MGM Medical College, [1]Department of Pharmacology, Modern Institute of Medical Sciences, Kanadia, [2]Department of Pharmacology, Bundelkhand Medical College, Sagar, Madhya Pradesh, India

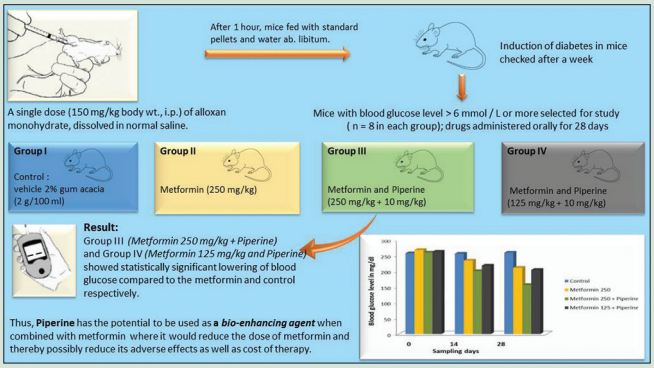
ABSTRACT

Background: Diabetes mellitus is the most rampant metabolic pandemic of the 21st century. Piperine, the chief alkaloid of *Piper nigrum* (black pepper) is widely used in alternative and complementary therapies has been extensively studied for its bio-enhancing property. **Objective:** To evaluate the bio-enhancing effect of piperine with metformin in lowering blood glucose levels in alloxan-induced diabetic mice. **Materials and Methods:** Piperine was isolated from an extract of fruits of *P. nigrum*. Alloxan-induced (150 mg/kg intraperitoneal) diabetic mice were divided into four groups. Group I (control 2% gum acacia 2 g/100 mL), Group II (metformin 250 mg/kg), Group III (metformin and piperine 250 mg/kg + 10 mg/kg), and Group IV (metformin and piperine 125 mg/kg + 10 mg/kg). All the drugs were administered orally once daily for 28 days. Blood glucose levels were estimated at day 0, day 14, and end of the study (day 28). **Results:** The combination of piperine with therapeutic dose of metformin (10 mg/kg + 250 mg/kg) showed significantly more lowering of blood glucose level as compared to metformin alone on both 14th and 28th day ($P < 0.05$). Piperine in combination with sub-therapeutic dose of metformin (10 mg/kg + 125 mg/kg) showed significantly more lowering of blood glucose as compared to control group and also showed greater lowering of blood glucose as compared to metformin (250 mg/kg) alone. **Conclusion:** Piperine has the potential to be used as a bio-enhancing agent in combination with metformin which can help reduce the dose of metformin and its adverse effects.

Key words: Bio-enhancing effect, Diabetes, Metformin, Piperine

SUMMARY

- Piperine is known for its bioenhancing property. This study evaluates the effect of piperine in combination with oral antidiabetic drug metformin. Drugs were administered for 28 days in alloxan induced diabetic mice and blood glucose lowering effect was seen. Results showed significantly better effect of combination of piperine with therapeutic dose of metformin in comparison to metformin alone. Piperine in combination with subtherapeutic dose of metformin also showed better effect than therapeutic dose of metformin. Piperine, thus shows potential to be used as bioenhancer in combination with metformin.



Correspondence:
Dr. Shubham Atal,
Department of Pharmacology,
MGM Medical College, A. B. Road,
Indore - 452 001, Madhya Pradesh, India.
E-mail: shubham.atal@gmail.com
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INTRODUCTION

Diabetes mellitus is the most severe metabolic pandemic to hit the globe in the 21st century. It was estimated that in the year 2000, 171 million people had diabetes which is estimated to get doubled by the year 2030.[1] It is characterized by an absolute or relative deficiency in insulin secretion and/or insulin action associated with chronic hyperglycemia. As a consequence of metabolic derangement in diabetes, various complications develop. Although, several oral anti-diabetic therapies such as biguanides, sulfonylureas and thiazolidinediones are in use along with insulin for the treatment of diabetes mellitus, there are certain limitations due to high cost and side effects such as development of hypoglycemia, weight gain, gastrointestinal disturbances, liver toxicity, etc.[2,3] Hence, medicinal plants are being looked up once again for the treatment of diabetes. Many useful herbs introduced in pharmacological and clinical trials have confirmed their blood glucose lowering effects.[4]

Black pepper (*Piper nigrum* Linn.) is a native South Indian spice. It is commonly found at the Malabar Coast of India and is well known due to its culinary and medicinal properties. It has been cited in Ayurveda treatment of fevers, gastric, respiratory and urinary diseases and used externally to treat rheumatism, neuralgias, and boils.[5] Piperine is the chief alkaloid of *P. nigrum*. The chemical structure of piperine is N-piperoylpiperidin; (E, E)-1-(5-[1,3-benzodioxol-5-yl]-1-oxo-2,4-pentadienyl)-piperidine.

It has been extensively evaluated for its antidepressant, anticonvulsant, antioxidant, anti-mutagenic, hepatoprotective, and various endocrine activities.[6] Another beneficial property that has been widely studied since a long time is the bio-enhancing property[7] of piperine. A "bio-enhancer" is an agent capable of enhancing bioavailability and bioefficacy of a particular drug with which it is combined, without any typical pharmacological activity of its own at the dose used.[8]

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Piperine has been studied extensively and reported to enhance bioavailability of many drugs by promoting rapid absorption of drugs and nutrients[9] or by inhibiting several cytochrome P450 enzymes and phase II reactions.[10] Table 1 show the drugs for which piperine has been found to increase bioavailability. This bio-enhancing property is beneficial to reduce the drug load and improve patient's quality of life [Table 1].

In view of this, the present study was undertaken to evaluate the bio-enhancing effect of piperine with metformin in lowering glucose level in alloxan-induced diabetes.

MATERIALS AND METHODS

Isolation of piperine

Piper nigrum extract was obtained from Amsar (P) Ltd., Indore, Madhya Pradesh, India, which contained 95% of piperine. One gram of the extract was dissolved in 5 mL of ethyl alcohol and 10 mL of 10% w/v of alcoholic potassium hydroxide added with constant stirring. It was then filtered and allowed to stand overnight. The yellow needle shaped crystals of piperine were separated the next day.[11] The purity of the isolated piperine was verified by checking its melting point (range 128–131°C), treating it with concentrated sulfuric acid (blood red color obtained), by thin layer chromatography on which a single spot was obtained and ultraviolet spectrophotometry which showed absorption maxima at 343 nm characteristic for piperine.[12,13]

Drugs and chemicals

Alloxan (Lobochem Ltd. India), Metformin (USV limited Ltd. India), potassium hydroxide pellets (Ranbaxy Pharma. Ltd. India), ethanol (Bengal chemicals, India), gum acacia (Himedia Lab, India), sulfuric acid (Ranbaxy Pharma Ltd. India), glycomet tablet (Metformin 500 mg, USV Ltd. India), and glucose estimation kit (Accu-Check Sensor, Roch USA) were purchased from their respective representatives.

Animals

Adult Swiss albino mice (20–30 g) of either sex were procured from the Central Animal House, MGM Medical College, Indore and acclimatized for a period of 7 days at room temperature (25 ± 2°C) and 50 ± 15% relative humidity. They were housed in a standard cage and maintained on standard pellets and water *ab libitum*. The animals described as "fasted" were deprived of food for 16 h, but had free access to water. The study was carried out in the Department of Pharmacology, MGM Medical College, Indore, Madhya Pradesh, India. The study protocol was approved by the Institutional Animal Ethics Committee.

Preparation of drugs for animal experiment

Piperine and other drugs to be given orally and intraperitoneally intraperitoneal (i.p.), were dissolved in 2% gum acacia to maintain uniformity of the solvent and respective drug solutions were prepared.

Table 1: Drugs for which piperine has been found to increase bioavailability

Name of the drugs	
Phenobarbitone	Curcumin (from turmeric)
Rifampicin, isoniazid	Tetracyclines
Pyrazinamide	Nimesulide, indomethacin
Beta lactams	Theophylline
Cefotaxime	Propranolol
Amoxicillin	Phenytoin, carbamazepine
Fluoroquinolones	Nevirapine

Induction of diabetes

A single dose (150 mg/kg body weight, i.p.) of alloxan monohydrate, dissolved in normal saline was used for induction of diabetes in mice after overnight fasting. After 1 h of alloxan administration, the animals were fed with standard pellets and water *ab libitum*. The animals were stabilized for a week and animals showing blood glucose level (estimated by glucometer) more than 6.0 Mmol/L or more (100–300 mg/dl; moderately diabetic)[14] were selected for the study.

Experimental design

A subacute study model involving once daily administration of drugs for a period of 4 weeks (28 days) was used. The "fasted" mice were divided into four groups, each group containing eight animals ($n = 8$):

Group I: Control: vehicle 2% gum acacia (2 g/100 mL)
Group II: Standard: Metformin (250 mg/kg)
Group III: Metformin and piperine (250 mg/kg + 10 mg/kg)
Group IV: Metformin and piperine (125 mg/kg + 10 mg/kg)

The dose of piperine (10 mg/kg) to be given in combination with metformin was selected on the basis of a previous study on the "*per se*" activity of piperine alone on lowering blood glucose level[15] which showed that piperine itself has no significant effect on blood glucose level at this dose. Similarly, another pilot study was carried out to establish that metformin at a dose of 125 mg/kg, which is half of its standard dose, also does not cause a significant lowering of blood glucose level. This dose was thus considered sub-therapeutic.

The drugs were administered orally to all animals in the four groups at the stipulated doses. The dosing was done in the same way for a total of 28 days at the same time daily. Samples were taken using the tail clip method[16] and blood glucose levels were estimated on day 0 (baseline), day 14 (mid-study), and day 28 (end of study). At each blood sampling, appropriate tail care and antiseptic measures were followed. At the end of the study, further drug administration was stopped.

Statistical analysis

Data were expressed as mean ± standard error of mean and statistical analysis was carried out by one-way ANOVA followed by multiple Tukey's comparison test using SPSS Software version 20.0 (IBM Corporation). $P < 0.05$ was considered to be statistically significant.

RESULTS

The results of interaction of piperine with metformin in its therapeutic and sub-therapeutic dose in lowering glucose level showed that both the combination groups of metformin with piperine and showed improved lowering of blood glucose as compared to the control group on day 14 and 28 ($P < 0.05$) and the standard group of metformin alone.

The study showed that the combination of piperine with standard dose of metformin (10 mg/kg + 250 mg/kg) that is Group III showed better results in lowering blood glucose levels at mid-study interval and end of the study; day 14 and 28 compared to metformin alone, that is Group II and this was statistically significant ($P < 0.05$). This indicates that piperine significantly enhances the activity of metformin used in its standard dose.

The piperine in combination with half of the standard dose (sub-therapeutic dose) of metformin (125 mg/kg) that is Group IV also showed statistically significant lowering of blood glucose compared to the control. This combination showed greater lowering of blood glucose of 17% as compared to 12.5% of metformin alone (250 mg/kg) that is Group II on day 14, although not statistically significant ($P > 0.05$), whereas the results of these two groups were comparable on day 28 [Table 2]. This indicates that piperine also improves the activity

Table 2: Interaction of piperine with metformin in its therapeutic and sub therapeutic dose in lowering blood glucose level in diabetic mice

Drug treatment	Dose (per oral)	Blood glucose level in mg/dL		
		Day 0	Day 14	Day 28
I. Control 2 % gum acacia	10 mL/kg	257.00±7.95	255.67±10.24	258.63±10.26
II. Metformin	250 mg/kg	267.33±7.32	233.50±8.90	210.67*±7.62
III. Metformin + piperine	250+10 mg/kg	259.00±7.19	200.17*,#±8.04	155.83*,#±4.50
IV. Metformin + piperine	125+10 mg/kg	261.83±6.88	217.67*±7.68	204.00*±3.79
One-way ANOVA	F	0.37	7.14	35.88
	P	>0.05	<0.05	<0.05

One-way ANOVA followed by multiple Turkey's comparison test. Values are mean±SEM, *n*=6 in each group, df=3, 20. **P*<0.05 as compared to control, #*P*<0.05 as compared to metformin. ANOVA: Analysis of variance; SEM: Standard error of mean; Df: Degree of freedom

of a sub therapeutic dose of metformin quite favorably although not statistically significant.

DISCUSSION

The results obtained in the study could well be attributed to the bio-enhancing property of piperine when used with metformin.

A "bio-enhancer" is defined as an agent of herbal origin or any phytomolecule, which is capable of enhancing bioavailability and bioefficacy of a particular drug or nutrient with which it is combined, without any typical pharmacological activity of its own at the dose used. The term bioavailability enhancer was first coined by Indian Scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine) who discovered and scientifically validated piperine as the world's first bioavailability enhancer in 1979.[8,17]

Various studies have indicated that piperine has a significant bio-enhancing effect with different categories of drugs. Some generalized mechanisms behind this effect which have been proven or proposed are:

Increased absorption

Gastrointestinal absorption is restricted with a number of drugs resulting in a low oral bioavailability. A number of mechanisms are hypothesized regarding the enhancement of absorption of various drugs by piperine. There may be:

a. The increase in solubility - Piperine increases the formation of the micelle, which is essential for the absorption of lipids and lipid soluble drugs by increasing secretion or synthesis of bile acids and inhibition of bile acid metabolism. Bile acid helps in the formation of the micelle[18]
b. The increase in the blood supply - Study done by Annamalai *et al*[9] suggested that trikatu enhances absorption of drugs from the digestive tract, by enhanced gastrointestinal blood flow
c. Epithelial cell modification to increase the permeability - Piperine also interacts with intestinal epithelial cells and stimulate gamma-glutamyltranspeptidase activity and increases amino acid uptake by epithelial cells, and thereby increasing the uptake of drugs by GI epithelium.[19]

Ultrastructural studies with piperine have also shown an increase in microvilli length.[20]

Decreased metabolism

Piperine has been found to be a nonspecific inhibitor of drug and xenobiotic metabolism. It appears to inhibit many different cytochrome P-450 isoforms as well as UDP-glucuronyltransferase and hepatic arylhydrocarbon hydroxylase. It inhibits another metabolic step, glucuronidation in isolated intestinal cells by inhibiting the enzyme UDP-glucose dehydrogenase.[21,22]

Piperine has been shown to inhibit certain hydroxylation, demethylation, deethylation and deamination processes, and *in vitro* studies with hepatic microsomal suspensions have found that piperine inhibited a variety of mixed function oxygenases.[23]

A recent study has found piperine to be a relatively selective inhibitor of cytochrome P450 (CYP P450) enzyme isoforms: CYP1A2, CYP1A1, CYP2D6, CYP3A4, and CYP2C8.[24]

Reduced efflux of the *drugs* from the site of action

The study of Bhardwaj *et al*.,[25] indicates that piperine inhibits p-glycoprotein, one of the major efflux pumps and thereby increase the duration of stay of the active drug at the target cell.

Increased entry in the cell by inhibiting solubilizer attachment

Solubilizer attachment prevents substances from entering in the cells by linking them chemically to a highly water-soluble substance. Piperine has been reported to inhibit glucuronic acid, an important solubilizer; substances bound to which are usually excreted either into the urine or into the small intestine.[26]

As mentioned earlier, a previous study showed that at dose of 10 mg/kg there is no significant lowering of blood glucose levels by piperine *per se*, however such an effect is observed subacutely at a higher dose of 20 mg/kg.[16] There could be partially selective activity of piperine on β_3 receptors, which results in increased thermogenesis and lipolysis, and increased levels of insulin receptors. Recent studies with selective agonists for the β_3-adrenergic receptor have found significant positive effects on energy expenditure and fat metabolism in adult humans and primates.[27] The ultimate effect of thermogenesis is to increase the demand for substrates such as glucose due to enhanced metabolism at the cellular level. This mechanism is akin to the action of biguanides such as metformin, which decrease glucose levels in the blood by enhancing its peripheral utilization or its consumption.[28]

Thus, this result with metformin is an excellent demonstration of the bio-enhancing effect of piperine. Metformin has a relatively low oral bioavailability of 40–60% and is metabolized by CYP 450 family of enzymes (CYP2C11, CYP2D1),[29] thus allowing piperine to potentially enhance its bioavailability and effect to a significant level. The mechanism of action of metformin includes increasing the peripheral utilization of glucose, increasing its uptake from the blood. So, there could some additive action provided by piperine itself at this lower dose, contributing further to a significantly enhanced therapeutic effect. This combination could allow for a reduction in dosage of metformin, similar to the commercially available combination of piperine with rifampicin, thereby reducing its adverse effects and also the cost of therapy to patients.

CONCLUSION

From the results obtained in the study, it is clearly evident that piperine has the potential to be used as a bio-enhancer when combined with metformin and therefore can be effectively included in the treatment of diabetes where it would help to reduce the dose of metformin, its adverse effects and cost of therapy. Piperine can also provide additional benefits in a disease like diabetes by virtue of its antioxidant and anti-obesity properties. Further studies need to be conducted to further explore this potential of piperine in the treatment of diabetes as a bio-enhancer with metformin and with other oral anti-diabetic drugs too.

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Conflicts of interest

There are no conflicts of interest.

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ABOUT AUTHORS

Shubham Atal

Dr. Shubham Atal (MBBS, MD) is an Assistant Professor in the department of Pharmacology, M.G.M. Medical College, Indore, a leading government institute in central India. His research interests include pre clinical studies in phytochemicals, BA/BE studies and pharmacovigilance. He was a gold medallist in Pharmacology, holds a Professional Diploma in Clinical research, a Masters in Pharma Business Management and also has an experience of working as a Manager in Medical Services division of Troikaa Pharmaceuticals Ltd., Ahmedabad. Currently, he is the co – ordinator of the Insitutional Review Board of his college. He has numerous scientific presentations in various conferences and publications in reputed journals to his credit.

Sarjana Atal

Dr. Sarjana Atal (MBBS, MD) is an assistant professor in pharmacology at Modern Institute of Medical Sciences, Indore. Her primary interests are in academics and medical writing. She has done a diploma course in clinical research also. She is head of the pharmacovigilance unit of her institute. She has presented her research in national conferences and authored drug review articles.

Savita Vyas

Dr. Savita Vyas (M. Pharm, PhD), is an associate professor in pharmaceutical chemistry at M.G.M. Medical College, Indore. She has mentored numerous postgraduate students including PhDs. Her primary research interests are in phytopharmacology and she has numerous publications in the field to her credit. She is member of research advisory board of her institute.

Pradeep Phadnis

Dr. Pradeep Phadnis (MBBS, MD) is an associate professor in pharmacology at Bundelkhand medical college, Sagar. He has previously taught at M.G.M. medical college, Indore for more than 30 years. He has served as guide for many post graduate students and helping them excel in their residency. He has many publications to his credit in various fields of pharmacology and holds keen interest in sports too.