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EVALUATION OF THE EFFECT OF PIPERINE *PER SE* ON BLOOD GLUCOSE LEVEL IN ALLOXAN-INDUCED DIABETIC MICE

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Abstract: Diabetes mellitus is a metabolic disorder and emerging pandemic of the 21st century. Piperine, the chief alkaloid present in *Piper nigrum* (black pepper), has a wide array of uses in alternative and complementary therapies. The effect of piperine on blood glucose level was studied in alloxan-induced diabetic mice in acute and subacute study models. Piperine was isolated from the fruits of *Piper nigrum* crude extract. Diabetes was induced using alloxan in albino mice which were then randomly divided into 5 groups (n = 6). In acute study, drugs were administered orally as: control (2% gum acacia, 10 mL/kg), standard (metformin 150 mg/kg), P10 (piperine 10 mg/kg), P20 (piperine 20 mg/kg) and P40 (piperine 40 mg/kg). Drug intervention for subacute study consisted of once daily oral administration for 14 days of 2% gum acacia 10 mL/kg, metformin 250 mg/kg, and piperine 5, 10 and 20 mg/kg, respectively, in the control, standard and P5, P10, P20 groups . Blood glucose levels were estimated before and at 1, 2, 3 and 4 h post dosing, respectively, in the acute study and on day 7 and 14 in the subacute study. Results of acute study showed that at 2 h post-dosing piperine at high dose of 40 mg/kg showed significant rise in blood glucose level ($p < 0.05$) in comparison to control group. In contrast, a significant blood glucose lowering effect was seen with piperine at dose of 20 mg/kg on day 14 ($p < 0.05$) in the subacute study**.** In summary, we suggest that subacute administration of piperine has statistically significant antihyperglycemic activity while acutely it raises blood glucose at high doses. Further investigations are needed to consider it as prospective anti-diabetic agent at appropriate dosage.

Keywords: piperine, diabetes, alloxan, albino mice, metformin

Diabetes mellitus is the most severe metabolic disorder characterized by absolute or relative insufficiency in insulin secretion and/or its action. In the year 2000, it was estimated that 171 million people had diabetes, which is estimated to get doubled by the year 2030 (1). As a consequence of metabolic derangement in diabetes, various complications develop. All oral antidiabetic agents have side effects. Sulfonylureas are associated with weight gain and hypoglycemia. Metformin's major side effects are seen in the gastrointestinal tract, with nausea, cramps, and diarrhea. The major side effect of α-glucosidase inhibitors is flatulence. The major problems with the thiazolidinediones are those of fluid retention, weight gain, and a normochromic, normocytic, dilutional anemia (2).

Parallel to this, the holistic approach of herbs have accelerated the global efforts to harness and harvest medicinal plants having multiple beneficial effects. Some pre-clinical and clinical studies have confirmed their hypoglycemic effect and actions like repair of β cells of islets of Langerhans (3). A recent study has reported the hypoglycemic activity of piperine in normal mice (4). In view of these facts, the present study was undertaken to evaluate the effect of piperine on blood glucose levels in alloxan-induced diabetic mice.

Black pepper (*Piper nigrum L.*) a native South Indian spice, found at the Malabar Coast of India and the Islands of Sri Lanka, belonging to the family Piperaceae is widely used in human diet. Historically, the use of black pepper has been in practice by Ayurvedic physicians in India with potentially beneficial actions (5). Piperine, the chief alkaloid of *Piper nigrum* has been extensively evaluated for its antidepressant (6), anticonvulsant (7), antioxidant (8, 9), antimutagenic (10), hepatoprotective (11) endocrine (12) and several other activities.

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MATERIALS AND METHODS

Drugs and chemicals

Piper nigrum crude extract was obtained from Amsar (P) Ltd., Indore (MP). Alloxan monohydrate (Lobochem Ltd., India), potassium hydroxide pellets (Ranbaxy Pharmaceuticals Ltd., India), ethanol (Bengal Chemicals, India), metformin tablets (USV Ltd., India), and glucometer (Accu-Chek sensor, Roche, USA) were purchased locally. All the treatments were prepared in 2% gum acacia (vehicle) and administered per oral (*p.o.*) with the help of oral gavage.

Animals

A total of 60 male Swiss albino mice weighing between 20–30 g were used. They were procured from the Central Animal House, M.G.M. Medical College, Indore. They were kept under standard conditions of temperature and humidity with a 12:12 h light : dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. The animals described as 'fasted' (on days of sampling) were deprived of food for 16 h, but had free access to water. The study was carried out in the Department of Pharmacology and the study protocol was approved by the Instituional Animal Ethics Committee (IAEC), M.G.M. Medical College, Indore, M.P. Animal handling was performed in accordance to Good Laboratory Practice (GLP).

Isolation of piperine (13)

The *Piper nigrum* crude extract was dissolved in ethanol and 10 mL of 10% w/v of alcoholic potassium hydroxide was added with constant stirring. The mixture was then filtered and allowed to stand overnight. The yellow needle shaped crystals of piperine were separated (Fig. 1). The purity of the isolated piperine was verified by checking its melting point (range 128–131^{o}C), by thin layer chromatography and ultraviolet spectrophotometry (absorption maxima at 343 nm for piperine) (14).

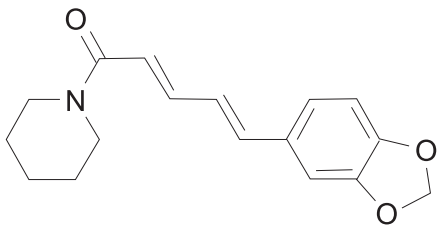

Figure 1. Structure of piperine [(E,E)-1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-piperidine]

Induction of diabetes

Diabetes was induced by a single dose of alloxan monohydrate 150 mg/kg, intraperitoneally (*i.p.*) to overnight-fasted mice. After 1 h of alloxan administration, the animals were fed with standard pellets and water *ad libitum*. Seventy-two hours later, the fasting blood glucose level in the mice was determined through tail clipping method (15) using a pre-calibrated Accu-Chek sensor glucometer (Roche, USA). Diabetes was further confirmed after 8 days and animals with fasting blood glucose of 6.0 mmol/L or more (100–300 mg/dL; moderately diabetic) were considered appropriate and employed in the study (16).

Experimental design

The fasted mice were randomly divided for the two study models i.e., acute and subacute models.

Acute study

A single day, single dose study with serial sampling of blood was employed. The fasted mice were divided into 5 groups of 6 animals each: Control – 2 % w/v gum acacia (10 mL/kg); Standard – metformin (150 mg/kg), P10 – piperine (10 mg/kg), P20 – piperine (20 mg/kg), P40 – piperine (40 mg/kg).

The drugs were administered orally to all the groups at the stipulated doses and the time of dosing was noted for each group. The blood samples were collected using tail clip method and glucose levels were measured before dosing (pre-dose, 0 hour) and post-dosing at 1, 2, 3 and 4 h, respectively, using a tail clip method as described previously (section – Induction of diabetes).

Subacute study

In the subacute study model, the drugs were administered orally once daily for 14 days. The fasted mice were divided into 5 groups (n = 6): Control – vehicle 2 % w/v gum acacia (10 mL/kg), Standard – metformin (250 mg/kg), P5 – piperine (5 mg/kg), P10 – piperine (10 mg/kg) and P20 – piperine (20 mg/kg).

The drugs were administered orally to all groups at a pre-fixed time once daily for 14 days. The fasting blood glucose levels were estimated using glucometer at three intervals, i.e., day 0 (before start of the study), day 7 (mid study interval) and day 14 (end of the study).

Statistical analysis

Results were analyzed by SPSS Version 17 and expressed as the mean ± SEM. One way analysis of variance (ANOVA) followed by multiple Tukey's

Table 1. Acute effect of piperine *per se* on blood glucose level in alloxan-induced diabetic mice at different doses.

Drug treatment	Dose (*p.o.*)	Blood glucose level in mg/dL				
		0 h	1 h	2 h	3 h	4 h
Control 2% gum acacia	10 mL/kg	168.00 ± 4.18	161.17 ± 4.28	159.50 ± 4.94	154.50 ± 6.54	144.33 ± 6.10
Metformin	150 mg/kg	158.83 ± 4.22	112.17* ± 7.65	88.17* ± 3.339	73.83* ± 1.33	88.00* ± 1.65
(P 10)	10 mg/kg	162.33 ± 5.12	152.33 ± 7.65	148.67 ± 6.58	151.33 ± 5.44	146.67 ± 5.05
(P 20)	20 mg/kg	160.83 ± 6.59	161.33 ± 8.18	151.17 ± 7.44	140.33 ± 7.76	143.67 ± 6.37
(P 40)	40 mg/kg	163.00 ± 4.34	163.17 ± 4.20	184.33* ±3.53	162.17 ± 5.62	152.17 ± 4.99
One way ANOVA	F p	0.47 > 0.05	12.92 < 0.05	42.79 < 0.05	38.76 < 0.05	26.76 < 0.05

One way ANOVA followed by multiple Tukey's comparison test. Values are the mean ± SEM, n = 6 in each group, df = 4, 25; * $p < 0.05$ as compared to control.

Table 2. Subacute effect of piperine *per se* on blood glucose level in alloxan-induced diabetic mice at different doses.

Drug treatment	Dose (*p.o.*)	Blood glucose level in mg/dL		
		Day 0	Day 7	Day 14
Control 2 % Gum acacia	10 mL/kg	248.67 ± 9.09	246.50 ± 10.23	240.50 ± 8.59
Metformin	250 mg/kg	251.50 ± 7.17	205.67* ± 4.84	194.17* ± 6.91
(P 5)	5 mg/kg	249.33 ± 10.45	247.83 ± 9.77	247.33 ± 8.39
(P 10)	10 mg/kg	260.33 ± 8.41	247.17 ± 4.82	242.33 ± 5.61
(P 20)	20 mg/kg	250.67 ± 7.11	221.00 ± 7.99	204.00* ± 7.95
One way ANOVA	**F** **p**	0.31 > 0.05	6.00 < 0.05	10.60 < 0.05

One way ANOVA followed by multiple Tukey's comparison test. Values are the mean ± SEM, n = 6 in each group, df = 4, 25. * $p < 0.05$ as compared to control.

comparison test for both acute and subacute studies; $p < 0.05$ was taken as significant value.

RESULTS

In the acute study (Table 1), administration of piperine at the dose of 40 mg/kg showed significant rise in blood glucose level at 2 h (184.33 mg/dL), as compared to the control group (159.50 mg/dL, $p < 0.05$). It also showed rise in blood glucose level at 3 and 4 h as compared to control group, but not significant ($p > 0.05$).

Piperine at doses of 10 and 20 mg/kg did not show any significant effect on blood glucose levels compared to control group. Metformin showed significant lowering of blood glucose as compared to control group as well as piperine groups at all time points after 0 h ($p < 0.05$).

In the subacute study (Table 2), piperine given at the dose of 20 mg/kg showed significant lowering of blood glucose level (204.00 mg /dL) on day 14 as compared to control group (240.50 mg/dL, $p < 0.05$). It also showed lowering of blood glucose (12%), although not significant, as compared to con-

trol group at day 7 ($p > 0.05$). There was no significant effect on blood glucose levels at the doses of 5 and 10 mg/kg ($p > 0.05$).

DISCUSSION

It has been shown in a study conducted in normal mice by Panda and Kar (4) that piperine has hypoglycemic effect. Thus, it could have a potential as an antidiabetic agent. Keeping this in view, the present study was designed to evaluate the effect of piperine on blood glucose levels in diabetic mice. In our acute study, piperine *per se* showed no effect on lowering the blood glucose level in diabetic mice at doses of 10 and 20 mg/kg (Table 1). In fact, piperine at the dose of 40 mg/kg showed significant rise in blood glucose levels at 2 h after drug administration ($p < 0.05$) with slight rise at 3 and 4 h too (not significant).

Piperine has been shown to have agonistic activity on the β adrenergic receptors as documented by Majeed et al. in their patent filed on piperine (17). The stimulation of β_2 receptors results in an increase in the blood glucose level due to glycogenolysis in liver and skeletal muscles and gluconeogenesis in liver (18, 19). So, the acute effects of piperine, especially at higher doses, could be attributed to a selective or relatively strong agonism at these receptors.

The subacute study showed that while piperine did not show any significant effects at doses of 5 and 10 mg/kg, respectively, there was significant lowering of blood glucose levels after 14 days ($p < 0.05$) and some lowering at day 7, although not significant (12%) when piperine was given at 20 mg/kg (Table 2). There could be partially selective activity of piperine on β_3 receptors upon subacute administration, which results in increased thermogenesis and lipolysis, and increased levels of insulin receptors (20). Recent studies with selective agonists for the β_3-adrenergic receptor expressed mainly in brown adipocytes and mediating induction of UCP-1, have found significant positive effects on energy expenditure and fat metabolism in adult humans and primates (21).

The ultimate effect of thermogenesis is to increase the demand for substrates like glucose due to enhanced metabolism at the cellular level. This mechanism could be similar to one of the mechanisms of action of biguanides like metformin, which decreased glucose levels in blood by enhancing its peripheral utilization or its consumption (22).

The fact that piperine did not show such an effect at doses of 5 and 10 mg/kg in the subacute studies, probably points to the fact that the mechanism(s) mentioned above do not come into operation at the lower doses of piperine. Such a beneficial effect also needs repeated administration for at least a few days as is evident from the non significant effects on blood glucose levels in the acute studies, except at the higher dose of 40 mg/kg.

CONCLUSION

It is apparent from the above results that piperine has the potential to be used as an antidiabetic agent, if given repeatedly at the appropriate doses over a period of time. Its acute effects of raising blood glucose could also be used beneficially by using it in combination with known antidiabetics to counteract their adverse effect of hypoglycemia. Further studies are needed to explore such combination effects.

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