



EVALUATION OF ANTI DIABETIC ACTIVITY OF SEEDS OF BLACK PEPPER IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Parijatha Bandigari*, Ashma Mohammed, Sushma Arikilla, Bhaskar Chanduri, Shruthi Yemba and Krishna Mohan Chinnala

School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, Telangana, India - 500088.

*Corresponding Author: Parijatha Bandigari

School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad, Telangana, India - 500088.

Article Received on 21/03/2018

Article Revised on 12/04/2018

Article Accepted on 02/05/2018

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 streptozotocin induced diabetic rats in subacute study models. Piperine was isolated from the fruits of *Piper nigrum* crude extract. Diabetes was induced using streptozotocin in albino rats which were then randomly divided into 5 groups (n = 6). Drug intervention for subacute study consisted of once daily oral administration of distilled water 10 mL/kg, metformin 150 mg/kg, piperine 20 and 50 mg/kg, in the control, standard, P20, and P50 groups respectively for 14 days. Blood glucose levels were estimated before administration of drug and on day 1, 7 and 14 in the subacute study respectively. A significant blood glucose lowering effect was seen with piperine at dose of 50 mg/kg on day 14 ($p < 0.05$) in the subacute study. In summary, we suggest that administration of piperine at the dose 50 mg/kg has statistically significant antihyperglycemic activity. Phytochemical investigation revealed the presence of alkaloids, as the major constituents in the plant *Piper nigrum* (black pepper). The results suggest that piperine (50mg/kg) showed antidiabetic activity in streptozotocin induced diabetic rats.

KEYWORDS: Diabetes, Piperine, *Piper nigrum*, Streptozotocin and Metformin.

INTRODUCTION

Black pepper

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. Piperine, the chief alkaloid of *Piper nigrum* has been extensively evaluated for its antidepressant, anticonvulsant, antioxidant^[1], ant mutagenic, hepatoprotective, anti-diabetic, anti-fertility^[2] and anti-hypertensive activity^[3]. The genus *Piper*, it is most closely related to other Asian species such as *Piper caninum*.



Figure 1: Black pepper plant with unripened fruits.

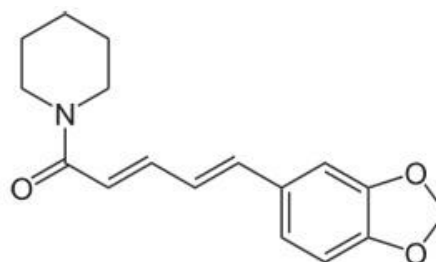


Figure 2: Molecular structure of piperine.

Properties of piperine**Table 1: Properties of piperine.**

Properties	
Chemical formula	C ₁₇ H ₁₉ NO ₃
Molar mass	285.34 g·mol ⁻¹
Density	1.193 g/cm ³
Melting point	130 °C (266 °F; 403 K)
Boiling point	Decomposes
Solubility in water	40mg/L
Solubility in alcohol	1g/15ml
Solubility in ether	1g/36ml
Solubility in chloroform	1g/1.7ml

MATERIALS AND METHODS**Plant material**

Leaves of *Piper nigrum* was collected from the local area of Hyderabad (India) in the month of January and authenticated by the Department of Botany, Osmania University, Hyderabad, India.

Experimental Animals

Wistar rats (150-200gm) of male sex were procured from Sainath Agenesis, (CPCSEA Reg No: 282/99/CPCSEA) Hyderabad, Telangana. Animals were housed at CPCSEA approved (Reg no.1832/PO/Re/S/15/CPCSEA) in animal house facility of School of Pharmacy, Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hr light and 12 hr dark cycle) and had free access to commercial pellet diet (Hindustan lever Ltd, Bombay, India) with water *ad libitum* [4]. The animal house temperature was maintained at 25 ± 2°C with relative humidity at (50 ± 15%). The study was approved by the Institutional Animal Ethics Committee (004/IAEC/NNRG/2016), Nalla Narasimha Reddy Education Society's Group of Institutions. Ethical norms CPCSEA were strictly followed during all the experiments.

Drugs and Chemicals

All the chemicals used in the study were of analytical grade the following chemicals were used for the experimental study.

Table 2: List of Drugs and chemicals.

S. No.	Chemicals	Source
1	<i>Piper nigrum</i>	SD fine chemicals
2	Metformin	SISCO
3	Streptozotocin	SISCO
4	Ethanol	SD fine chemicals
5	alcoholic KOH	SD fine chemicals
6	Acetone	SD fine chemicals
7	Hexane	SD fine chemicals

Methods**Extraction of Piperine from Black Pepper seeds**

- 1) 50 gms of black pepper powder, extract with 625 ml of 90% ethanol in Soxhlet extraction [5] for 3 hrs.
- 2) The solution is filtered and concentrated under water bath at 60°C.
- 3) Alcoholic KOH is added to the filtrate residue and after awhile decanted from a insoluble residue to alcoholic solution is left overnight, where under yellow needles [6] of piperine separated out.
- 4) Melting point is 125-126° C.

Preliminary Phytochemical Analysis

In preliminary phytochemical screening, the ethanolic extract was tested for carbohydrates, alkaloids, glycosides, sterols, phenolic compounds, tannins, flavonoids, saponins, proteins and amino acids using standard procedure. The ethanolic extract of leaves of *Piper nigrum* was subjected to preliminary phytochemical screening.

Test for Alkaloids

The ethanolic extract was treated with diluted HCl acid and filtered. The filtrate was treated with various alkaloidal agents.

Mayer's Test: Sample was treated with Mayer's reagent; appearance of cream colour indicates the presence of alkaloids.

Drangendroff's Test: Sample was treated with Drangendroff's reagent [7]; appearance of reddish brown precipitate indicates the presence of alkaloids.

Hager's Test: Sample was treated with Hager's reagent; appearance of yellow colour indicates the presence of alkaloids.

Wager's Test: Sample was treated with Wager's reagent; appearance of brown precipitate indicates the presence of alkaloids.

Test for Carbohydrates

The ethanolic extract was treated with 3ml of α-naphthol in alcohol and Conc. Sulphuric acid was carefully added to side of the test tube. Formation of a violet ring at the junction of two liquids indicates presence of carbohydrates.

Fehling's Test: To the sample Fehling's A and B were added and heated for 2 min. appearance of reddish brown colour indicates presence of reducing sugars.

Benedict's test: To the sample Benedict's was added and heated appearance of reddish orange precipitate indicates presence of reducing sugar.

Barfoed's Test: The sample was treated with Barfoed's reagent and heated appearance of reddish orange precipitate indicates the presence of reducing sugar.

Test for Proteins

Biuret's Test: To the Ethanolic extract, copper sulphate solution followed by sodium hydroxide was added; a violet colour precipitate indicates the presence of proteins.

Million's Test: To the Ethanolic extract, Million's reagent was added; appearance of pink colour indicates presence of proteins.

Test for Steroids**Libermann Bruchard's Test**

The Ethanolic extract was treated with Conc. Sulphuric acid and glacial acetic acid followed by acetic anhydride, a violet ring appears at the junction of the liquids appearance of green colour in the aqueous layer indicates the presence of steroids.

Test for Sterols

The Ethanolic extract was treated with 5% KOH solution; appearance of pink colour indicates the presence of sterols.

Test for Phenols

The Ethanolic extracts were treated with neutral ferric chloride solution^[8]; appearance of violet colour indicates the presence of phenols.

The Ethanolic extract was treated with 10% sodium chloride solution; appearance of cream colour indicates presence of phenols.

Test for Tannins

The Ethanolic extract was treated with 19% lead acetate solution appearance of white precipitate indicates the presence of tannins.

The Ethanolic extract was treated with aqueous bromine water; appearance of white precipitate indicates the presence of tannins.

Test for Flavonoids

5ml of the Ethanolic extract solution was hydrolyzed with 10% sulphuric acid and cooled. It was then extracted with diethyl ether and divided into 3 portions in three separate test tubes. One ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide and 1ml of diluted ammonia solutions were added to the first, second and third test tubes respectively. Development of yellow colour in each test tube indicates the presence of Flavonoids.

Shinoda's Test

The Ethanolic extract was dissolved in alcohol, to which a piece of magnesium followed by drop wise addition of Conc. HCl and heated. Appearance of magenta colour indicates the presence of Flavonoids.

Test for Gums and Mucilage

The Ethanolic extract was treated with 25ml of absolute alcohol and then the solution was filtered. The filtrate was examined for its swelling properties.

Test for Glycosides

A pinch of Ethanolic extract was dissolved in glacial acetic acid and few drops of ferric chloride was added followed by the addition of Conc. Sulphuric acid. Formation of red ring at the junction of the two liquids indicates the presence of glycosides.

Test for Saponins

Foam test: One ml of the Ethanolic extract was diluted to 20 ml with distilled water; formation of foam in the upper part of the test tubes indicates the presence of saponins.

Test for Terpenes

The Ethanolic extract was treated with tin and thionyl chloride; appearance of pink colour indicates the presence of terpenes.

Toxicological Evaluation**Acute oral toxicity study**

The acute oral toxicity procedure was followed according to OECD 423 guidelines. The acute toxic class method is a stepwise procedure with 3 animals of a single sex per step. Depending on the mortality and/or morbidity status of the animals,^[9] on the average 2-4 steps may be necessary to allow the judgment on the acute toxicity of the test substance. This procedure results in the use of a minimal number of animals while allowing for acceptable data based scientific conclusion. The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemicals which cause the acute toxicity.

Experimental Procedure**Qualitative chemical test**

Isolated piperine was tested by the standard procedures. The isolated piperine showed the presence of alkaloids. Wistar albino rats (150-200 g) of either sex were used. Animals maintained under standard environmental conditions and had free access to feed and water *ad libitum*.

Streptozotocin-induced diabetes

The albino rats weight of 150-200 g of either sex allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intra peritoneal injection of streptozotocin 50 mg/kg body weight^[10]. After 18 hours of injection of streptozotocin, diabetes was confirmed by testing blood sugar level more than 250 mg/dl were selected for the further study. Animals maintained for four days in diabetic condition for well establishment of diabetes.

Animal Grouping and drug administration

They were divided into five groups.

Group 1 (control): Animals were administered distilled water^[11] orally.

Group 2 (diabetic control): Treated with streptozotocin (50mg/ kg, I.p)

Group 3 (standard): Treated with standard metformin, (150mg/ kg, orally)

Group 4 (Test No.1): Treated with isolated piperine from *piper nigrum* (20 mg/kg b.w)^[10]

Group 5 (Test No.2): Treated with isolated piperine from *piper nigrum* (50 mg/kg b.w)

Assessment of Anti-diabetic Activity Effects of consumed piperine on blood-glucose level of rats.

The blood samples were collected from the tail vein of the rat and blood glucose levels was estimated at 1st, 7th,

and 14th days after piperine administration by using One touch basic glucose strips ^[12].

Piperine Effect on body weight of Streptozotocin induced diabetic rats

Body weight and fasting blood glucose were monitored on day1, 7 and 14.

RESULTS AND DISCUSSION

Melting point

Melting point of the piperine was determined ^[13] as 130°C.

Phytochemical Screening

Table 3: Results obtained from Phytochemical screening.

S. No.	Name of the chemical test	RESULTS
I.	Amino acids and proteins	
1.	Ninhydrin test	-
2.	Biuret test	-
II.	Steroids and Triterpenoids	
1.	Liebermann and Burchard reaction	-
2.	Salkowski test	-
III.	Alkaloids	
1.	Dragendorff's test	+
2.	Mayer's test	+
IV.	Saponins	
1.	Foam test	+
V.	Flavonoids	
1.	Ammonia test	+
2.	Shinoda test	+
VI.	Tannins	
1.	Extract with FeCl ₃	+

Key (+) = Presence, (-) = Absence.

Piperine Effect on body weight of streptozotocin induced diabetic rats.

Body weight and fasting blood glucose were monitored ^[14] on day 1, 7 and 14. Results of the effect of Piperine on body weight is given in table-7 and fig-15.

Table 4: Effect of Piperine on body weight of animals.

S. No.	Groups	Body weight		
		0 Day	5 Day	10 Day
1.	Normal	168.00±4.76	169.16±5.30	169.66±5.03
2.	Diabetic	180.66±4.92	161.50±5.09	154.66±3.89
3.	Metformin	192.06±4.93	188.83±4.35	202.50±2.12
4.	Piperine 20mg/kg	194.33±3.73	188.50±3.84	191.50±4.25
5.	Piperine 50mg/kg	195.66±4.47	198.83±6.04	198.00±4.36

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

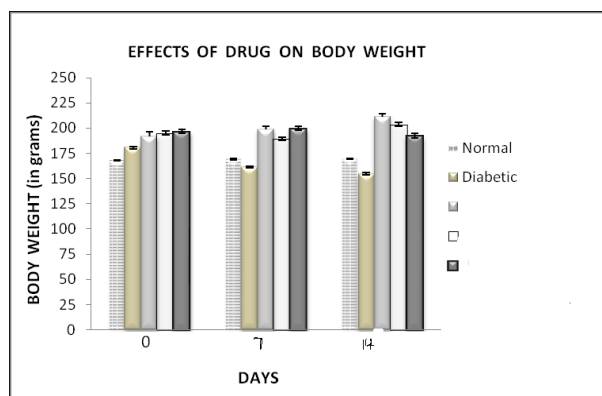


Figure 3: Effect of Piperine on body weight of diabetic rats.

Table 5: Effect of piperine on fasting blood glucose level.

S. No.	Groups	Fasting blood sugar level		
		0 Day	5 Day	10 Day
1	Normal	102.50±5.70*	106.00±4.69*	107.83±6.41*
2	Diabetic	248.16±4.16	253.50±2.94**	263.16±3.09**
3	Metformin	246.83±2.72	189.5±2.60**	111.33±1.49**
4	Piperine 20mg/kg	260.66±1.66	158.16±2.49***	132.50±3.37***
5	Piperine 50 mg/kg	256.00±0.71	146.00±2.99***	121.83±3.14***

Effect on fasting blood glucose level

On treatment with piperine the fasting mean blood glucose levels on 0 day (after being diabetic) i.e. 246.83±2.72 mg/dl, 260.66±1.66 mg/dl and 246.00±0.71 mg/dl reduced to 124.33±1.49 mg/dl, 118.50±3.37 mg/dl and 131.83±3.14 mg/dl respectively on 14th day. Blood glucose was estimated using a commercial diagnostic kit. (Accu check active glucometer).

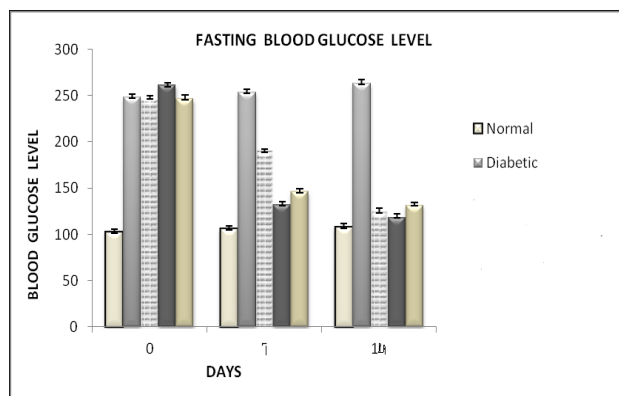


Figure 4: Effects of piperine on fasting blood glucose levels of diabetic rats.

On treatment with Metformin and piperine (20,50 mg/kg) the fasting mean blood glucose levels on 0 day (after being diabetic) i.e. 246.83±2.72 mg/dl, 260.66±1.66 mg/dl and 256.00±0.71 mg/dl reduced to 111.33±1.49 mg/dl, 132.50±3.37 mg/dl and 121.83±3.14 mg/dl respectively on 14th day. Blood glucose was estimated using a commercial diagnostic kit. (Accu check active glucometer).

On treatment with Metformin and piperine (20,50 mg/kg) the mean body weights of rats on 0 day (after being diabetic) i.e. 192.06±4.93, 194.33±3.73 and 195.66±4.47 increased to 202.50±2.12, 191.50±4.25 and 198.50±4.36 respectively on 14th day. Results of the effect on body weight are given in table 4 and fig3.

Antidiabetic Activity Effects of consumed piperine on blood-glucose level of rats.

The blood samples were collected from the tail vein of the rat and blood glucose levels was estimated at 1st, 7th, and 14th days after piperine administration by using one touch basic glucose strips.

DISCUSSION

In present study the experimental groups streptozotocin induced diabetic rats were treated with Black pepper seed extract (piperine). The present study of piperine showed significant effects at doses 20 mg/kg and 50 mg/kg respectively, there was significant lowering of blood glucose levels after 14 days ($p < 0.05$) and some lowering at day 7. 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. The experiment was conducted in accordance with parallel design i.e. each group received single formulation, single time. After completion of the study protocol, it was found that the blood glucose level and body weight improved significantly as compared to diabetic control. Streptozotocin induced diabetic rats exhibited decreased body weight, polyphagia, polydipsia associated with decrease in endogenous insulin and hyperglycaemia. Treatment with extracts to diabetic rats increases body weight and also decrease in elevated blood sugar level. These effects may be attributed to either inhibition of increase in insulin output or inhibition of intestinal absorption of glucose or increase in glucose metabolism or combination of all. Administration piperine increases body weight in streptozotocin diabetic rats. The ability to protect body weight loss seems to be as a result of its ability to reduce hyperglycaemia.

CONCLUSION

The herbals occupied a distinct place in the life right from the primitive period to till date and provided information on the use of plants or plant products as medicine. Use of medicinal plants in the management of

illness is due to their phytochemical constituents. The present data demonstrated that piperine having the anti-diabetic activity. It can be a safe supplementary therapy for a long term and effective management of diabetic patients if given repeatedly at the appropriate doses over a period of time. Further, systematic studies in humans *in vitro* and *in vivo* are also needed to identify the effect and mechanism of piperine as an anti diabetic agent. Further studies are needed to explore combination effects.

ACKNOWLEDGEMENTS

The authors are thankful to the Management of Nalla Narasimha Reddy Education Society's Group of Institutions, Hyderabad for providing necessary facilities to carry out the research work in a successful manner.

REFERENCES

- Arvind Manohar Mujumdar, Jayant Nilkanth Dhuley, Vinay Kumar Kesav Deshmuk, Palghathari Haranraman, Suresh Ramnath Naik Anti-inflammatory activity of piperine in rats. *Japanese journal of medical science and biology*, 1990; 4(3): 95-100.
- Evan Prince Sabina, Annie Deborah Harris Souriyana, Deborah Jackline, Mahaboob Khan Rasool. Piperine, an active ingredient of black pepper attenuates acetaminophen-induced hepatotoxicity in mice. *Asian Pacific Journal of Tropical Medicine*, 2010; 3(12): 971-976.
- G. Bano.R. K., Raina.U., Zutshi.K. L., Bedi.R. K., Johri.S. C. Sharma. Effect of piperine on bioavailability and pharmacokinetics of propranolol and theophylline in healthy volunteers. *European Journal of Clinical Pharmacology*, 1991; 41(6): 615-617.
- Ganiyu Obob, Ayokunle O. Ademosun, Oluwatoyin V. Odubajo, and Ifeoluwa A. Akinbola. Antioxidative Properties and Inhibition of Key Enzymes Relevant to Type-2 Diabetes and Hypertension by Essential Oils from Black Pepper. *Advances in Pharmacological Sciences*, 2013; 2(1): 92-98.
- Hyejeong Jwa, Youngshim Choi, Ui-Hyun Park, Soo-Jong Um, Seung Kew Yoon, Taesun Park. Piperine, an LXR α antagonist, protects against hepatic steatosis and improves insulin signaling in mice fed a high-fat diet. *Biochemical Pharmacology*, 2012; 84 (11): 1501-1510.
- Jintanaporn Wattanathorn, Pennapa Chonpathompikunlert, Orathai Tankamnerdthai. Piperine, the potential functional food for mood and cognitive disorders. *Food and Chemical Toxicology*, 2008; 46(9): 3016-3110.
- K.Selvendiran. J.Prince. Vijeya Singh. K.Baba Krishnan. D Sakthisekaran. Cytoprotective effect of piperine against benzo α pyrene induced lung cancer with reference to lipid peroxidation and antioxidant system in Swiss albino mice. *Elsevier Fitoterapia*, 2003; 74(2): 109-115.
- LM Lichtenberger, JJ Romero, OR Carryl. Effect of pepper and bismuth subsalicylate on gastric pain and surface hydrophobicity in the rats. *Pharmacology wiley online library*, 1998; 78(6): 1025-1035.
- Milan Suhaj. Jana Rácová. Martin Polovka. Vlasta Brezová. Effect of γ -irradiation on antioxidant activity of black pepper (*Piper nigrum* L.). *Elsevier*, 2006; 97(4): 696-704.
- Md. Gayasuddin, Md.Parvez, Mohd Iqbal, G. Venkataiah. Effect of ethanolic extract of piper nigrum Linn. fruits on midazolam induced hypnosis in rats. *International Journal of Pharmacology & Toxicology*, 2013; 3(1): 5-8.
- M.S. Alhumayyd, I.A. Bukhari, A.A. Almotrefi. Effect of piperine, a major component of black pepper, on the pharmacokinetics of domperidone in rats. *J Physiol Pharmacol*, 2014; 50(3): 620-625.
- Pawin Pongkorsakol. Preedajit Wongkrasant. Saowanee Kumpun. Varanuj Chatsudthipong. ChatchaiMuanprasat. Inhibition of intestinal chloride secretion by piperine as a cellular basis for the anti-secretory effect of black peppers. *Pharmacological Research*. 2015; 100(6): 271-280.
- Pennapa Chonpathompikunlert, Jintanaporn Wattanaathorn, Supaporn Muchimapura. Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease. *Food and Chemical Toxicology*, 2010; 48(3): 798-802.
- Qing-Qiu Mao. Zhen Huang. Xiao-Ming Zhong. Yan-Fang Xian. Siu-Po Ip. Piperine reverses the effects of corticosterone on behaviour and hippocampal BDNF expression in mice. *Neurochemistry International*, 2014; 74: 36-41.