Journal of Science and Technology, 6(1 & 2): 73-79, December 2016

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITIES OF DIFFERENT FRACTIONS OF EXTRACT OF *PEPEROMIA PELLUCIDA (L.)* IN ALLOXAN INDUCED DIABETIC MICE

CHAND SULTANA[1*], NETISH KUMAR KUNDO[1], MD. NURUL ISLAM[1], MOHAMMAD MOSLEH UDDIN[2] and MIR IMAM IBNE WAHED[3]

[1]Department of Pharmacy, Mawlana Bhasani Science and Technology University, Tangail-1902, Bangladesh; [2]Department of Biochemistry and Molecular Biology, Mawlana Bhasani Science and Technology University, Tangail-1902, Bangladesh; [3]Department of Pharmacy, Rajshahi University, Rajshahi-6205, Bangladesh

Abstract

The present study was undertaken to explore the antidiabetic and antihyperglycemic activities of different fractions of methanolic extract of *Peperomia pellucida* (PP). Metformin HCl (100 mg/kg body weight, op.) was used as a standard antidiabetic agent. The result indicated that in diabetic mice, the extracts significantly ($p<0.05$) improved 63.31%, 60.34% and 72.87% accordingly at the dose of 500 mg/kg body weight (b. wt.) glucose tolerance in groups-DHPP, DCTPP and DCLPP, respectively. Short-term treatment with extracts had no effect on body weight to organ weight ratio; however, it significantly lowered liver weight in groups DHPP, DCTPP and DCLPP. Administration of extracts greatly reduced both the serum cholesterol, triglycerides and phospholipids levels in groups DHPP, DCTPP and DCLPP was compared to DC mice. The present investigation established the pharmacological evidence to support the folklore claim that the extracts have anti-diabetic and hypolipidemic potential.

Keywords: *Peperomia pellucida*, serum cholesterol, triglycerides and phospholipids

Introduction

Diabetes is a global disease with huge adverse impacts on health and mortality particularly of cardiovascular disorders. Diabetes mellitus (DM) is the major clinical disorder affecting nearly 10% of the populations all over the world (Burke, 2003).The prevalence of the DM is increasing rapidly in the developing countries than in the developed country. There are an estimated 246 million people with diabetes in the world, of whom about 80% reside in developing countries (Sicree, 2006). Patient with diabetes have an increased risk of coronary heart disease, peripheral vascular disease, strokes and may account for more than 65% death among people with diabetes (Stamler, 1993 and Foulis, 1986). Multiple pathophysiologic mechanisms play a role in the risk of cardiovascular events in the metabolic syndrome including glucose intolerance, hyperglycemia, hypertension, dyslipidemia, atherosclerosis that are caused primarily by insulin resistance (Reaven, 1988; Park, 2003). In particular hyperglycemia may contribute to diabetic complications by altering vascular cellular

* Corresponding author: E-mail:drmirimam@gmail.com

metabolism, vascular matrix molecules and circulating lipoproteins. However, an abnormal lipid profile was found to be a more significant risk factor than either hypertension or diabetes alone (Assmu, 1988). The burden of death and disability from diabetes and its related complications remain great due to their therapeutic failure as well as the potential for induction of hypoglycemia. Traditional medicines are used to reduce blood glucose level as well as have beneficial effects on complication of diabetes (Dixit, 2006). Therefore, natural agents having hypoglycemic and hypolipidemic properties would be better for the management of diabetes.

Peperomia pellucida (L.) Kunth (Family-Piperaceae), locally known as LuchiPata, is an annual herb that is widely distributed in many South American and Asian countries. The plant is refrigerant and its leaves are used in the treatment of headache, fever, eczema, abdominal pains and convulsions (Ghani, 1998). According to Manila Medical Society, *Peperomia pellucida* is used to relieve arthritic pains but it may cause CNS depression (Calimag, 2007). Antibacterial and anti-inflammatory activities (Arrigoni-Blank, 2004; Aziba, 2001) and isolation of antifungal and anticancer constituents (Ragasa, 1998; Xu S, 2006) from this plant have also been reported. In the present study, we investigate the hypoglycemic and hypolipidemic activities of *Peperomia pellucida.*

Materials and Methods

Plant Materials

Fresh plants (Whole plant) of *Peperomia pellucida* Linn. were collected from Rajshahi in April 2014 and the plant authenticity was confirmed from the Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen no. DACB-55123 was maintained in our laboratory for future reference.

Extraction

The collected plant materials were cleaned, sun dried and pulverized. The whole plants (500 g) powdered separately soaked in 2.0 liters of methanol at room temperature for 7 days. The extract was filtered through fresh cotton bed and finally with Whatman filter paper number 1. The filtrates were concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 g) of the concentrated methanol extracts was fractionated by the modified Kupchan partitioning protocol (Vanwagenen, 1993) and the resultant partitionates were evaporated to dryness to yield *n*-hexane (HPP), carbon tetrachloride (CTPP), chloroform (CLPP) and aqueous (AQPP) soluble materials. The residue were then stored in a refrigerator until further use.

Drugs and Chemicals

The active drug, metformin hydrochloride was the generous gift from Square Pharmaceuticals Ltd; Pabna, Bangladesh. Total cholesterol (TC) and triglyceride (TG) wet reagent diagnostic kits were purchased from Crescent diagnostic kits. Alloxan was

purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. All other chemicals and solvents were of analytical grade.

Animals

Nine weeks old male Swiss Albino mice (weight, 25-28g) were purchased from ICDDRB, Dhaka, Bangladesh and housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%) with water *ad. libitum*. The animals used in this study were cared in according to guidelines of animal experiment.

Phytochemical Screening

Phytochemical analysis was performed according to the standard methods described by Nayek and Pereira 2006.

Antihyperglycemic Test

Antihyperglycemic test was performed according to standard method. All mice were divided into four groups comprising five mice in each group. Groups were named as DC (Diabetic Control group receiving vehicle 0.5% methyl cellulose), DS (Diabetic Standard group mice were receiving metformin HCl, 150 mg/kg), DHPP (Diabetic *n*-hexan *Peperomia pellucida* group mice received 500 mg/Kg body weight n-hexan PP extract), DCTPP (Diabetic Carbon tetrachloride *Peperomia pellucida* group mice received 500 mg/Kg body weight carbon tetrachloride PP extract) and DCLPP (Diabetic Chloroform *Peperomia pellucida* group mice received 500 mg/kg body weight carbon tetrachloride PP extract). All extracts were dissolved in 0.5% methyl cellulose. After fasting 16 hours, diabetes was induced to all groups by intra-peritoneal injection (IP) of alloxan monohydrate (150 mg/kg) dissolved in saline. After 72 hours, blood glucose levels were measured from tail-vein blood of all groups by glucometer considered as 0 day and blood glucose level higher than 11.5 mmol/l considered as diabetic. 0.5% methyl cellulose, standard drug metformin (150 mg/kg) and extracts (500 mg/kg accordingly) were administered once daily for seven days to respective mice groups. Blood glucose content was measured after 1^{st}, 3^{rd} and 7^{th} days by glucometer.

Blood and Organs Sampling

The body weight of mice of each groups were measured before and after week-long antihyperglycemic test with drugs. The mice were sacrificed by anesthetizing with pentobarbital (5mg/kg, i.p.), blood samples were withdrawn from aorta of heart using a syringe and kept into an EDTA containing tube. Heart, liver and kidney were excised and cleaned of the surrounding tissues. The organ weights were measured immediately and the organ weight to body weight ratio were calculated. Finally, the blood and tissue samples were preserved in refrigerator at –40°C for biochemical estimations.

Estimation of Serum Cholesterol, Triglyceride and Phospholipids in Diabetic Mice

Serum samples were obtained by centrifugation of blood at 4000 rpm for 10 minutes. The concentration of TC and TG were measured by UV-spectrophotometer, using wet reagent

diagnostic kits according to the manufacturer's protocol. Total lipid was extracted from the liver and kidney tissue according to the method of Folch et al, 1957. Phospholipids were estimated by the method of Bartlette, 1959; by digestion with perchloric acid and the phosphorous liberated was estimated by the method of Fiske and Subbarow, 1952.

Statistical Analysis

The results were expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed by using ANOVA followed by Tukey's test using Graph pad Prism Software version 5.03. $p < 0.05$ were considered as statistically significant.

Results and Discussion

Antihyperglycemic Effect of PP Extracts in Diabetic Mice: Hypoglycemic test was done with extract which give more improvement result compared with Group-DC (Table-2). After 7 days mice treated with extracts in Group-DHPP, Group-DCTPP and Group-DCLPP (Diabetic mice were received *n*-Hexan, Carbontetra chloride and Chloroform extracts of 500 mg/Kg body weight accordingly). Glucose levels were significantly lowered 63.31%, 60.34% and 72.87%, respectively showed in Table 2.

Table 1. Phytochemical screening of the extract

Plant extract	Alkaloids	Saponins	Flavonoids	Tanins	Triterpenoids
HPP	+	+	+	+	+
CTPP	+	_	+	_	+
CLPP	+	+	+	_	+

Note: + = presence, - =Absence

Table 2. Anti-hyperglycemic effect of PP extracts in diabetic mice

Groups	0 day	1st day	3rd day	7th day
DC	20.75±1.4	18.5±2.1	18.8±2.1	19.53±2.5
DS	18.75±1.35	13.3±1.3	10.3±1.0*	4.6±1.*
DHPP	19.35±0.6	13.8±1.2	11.2±2.1	7.1±0.5*
DCTPP	17.15±2.5	12.7±1.1	9.5±1.1*	6.8.±1.3*
DCLPP	21.75±1.2	14.75±1.5	9.2±1.0	5.9±1.3*

Note: Values were expressed in Mean ± SEM. *p<0.05 indicates significant changes compared with diabetic control group.

Effect of extracts on the change of body weight, organ weight (heart, liver and kidney) and organ weight to body weight ratio changes: Table 3 shows no significant changes in the body weight among experimental animal after seven days treatment with extract. The results revealed that heart weight to body weight and kidney weight to body weight ratio did not alter significantly in experimental mice compared to DC group. Although the

liver weight to body weight ratio were severely altered or increase in DC group, after treatment. It was significantly decreased in DHPP, DCTPP and DCLPP groups compared to the DC group mice although total food intake was not different.

Table 3. Effect of PP extracts on body weight and body weight to organ weight ratio in diabetic mice

Groups	Initial Body weight (g)	Final Body weight (g)	HW/BW (g/kg)	LW/BW (g/kg)	KW/BW (g/kg)
DC	29.1±1.20	36.±1.20	0.87±0.44	9.1±1.3	1.6±0.21
DS	32±1.7	27±2.12	0.71±0.32	4.21±0.11*****	1.1±0.16
DHPP	28±1.12	28.4±1.11	0.72±0.17	4.9±0.20*****	1.2±0.14
DCTPP	32.33±1.8	30±0.90	0.74±0.08	6.0±0.10*****	0.99±0.3
DCLPP	33.5±2.2	31.1±1.8	0.62±0.12	3.8±0.30*	0.88±0.13

Note: Values were expressed in Mean ± SEM. Control group received 0.5% Methyl cellulose and standard group received metformin 150 mg/kg. *p<0.05 indicate significant changes compared with diabetic control.

*Effects of PP Extracts on Total Cholesterol, Triglyceride and Phospholipid in Diabetic mice***:** Comparison of serum lipid contents in control group and experimental groups of mice are shown in Table 4. When PP extracts and metformin were administered in diabetic mice a significant decrease ($p<0.05$) of the levels of total cholesterol, triglycerides, and phospholipids was observed in DHPP, DCTPP and DCLPP groups compared to DC. Administration of PP extracts and metformin, mice tend to bring the levels of hepatic lipids to near normal level.

Table 4. Levels of total cholesterol, triglycerides and Phospholipids in serum of diabetic mice after treatment with extracts or drugs

Groups	Total Cholesterol mg/dl	Triglycerides mg/dl	Phospholipid mg/dl
DC	190±1.32	211±2.5	173.5 ± 13.4
DS	86.6±1.15*	88.86±1.52*	91.7 ± 8.5*
DHPP	110.57±1.24*	105.6±1.11*	138.5 ± 6.4*
DCTPP	99.8± 1.44*	100.7±1.04*	128.1±1.8*
DCLPP	105.3±1.75*	98.8±2.01*	100.1±1.1*

Values were expressed in Mean ± SEM. Control group received 0.5% Methyl cellulose and standard group received Metformin 150 mg/kg. *p<0.05 indicate significant changes compared with diabetic control group.

The experiment showed that the antihyperglycemic showed of extracts on diabetic mice showed lower blood glucose level (Table 2). After 7th day's treatment, DHPP, DCTPP and DCLPP groups showed significant decrease in blood glucose concentration which were 63.31%, 60.34% and 72.87% respectively. Extracts may have the properties to stimulate ß-cell for the secretion of insulin and are most effective for controlling diabetes due to presence of hypoglycemic alkaloid, saponin and flavonoid (Table 1). The extracts might be promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis (Socher, 2006). Short-term treatment with extracts had no effect on body weight to organ

weight ratio; however, it significantly lowered liver weight in DHPP, DCTPP and DCLPP groups compared to DC (Table 3).

In hyperglycemic mice there was a significant increase in lipids (total cholesterol and triglycerides). The most common lipid abnormalities in diabetes are hypercholesterolemia and hypertriglyceridemia. Oral administration of different fraction of PP extracts resulted in a significant reduction of serum lipids level in mice, viz total cholesterol and triglycerides. Flavonoids are known for their diverse activities including hypolipidemic and antioxidant activities (Ray, 2011). DHPP, DCTPP and DCLPP extracts contain flavonoids and other different compounds such as saponin, tannin, triterpenes and alkaloids (Table 1). With respect lipid lowering capacity of this plant extract, it may be proposed that the constituents of the plant extracts may act as inhibitors for enzyme such as hydroxy-methyl-glutaryl-CoA reductase, which participates in de novo cholesterol biosynthesis (Table 4) as has been suggested for some plants earlier (Ray, 2011). The increase concentration of free fatty acid in liver and kidney may be due to lipid breakdown and this may cause increased generation of NADPH dependent microsomal lipid proxidation during diabetes. As a result liver and kidney phospholipids were increased in diabetic mice (Gebherdt, 1996). Administration of different fraction extracts significant decrease the level of tissue free fatty acids and phospholipids.

Conclusion

Based on results, we conclude that the plant extracts of *Peperomia pellucida* (Whole Plant) has blood glucose and lipid lowering activities. However, further studies are needed to isolate active compounds responsible for these pharmacological activities and also necessary to examine underlying mechanism of antidiabetic and lipid lowering effects of *Peperomia pellucida.*

Acknowledgements

The authors would like to express thanks, to the Department of Pharmacy, Rajshahi University, Rajshahi and Mawlana Bhasani Science and Technology University, Bangladesh for providing lab facilities and International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB) for supplying animals (mice) for the research work of *Peperomi apellucida*.

References

Abdul, G., (2003). Medicinal plants and Bangladesh with chemical constituents and uses. *Asiatic Society of Bangladesh*, **2**: 235

Afanas'ev, I.B., E.A. Ostrachovitch, N.E. Abramovaand, L.G. Korkina, (1995). Different antioxidant activities of biflavonoidrutin in normal and iron overloading rats. *Biochem. Pharmacol*, **80**: 627.

Arrigoni-Blank, M.F., E.G. Dmitrieva, E.M. Franzotti, A.R. Antoiolli, M.R. Andrade and M. Marchioro, (2004). Anti-inflammatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae). *J Ethnopharmacol*, **91**: 215-218.

Assmau, G. and Schultett, (1988). The prospective cardiovascular munster (PROCAM) study. Prevelance of hyperlipidimia to coronary heart disease. *As Heart J*, **116**: 1713-1724.

Aziba, P.I., A. Adedeji, M. Ekor, and O, Adeyemi, (2001). Analgesic activity of *Peperomia Pellucida* aerial parts in mice. *Fitoterapia*, **72**: 57-58.

Bartlette, G.R., (1959). Phosphorous assay in column chromatography. *Journal of Biological Chmestry*, **234**: 466-468.

Burke, J.P., K.Williams, M.V.Narayan, (2003). *Diabetes Care*, **26**: 99-2004.

Calimag, M.M.P., (2007). Herb-drug interactions. Manila, Philippines, Manila Medical Society.

Dixit, P.P., J.S. Londhe, S.S. Ghashadi, and T.P.A. Devasagayam, (2006). Antidiabetic and related benificial properties of Indian medicinal plants in herbal drug research. A twenty first century perspective. Jaypee brother's medical publisher Limited, New Delhi, India, 377-386 pp.

Fiske, C.H. and Y. Subbarow, (1925). The colorimetric determination of phosphorous. *Journal of Biological Chemistry*, 66: 375-400.

Folch, C.H., M. Lees and G.H.S. Stanley, (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*. **226**: 497-509.

Foulis, A.K., C.N. Liddle *et al*., (1986). The histopathology of the pancreas in type -1 DM, A 25 year in UK. *Diabetologia*, **29**: 267-274.

Gebhardt, R. and H. Beck, (1996). Differential inhibitory effects of garlic derived Organosulfer compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. *Lipids*, **31(12)**: 1269-1276.

Nayak, B.S and L.L.M.P. Pereira, (2006). *Catharanthus roseus* flower extract has wound-heating activity in Sprague Dawley rats. *BMC Complement and Alt. Medicine*, **6(41)**: 1-6.

Park, Y., S. Kuan, L. Palaniappau *et. Al*., (2003). The metabolic syndrome: Prevalence and association risk factor findings in the population from the Third National Health and Nutrition Examination Survey. *Arch intern Med*, **163**: 427-436.

Ragasa, C.Y., M. Dumato, and J.A. Rideout, (1998). Antifungal compounds from *Peperomia pellucida. ACGC Chem Res Commun*, **7**:54-61.

Ray, B., J. Jena, S. Biswal and B. Rath, (2011). Antidiabetic properties of *Stevia rebaudiana* leaf estract. *Int R J Pharm Sci*, **2(1)**: 3-4.

Reaven, G.M., (1998). Role of insulin resistance in human disease. *Diabetes*, **37**: 1595-1607.

Sicree, R., J. Shaw and P, Zimmet, (2006). *Diabetes Atlas*,**15**-102.

Socher, M., N.Z. Baquer and P. Mclean, (1995). Comperative studies on the change in activities of enzymes of glucose metabolism. *Glucose under utilization in diabetes*, **4(5)**: 333-337.

Stamler, O., J.D. Vaccaro and Neaton, (1993). Diabetes, other risk factors and 12 year cardiovascular mortality for meant screened in the multiple risk factor intervention trial. *Diabetes Care*, **15**: 434-444.

Toronoky K. *et. al*., (1963). Spectrophotometric determination of glycogen with O-toludine. *Clin Chim Acta*. **8**: 627-628.

Vanwagenen, B.C., R. Larsen, J.H. Cardellina, D. Randazzo, Z.C. Lidert and C. Swithenbank, (1993). Ulosantoin, a potent insecticide from the sponge Ulosaruetzleri. J. org. Chem, 5: 335-337.

Xu, S., N. Li, M.M. Ning, C.H. Zhou, Q.R. Yang and M.W. Wang, (2006). Bioactive compounds from *Peperomia pellucida. J Nat Prod*, **69**:247-250.