Contents lists available at ScienceDirect

Journal of Acute Disease

journal homepage: www.jadweb.org

Document heading doi: 10.1016/S2221-6189(13)60074-8

Peperomia pellucida in diets modulates hyperglyceamia, oxidative stress and dyslipidemia in diabetic rats

Rabiat U. Hamzah[1*], Adebimpe A. Odetola[2], Ochuko L. Erukainure[3], Ademola A. Oyagbemi[4]

[1]*Department of Biochemistry, Federal University of Technology, Minna, Nigeria*
[2]*Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[3]*Food Technology Division, Federal Institute of Industrial Research, Lagos, Nigeria*
[4]*Department of Veterinary Physiology, University of Ibadan, Ibadan, Nigeria*

ARTICLE INFO

Article history:
Received 19 February 2012
Received in revised form 30 March 2012
Accepted 7 April 2012
Available online 20 November 2012

Keywords:
Peperomia pellucid
Antidiabetic
Antioxidant

ABSTRACT

Objective: To investigate the antidiabetic and antioxidant properties of *Peperomia pellucida* (*P. pellucida*) in alloxan-induced diabetic rats. **Methods:** Beside mouse chow, two diets were designed to contain 10%w/w and 20%w/w *P. pellucida* as supplements respectively. Diabetes was induced in groups of five male albino rats by a single intraperitoneal injection of alloxan. Two groups of diabetic rats and normo rats were each fed one of these diets respectively, while two other groups served as positive and negative controls respectively. A seventh group was fed pelletized mouse chow. **Results:** Diabetic rats on diets supplemented with 10%w/w and 20%w/w of *P. pellucida* for 28 d resulted in reduction of blood glucose level. The level of total serum cholesterol, triglycerides and LDL-cholesterol decreased significantly ($P<0.05$) with the supplementation diets compared to the untreated diabetic rats. Also treatment with glibenclamide and *P. pellucida* (10% and 20%w/w) led to increased activities of SOD, CAT and GSH respectively. There was significant ($P<0.05$) reduction in the level of HDL-cholesterol, Catalase, SOD activities and GSH concentration in diabetic untreated rats. The supplemented diets significantly ($P<0.05$) reduced lipid peroxidation, which was elevated in untreated diabetic rats. Significant decrease ($P<0.05$) in the activities of AST, ALT and ALP was also observed in rats fed *P. pellucida* supplemented diets. **Conclusions:** The results from this study indicate that *P. pellucida* has an antidiabetic and antioxidant properties in experimental diabetes mellitus and thus justifies the acclaimed traditional antidiabetic use.

1. Introduction

Diabetes mellitus is a major public health problem in the developed as well as developing countries. It is ranked as the seventh cause of death in the world and third when it's fatal complications are taken into consideration[1]. It is a disease characterized by chronic hyperglycaemia and glucosuria produced by an absolute or relative insufficiency of insulin. The ailment may result into the development of further metabolic and anatomic disturbances among which is lipemia, hypercholesterolaemia, weight loss, ketosis, arteriosclerosis, and gangrene, pathologic changes in the eye, neuropathy, renal disease and coma[2,3]. It is known that diabetes is a condition within the body where the beta cells of the islets of Langerhans in the pancreas do not produce enough insulin and/or the insulin receptors are not working properly. This results to an inadequate supply of insulin and therefore elevated blood glucose level. The symptoms of diabetes include increased blood glucose; increased appetite and thirst, unexplained weight loss, weakness, decreased blood pressure and blurred vision[4].

Diabetes is also associated with significant oxidative stress which has been reported to be a major contributory factor to several diabetic complications[5].Reactive oxygen species (ROS) are important part of the defense mechanisms against infection, but its excessive generation has been implicated in the pathogenesis of vascular disease[6-8]. Diabetic patients have an increased incidence of vascular disease and it has been shown that free radical activity is

*Corresponding author: Rabiat U. Hamzah, Department of Biochemistry, Federal University of Technology, Minna, Nigeria.
Tel: +2348035911608
E-mail: rabiune@yahoo.com;

elevated during diabetes[9,10]. Increased oxidative stress has also been proposed to be one of the major causes of the hyperglycemia-induced trigger of diabetic complications. Hyperglycemia stimulates ROS formation from a variety of sources. These sources include oxidative phosphorylation, glucose autooxidation, NAD(P)H oxidase, lipooxygenase, cytochrome P450 monooxygenases, and nitric oxide synthase. Normal levels of antioxidant defense mechanism is not sufficient for the eradication of free radical induced injury, therefore administrations of antioxidants from a natural origin have a promising role to play. Several antioxidants of plant material are experimentally confirmed and widely used as more effective agents against oxidative stress[11,12].

Peperomia pellucida (*P. pellucida*) (L.) HBK (Piperaceae) is popularly known in Nigeria as shiny bush or riri and is used locally for hypertension, diabetes and generally as tonic for healthy well being. It is an herbaceous plant with succulent alternate and ovate leaves, with terminal and axillary efflorescences, at the opposite side from leaves, developing well in loose and humid soil by the tree shadows[13]. In folk medicine, this species is employed on abscesses, furuncles, and skin sores, as well eye inflammation (conjunctivitis). Literature data confirmed the antimicrobial and analgesic effects including other activities, such as anti-inflammatory effect[14,15]. Phytochemical studies revealed the presence of dill-apiol and pellucidin A, in *P. pellucida*[16]. The present study aims at investigating the antidiabetic and antioxidant effect of *P. pellucida* in alloxan induced diabetic rats.

2. Materials and methods

2.1. Preparation of plant material

Fresh leaves of *P. pellucida* were collected from the environs of the University of Ibadan, Ibadan, Nigeria. They were authencitated at the Herbarium, Botany Department, University of Ibadan, Ibadan, Nigeria. The leaves were air dried under laboratory conditions and grinded to powdering form. The fine powder was stored in airtight containers at room temperature until use. 100 g and 200 g of *P. pellucida* were compounded with 900 g and 800 g of standard rat feed (Ladokun feeds, Ibadan) to get 10% and 20% w/w of the plant-supplemented diet respectively.

2.2. Chemicals

Alloxan was obtained from were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and drugs used in this experiment were of analytical grade and the purest quality available.

2.3. Animals

Male albino rats of Wistar strain weighing about 150-200 g obtained from the faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria were used for the study. They were fed on standard rat pellet diet (Ladokun Feeds, Nigeria) and water was provided *ad libitum*. They were maintained under standard laboratory conditions and were subjected to natural photoperiod of 12 h light: dark cycle. Group one animals were administered with physiological saline at 10 mL/kg body weight and served as control. Experimental protocols complied with the "Principle of Laboratory Animal Care" (NIH publication No 85-23) guidelines.

2.4. Alloxan Induced diabetes

Diabetes was induced by a single (I.P) injection of 100 mg/kg of alloxan monohydrate. After 72 h of alloxan injection, the diabetic rats (glucose level > 250 mg/dL) were separated and used for the study.

2.5. Preparation of reference drug

The reference drug, Glibenclamide (Clamide by hovid, Malaysia) was purchased from Danax Pharmaceuticals, a local chemist in Ibadan, Nigeria. It was administered orally to the group on standard drug daily. The drug was dissolved freshly in normal saline and appropriate volumes were given to the animals depending on their weight. The animals were given 600 μg/kg body weight of the active ingredient.

2.6. Experimental design

A total of 35 rats were used. The rats were randomly distributed into seven groups of five rats each.

Group A - received water; standard feed and served as Normal control (10 mL/kg bwt)

Group B - Normal + 10%w/w of *P. pellucida*

Group C - Normal + 20%w/w of *P. pellucida*

Group D - Diabetic control

Group E - Diabetic + 600 μg/kg body weight glibenclamide (standard drug)

Group F - Diabetic + 10%w/w *P. pellucida*

Group G - Diabetic + 20%w/w *P. pellucida*

The rats were treated for four weeks after which they were sacrificed by cervical decapitation. Plasma was collected after centrifugation of collected blood samples at 3 000 g for 10 min in MSC bench centrifuge (Beckman and Hirsch, Burlington, 10, USA).

The livers from animals were removed and rinsed in ice - cold isotonic, 1.15% KCl solution. The liver samples were homogenized in four times of ice - cold isotonic phosphate buffer, pH 7.4 and centrifuged at 10 000 g for 15 min to obtain the post mitochondria fraction (PMF). Both plasma and PMF aliquots were stored at -4 ℃ until use.

2.7. Biochemical analysis

Blood glucose was determined by the Glucose Oxidase method described by NCCLS[17]. Total plasma cholesterol was determined by Roeschlau *et al*[18], plasma triglycerides by enzymatic colorimetric method using Randox Kit[19]. Plasma HDL was assayed by the method of Lopes-Virella *et al*[20] plasma LDL[21] plasma alanine aminotransferase (ALT) and aspartate aminotransferase activity was determined by following the method of Reitman and Frankel[22],using Randox kit, Alkaline Phosphatase (ALP) activity was determined by the optimized DGKC method using Randox kit. The supernatant obtained from the centrifuged liver homogenate was used for the following biochemical assays: superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and lipid peroxidation (LPO)[23-26].

2.8. Statistical analysis

All values are expressed as mean±S.E.M. Data was analyzed by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison tests. Differences of means were considered significant at $P<0.05$ using Graph-Pad Prism software Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, "www.graphpad.com."

3.Results

The hypoglycemic effect of *P. pellucida* supplemented diet is shown in Figure 1.

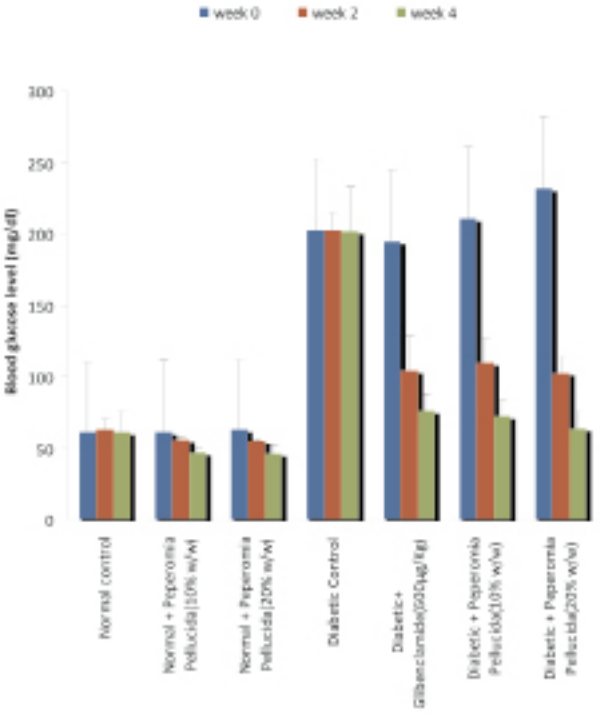

Figure 1. Effect of *P. pellucida* on blood glucose level in normal and alloxan induced diabetic rats. Results are expressed as Mean± Standard deviation, $n = 5$.
*$P<0.05$ between blood glucose level at week 0 and week 4 in each group; [a]$P<0.05$ between blood glucose level at week 4 of each group and end of normal control; [b]$P<0.05$ between diabetic control and diabetic groups.

An increase in blood glucose was observed in diabetic control rats compared to normal control while treatment with glibenclamide, *P. pellucida* at 10% w/w and 20% w/w diet supplementation resulted in a 62%, 64% and 68% reduction

Table 1
Effect of *P. pellucida* on lipid profile in normal and alloxan induced diabetic rats.

Group No	Treatment	Total cholesterol (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Group A	Normal control	17.60±4.51	9.60±0.89	6.40±2.19	8.80±4.64
Group B	Normal + 10%w/w *P. pellucida*	15.00±3.81	8.00±7.18	8.60±2.51	5.70±2.52[b]
Group C	Normal + 20%w/w *P. pellucida*	14.40±5.60	7.20±3.70	9.80±3.49	4.36±2.65*
Group D	Diabetic control	27.60±7.83*	21.00±3.67*	3.40±1.34*	20.52±7.15*
Group E	Diabetic + 600 μg/kg Gilben.	19.00±6.09**	12.40±5.86**	5.80±1.92**	10.52±3.52**
Group F	Diabetic + 10% w/w *P. pellucida*	17.50±5.30**	10.75±6.50**	6.75±2.36**	9.30±5.67**
Group G	Diabetic + 20% w/w *P. pellucida*	15.00±5.77**	8.75±5.69**	8.25±1.26**[c]	8.75±1.54**

Result are expressed as Mean±Standard deviation ($n = 5$). *$P<0.05$ All groups compared with Normal control; **$P<0.05$ All diabetic treated compared with Diabetic control; [b]$P<0.05$ the two normal treated with *P. pellucida* compared; [c]$P<0.05$ Diabetic treated with *P. pellucida* compared with Diabetic + Glibenclamide.

Table 2
Effect of *P. pellucida* on AST, ALT and ALP in alloxan-induced diabetic rats (unit/L).

Group No	Treatment	AST	ALT	ALP
Group A	Normal control	16.20±7.05	8.60±6.91	10.80±4.32
Group B	Normal + 10%w/w *P. pellucida*	11.20±5.26	6.40±3.78	8.80±2.95
Group C	Normal + 20%w/w *P. pellucida*	17.80±9.23	9.20±5.54	13.40±3.78[b]
Group D	Diabetic control	31.20±14.53*	18.20±3.27*	26.20±4.34*
Group E	Diabetic + 600 μg/kg Gilben.	22.80±10.18	13.00±5.24**	15.00±9.51**
Group F	Diabetic + 10% w/w *P. pellucida*	18.25±9.09	11.25±5.06**	12.25±3.30**
Group G	Diabetic + 20% w/w *P. pellucida*	17.50±8.50	10.00±6.78**	10.75±1.50**

Table 3
Effect of *P. pellucida* on lipid peroxidation(LPO), gutathione concentration(GSH), superoxide dismutase(SOD) and catalase activities(CAT).

Treatment group	LPO (mmol/mg protein)	GSH (μ g/mg protein)	SOD (unit/mg protein)	CAT (units/mg protein)
Normal control	2.03±0.72	4.98±0.69	1.42±0.19	1.56±0.58
Normal + 10% w/w *P. pellucida*	1.58±0.42	5.88±0.85	1.54±0.52	2.18±1.06
Normal + 20% w/w *P. pellucida*	2.82±1.15	6.24±0.86*	1.62±0.73	2.54±0.85*
Diabetic control	4.31±2.36*	1.76±0.55*	0.32±0.17*	0.58±0.37*
Diabetic + 600 μ g/kg Gilben.	3.34±2.67	2.58±0.57* **	0.62±0.20* **	0.86±0.24
Diabetic + 10% w/w *P. pellucida*	2.06±0.44**	3.00±0.57* **	1.19±0.52** [c]	1.10±0.32**
Diabetic + 20% w/w *P. pellucida*	1.84±0.58**	4.05±0.52* ** [ac]	1.37±0.47** [c]	1.38±0.28** [c]

in blood glucose level respectively.

It was observed that the levels of total cholesterol, triglycerides and LDL – cholesterol except HDL–cholesterol were significantly ($P<0.05$) higher in case of alloxan induced hyperglycemic animals (Diabetic control) when compared with normal control (group 1) animals while the values of the above mentioned plasma lipid parameters were near to normal in case of animals receiving glibenclamide and diet supplemented with 10% and 20% *P. pellucida*. However, HDL–cholesterol increased significantly in these groups compared to the diabetic control (Table 1).

Table 2 shows the activities of AST, ALT and ALP of experimental rats. Diabetic rats showed significantly more activities of plasma AST, ALT and ALP respectively compared to normal control. Treatment with all plant supplemented diet and glibenclamide significantly reduced the activity of AST, ALT and ALP in the diabetic control rats ($P<0.05$).

Table 3 show the effect of P.pellucida on lipid peroxidation, GSH concentration and the activity of antioxidant enzymes, SOD and CAT. A statistically significant ($P<0.05$) increase in lipid peroxidation was observed in diabetic control animals compared to normal control and these values were brought down to almost normal by the plant in diabetic rats and below normal value in non–diabetic rats. The two supplement (10%w/w and 20%w/w) reduced lipid peroxidation by 52% and 57% respectively more effectively than glibenclamide (23%). GSH concentration, SOD and CAT activities were reduced significantly in diabetic rats compared to the control; however supplementation with the 10%w/w, 20%w/w *P. pellucida* supplements and glibenclamide increased the activities of these antioxidants.

4. Discussion

Diabetes mellitus is one of the most common chronic diseases and is associated with hyperglycemia, hyperlipidemia, increased oxidative damage, glucosuria and other complications such as obesity and hypertension[27]. Alloxan, β – cytotoxin induces chemical diabetes in a wide variety of animal species by damaging the insulin producing pancreatic β – cell resulting in a decrease in endogenous insulin release which paves the way for the decreased utilization of glucose by tissues[9,28].

Our findings indicate that *P. pellucida* has antidiabetic and antioxidant activities. A general increase was observed in the level of blood glucose in the diabetic control rats. This increase is significant when compared to the normal control, normal rats fed with different percentages (10% and 20% w/w) of *P. pellucida* and the various diabetic rats treated with glibenclamide and *P. pellucida*. The hypoglycemic action was more pronounced with both 10% and 20% *P. pellucida* supplementation than glibenclamide treated group. This may therefore imply that the *P. pellucida* preparation at 10% and 20% w/w supplement are slightly more potent than glibenclamide at 600 μ g/kg body weight dose. Though no mechanism to the effect of *P. pellucida* on blood glucose has been proposed, it could be suggested that the plant might contain substances that mimic the action of insulin just like the sulphonylureas that promote insulin secretion by closure of K^+–ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion[29,30].

It is reported that the derangement of glucose, fat and protein metabolism during diabetes, results into the development of hyperlipidemia[31,32]. Diabetic rats were observed to have increased plasma lipids, which are responsible for several cardiovascular disorders[33]. The higher lipid levels seen in diabetic rats may be due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones[34]. Supplementation with both supplements of *P. pellucida* produced significant beneficial effects in the lipid profile in alloxan–induced diabetic rats, reducing triglycerides, total cholesterol, LDL, and increasing HDL, significantly. Thus, it can be concluded from this findings that the levels of total plasma cholesterol, triglycerides and LDL–cholesterol which are actually raised in diabetes can be lowered with *P. pellucida* supplementation; this antihyperlipidemic effect could represent a protective mechanism against the development of cardiovascular disorder such as atherosclerosis in diabetic patients.

The hepatic enzymes AST, ALT and ALP were used as biomarkers to check for early acute hepatic damage. The activities of AST and ALT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore,

elevated activities of ALT and AST in the circulation serve as indicators of hepatic damage. ALP acts as a marker for biliary function and cholestasis. In this study, all treatment groups with experimental plant preparation effectively reduced plasma AST, ALT and ALP activities in diabetic rats better than the standard drug, suggesting that the plants may prevent hepatic injury associated with diabetes.

Oxidative stress resulting from enhanced free radical formation and/or defects in anti-oxidants defense causes severe tissue damage which may lead to a number of diseases like coronary artery disease, atherosclerosis, cancer and diabetes. Increased oxidative stress in streptozotocin diabetic rats has been reported[35].This oxidative stress is also implicated in the development of diabetic complications[36]. Increased oxidative stress as measured by indices of lipid peroxidation and protein oxidation has been shown to be elevated in both type1 and type 2 diabetes even in patients without complications[37,38].

The results showed increased lipid peroxidation in the liver of diabetic control group. This may be because the tissues contain relatively high concentration of easily peroxidizable fatty acids. The increase in oxygen free radicals in diabetes could be primarily due to increase in blood glucose levels, which upon auto-oxidation generate free radicals and secondarily due to the effects of diabetogenic agent of alloxan[39]. In diabetes, hypoinsulinaemia increases the activity of the enzyme, fatty acyl coenzyme, coenzyme A oxidase, which initiates β-oxidation of fatty acids resulting in lipid peroxidation[9]. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity, and changing the activity of membrane-bound enzymes[36]. Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with various disease condition, atherosclerosis and brain damage[36]. Supplementation with *P. pellucida* and glibenclamide reduced the extent of lipid peroxidation in the diabetic treated group. This indicates that *P. pellucida* supplement may inhibit lipid peroxidation and thereby oxidative damage to tissues in diabetes.

Decreased glutathione (GSH) concentration in diabetes mellitus under *in vivo* concentration has been reported[40]. Supplementation with *P. pellucida* supplement significantly increased the glutathione content compared to diabetic control rats where the levels were significantly decreased. This depletion of GSH in diabetic rats can be attributed to GSH consumption by glutathione transferase for metabolic conjugation and to GSH oxidation for defense against the produced oxidative stress[6]. Elevated level of GSH treatment with *P. pellucida* therefore indicates recovery from oxidative damage done by alloxan diabetes on tissues.

SOD and CAT are the two scavenging enzymes that remove the toxic free radicals[41]. Superoxide dismutase is present in essentially every cell in the body and has been shown to play an important role in protecting cells and tissues against oxidative stress. Superoxide dismutases (SOD) remove the superoxide radical $O_2^{\bullet -}$ by accelerating its conversion to H_2O_2. Catalase is ubiquitous to most aerobic cells in animals and is especially concentrated in the liver and erythrocytes. It is known to decompose hydrogen peroxide into water and oxygen as shown in the equation:

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (1)$$

The result showed that there was a significant reduction in SOD and CAT activity in diabetic rats compared to normal control while all treated groups showed significant increase in activity with 20% w/w supplement showing greater increment compared to either group treated with the drug and the 10% w/w supplement. This decrease in the activity of SOD observed in diabetic rats is consistent with various reports already documented[40,41]. As proposed by Wohaieb and Godin[41], the reduction in SOD activity might be due to the direct damaging effect of free radicals on the enzyme. Therefore the activity of SOD, which is to remove the superoxide radical O_2^- by accelerating its conversion to H_2O_2 is inactivated. The ability of the plant preparation to increase catalase activity may be due to the induction of the enzyme. Catalase is haemoprotein and phytochemical screening of *P. pellucida* has also shown the presence of iron (Fe)[42]. Iron is essential for the function of catalase.

The results of the present study show that diet supplemented *P. pellucida* have an antidiabetic and antioxidant property in alloxan induced diabetic rats. This effect may be due to the presence of tannin, saponin, flavonoid and other constituents in the plant, which could act synergistically or independently in enhancing the activity of glycolytic and antioxidant enzymes. Therefore the acclaimed traditional antidiabetic use of this plant is justified in this study.

Conflict of Interest

There was no conflict of interest among the authors.

References

[1] Trivedi B, Mazumdar JD, Bhatt KG. Effect of Shilajit on blood glucoseand lipid profile in alloxan-induced diabetic rats. *Indian J Pharmacol* 2004; **36**: 373-376.

[2] Andrew IR, Scott BE, Clarke HH, Michael DE, Scott C. Microvascular complications in cystic fibrosis-related diabetes mellitus: a case report. *J Pancreas* 2000; **14**: 208-210.

[3] Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. *Diabetologia* 1990; **33**: 462-464.

[4] Nelson DL, Cox MM. *Leninger Principles of Biochemistry* (4th edn). 2000, p. 909-910.

[5] Kakkaar R, Kalra J, Mantha SV, Prasad K. Lipid peroxidaton and activity of antioxidant enzymes in diabetic rats. *Mol Cell Biochem* 1995; **151**: 113-119.

[6] Halliwell B, Gutteridge JMC. *Free radicals in biology and*

medicine. Oxford: Clarendon Press; 1989.

[7] Hughes K, Choo M, Keperan P, Ong CN, Aw TC. Cardiovascular risk factors is non-insulin dependent diabetes compared to non-diabetic controls. A population based survey among Asians in Singapore. *Athero* 1998; **136**: 25-31.

[8] Neville A, Punchard F, Kelly J. *Free radicals. a practical approach*. Oxford: Oxford University Press; 1996, p. 1-7.

[9] Oberley LW. Free radicals and diabetes. *Free Rad Biol Med* 1988; **5**: 113-124.

[10]Garg MC, Bansal DD. Protective antioxidant effect of vitamin C and E in streptozotocin induced diabetic rats. *Indian J Expt Biol* 2000; **38**: 101-104.

[11]Battacharya SK, Satyan KS, Ghosal S. Antioxidant activity of glycowithanolides from *Withania somnifera*. *Indian J Expt Biol* 1997; **5**: 236-239.

[12]Manonmani G, Anbarasi K, Balakrishna K, Veluchamy G, Devi CS. Effect of *Terminalia arjuna* on the antioxidant defence system in alloxan induced diabetes in rats. *Biomed* 2002; **22**: 52-61.

[13]Carriconde C. Coraçãozinho, *Peperomia pellucida* H.B.K. In: De Volta Às Raízes, Olinda: Centro Nordestino de Medicina popular, *Olinda (Brazil)* 1997; **12**: 2-3.

[14]Aziba PI, Adedeji A, Ekor M, Adeyemi O. Analgesic activity of *Peperomia pellucida* aerial parts in mice. *Fitoterapia* 2001; **72**: 57-58.

[15]Bojo AC, Albano-Garcia E, Pocsidio GN. The antibacterial activity of *Peperomia pellucida* (L.) HBK (Piperaceae). *Asia Life Sci* 1994; **3**: 35-44.

[16]Bayma JD, Arruda MS, Muller AH, Arruda AC, Canto WC. A dimeric ArC2 compound from *Peperomia pellucida*. *Phytochem* 2000; **55**: 779-782.

[17]National Committee for Clinical Laboratory Standards, Interference Testing in Clinical Chemistry; Proposed Guideline, *NCCLS document* 1986; EP7 - P.

[18]Roeschlau P, Bernt E, Gruber JW. *Clin Chem Clin Biochem* 1973; **12**: 403-407.

[19]Tietz NW. *Clinical Guide to Laboratory Tests*, Second Edition. Philadephia: W. B. Saunders Company; 1990, p. 554-556.

[20]Lopes-Virella MF, Stone P, Ellis S. Cholesterol determination in high density lipoprotein separated by three methods. *Clin Chem* 1977; **23**: 882.

[21]Friedwald WT, Levy RL, Fredickson DS. Estimation of the concentration of low Density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499.

[22]Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic transaminases. *Am J Clin Pathol* 1957; **28**: 56-63.

[23]Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; **247**: 3170-3175.

[24]Sinha AK. Colorimetric assay of Catalase. *Analytical Biochem* 1972; **47**: 389-394.

[25]Beutler E, Duron O, Kelly BM. Improved method for determination of blood glutathione. *J Lab Clin Med* 1958; **61**: 882-888.

[26]Varshney R, Kale KK. Effect of calmoldulin antagonists on radiation induced peroxidation in microsomes. *Int J Radiat Biol* 1990; **58**: 733-743.

[27]Bierman EL, Amaral JAP, Balknap BH. Hyperlipidemia and diabetes mellitus. *Diabetologia* 1975; **25**: 509-515.

[28]Lenzen S, Panten U. Alloxan: history and mechanism of action. *Diabetologia* 1998; **31**: 337-342.

[29]Latha M, Pari L. Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. *Clin Exp Pharmacol Physiol* 1983; **30**: 38-43.

[30]Venkateswaran S, Pari L. Effect of *Coccinia indica* on blood glucose, insulin and hepatic key enzymes in experimental diabetes. *Pharm Bio* 2002; **40**: 165-170.

[31]Brown MS, Goldstein JL. Lipoprotein receptor in the liver. Control signals for plasma cholesterol traffic. *J Clin Invest* 1983; **72**: 743-747.

[32]Austin MA, Hokanson JE. Epidemiology of triglyceride, small dense low- density lipoprotein and lipoprotein as risk factors for coronary heart disease. *Med Clin North Am* 1994; **78**: 99-115.

[33]Alarcon-Aguilar FJ, Campos-Sepulveda AE, Xolalopa-Molina S, Hernandez-Galicia E, Roman-Ramos R. Hypoglycemic activity of *Ibervillea sonorae* root in healthy and diabetic mice and rats. *Pharma Biol* 2002; **40**: 570-575.

[34]Nikkhila EA, Kekki M. Plasma triglyceride transport kinetics in diabetes mellitus. *Metabolism* 1973; **22**: 1-22.

[35]Garg MC, Ojha S, Bansal DD. Antioxidant status of STZ diabetic rats. *Indian J Exp Biol* 1996; **34**: 264-266.

[36]Baynes JW. Reactive oxygen in the aetiology and complications of diabetes. In: Ioannides C, Flatt PR. (Ed). *Drug, Diet and Disease, Mechanistic Approach to Diabetes*. Hertfordshive: Ellis Horwood limited; 1995.

[37]Laaksonen DE, Sen CK. Exercise and Oxidative Stress in Diabetes Mellitus. In: Sen CK, Packer L and Hanninen O. *Handbook of Oxidants and Antioxidants in Exercise*. Amsterdam: Elsevier 2000, p. 1105-1136.

[38]Cederberg J, Basu S, Eriksson UJ. Increased rate of lipid peroxidation and protein carbonylation in experimental diabetic pregnancy. *Diabetologia* 2001; **44**: 766-774.

[39]Szkudelski T. The mechanism of alloxan and streptozotocin action in b cells of the rat pancreas. *Physiol Res* 2001; **50**: 536-546.

[40]Illing EKB, Gray CH, Lawrence RD. Blood glutathione and non-glucose reducing substances in diabetes. *J Biochem* 1951; **4**: 637.

[41]Wohaieb SA, Godin DV. Alterations in free radical tissue defense mechanisms in streptotozcin-induced diabetes in rat: effects of insulin treatment. *Diabetologia* 1987; **36**: 169.

[42]Egwuche RU, Odetola AA, Erukainure OL. Preliminary Investigation into the chemical properties of *Peperomia pellucida* (L.). *Res J Phytochem* 2011; **5**(1): 48-53.