International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 4, 2016

Original Article

ANTI-HYPERGLYCEMIC ACTIVITY OF HYDRO-ALCOHOLIC BARK EXTRACT OF *MANILKARA HEXANDRA* (ROXB) IN STREPTOZOTOCIN INDUCED DIABETIC RATS

TRISHNA DAS[1*], BHABESH DAS[2], DIPANKAR SAHA[1], SHANTI BHUSHAN MISHRA[3]

[1]Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati, India, [2]Department of Shalya Tantra, Government Ayurvedic College, Jalukbari, Guwahati, India, [3]Department of Pharmacognosy, United Institute of Pharmacy, Naini, Allahabad 211010 India
Email: trishna2684@gmail.com

Received: 05 Dec 2015 Revised and Accepted: 18 Feb 2016

ABSTRACT

Objective: *Manilkara hexandra* Roxb. (Family: Sapotaceae) is a common evergreen tree and commercial crop in India which is widely used as antibacterial, diuretic, anthelmintic, antioxidant and antidiabetic in folklore medicine in India. The objective of the study was to evaluate the antidiabetic activity of *Manilkara hexandra* bark in streptozotocin-induced diabetes in experimental animals.

Methods: Non-insulin-dependent diabetes mellitus (NIDDM) was induced in overnight fasted rats by an intra-peritoneal injection (i. p.) of 60 mg/kg streptozotocin. 50 % ethanolic extract of *M. hexandra*, 250 mg/kg or 500 mg/kg body weight was administered orally to the rats once daily for 21 d. The blood glucose level was assessed by a glucometer. The serum levels of cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were determined by using diagnostic kits.

Results: A significant reduction ($p<0.001$) in the blood glucose level was observed in diabetic animals treated with the different doses of the extract, compared to untreated diabetic rats. The drug posses good hypolipidemic effect by normalizing the lipid parameters. This also evidenced by histopathological examination of isolated organs viz. pancreas and kidney showing reduced the injuries induced by streptozotocin.

Conclusion: The result of this study thus shows that 50 % of the ethanolic extract at different doses possesses significant antidiabetic activity and potent hypolipidemic potential in diabetic conditions.

Keywords: Antidiabetic, Bark extract, Histopathology, *Manilkara hexandra*, Streptozotocin

INTRODUCTION

Diabetes mellitus is linked with prejudice glucose metabolism that escorts to a rise in free radical production and augmentation in the lipoprotein and triglyceride levels. The purported Indian Phenotype proposed to have inimitable biochemical as well as a clinical idiosyncrasy in the Indians of Asia. This assemblage of abnormalities is well thought-out to be one of the foremost factors contributing to raising pervasiveness of type 2 diabetes in Indians of Asia. The ultimate goal of diabetes therapy is to prevent micro-and macrovascular complications in order to improve life expectancy and quality of life [1]. The general consensus on treatment of type 2 diabetes is that lifestyle management is at the forefront of therapy options. In addition to exercise, weight control, and medical nutrition therapy, oral glucose-lowering drugs and injections of insulin are the conventional therapies. Since the most important pathological process during the development of diabetes involves three key organs, i.e., pancreatic islets, liver, and skeletal muscle, almost all anti-diabetic therapies are aimed at these organs.

Experimental diabetes in animals has endowed with an extensive approach into the physiologic and biochemical clutter of the diabetic state. In recent years, the plants provide a potential source of hypoglycemic drugs because many plants and plant-derived compounds have been used in the treatment of diabetes. The use of plants as a source of drugs and their validation is gaining increasing attention [2]. Because of this bleak forecast, research on anti-diabetic plants has been strongly encouraged by World Health Organization. *Manilkara hexandra* (Roxb.) Dubard is a large evergreen tree widely distributed throughout the greater parts of India and other tropical countries. The stem bark is astringent, febrifuge, sweet, tonic, and is used traditionally to treat a wide range of gastrointestinal symptoms.[3]Previously isolated constituents of the plant are Saponins, cinnamic acid, hentriacontane, taraxerol, quercitol, gallic acid, hexandrone, hexandrin, taraxerol, quercetin, quercitrin and beta-sitosterol [4, 5]. Although decoction of *M. hexandra* bark has been used to treat diabetes as a folk medicine, no scientific report exists for the use of this plant to treat diabetes. Therefore an attempt has been made for investigating its antihyperglycemic activity and effects on total lipid, cholesterol, and triglycerides as scientific evidence.

MATERIALS AND METHODS

Plant material

Fresh bark was stripped from the trunk of fully-grown tree *Manilkara hexandra* (Roxb.) Dubard (Sapotaceae) from BSI campus, Allahabad and authenticated by a taxonomist Dr. A S Sandhu, of National Institute of Pharmaceutical Education & Research (NIPER), Mohali with voucher specimen number NIP-NPM-CD-157.

Extract preparation

The freshly collected bark (2 kg) of *M. hexandra* were first air-dried and then dried in tray drier under control conditions and powdered. The powdered bark (1 kg) was macerated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of ethanol (50 % v/v) for 72 h by cold percolation method. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure, and thus, 120.0 g of solid residue (yield 12 % w/w) was obtained.

Phytochemical screening

Phytochemical screening of extract was carried out by standard procedures [6], which revealed the presence of chemical constituents like fixed oil, flavonoids, phenolic compounds phytosterol and saponins.

Experimental animals

Healthy adult Wistar albino rats weighing 200–250 g were used for the pharmacological studies. These animals were kept in polypropylene cages for a minimum of 5 d prior to oral administration

at the Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Azara, Guwahati animal room to allow for their acclimatization to the laboratory conditions. The animal room was ventilated with 12-h cycle of day and night light conditions, and the temperature maintained at approximately 25 ˚C. They were fed with standard rat pellet diet (Amrut, India) and water *ad libitum*. The protocol for this study was approved by the Girijananda Chowdhury Institute of Pharmaceutical Science Institutional Animal Ethical Committee (No. GIPS/IAEC/2012/06) Guwahati.

Oral acute toxicity study

The median lethal dose (LD_{50}) determination was done in rats by OECD guidelines 423 [7]. A single dose of the extracts (5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg) in an appropriate quantity of water was given orally by gavage to a different group of animals (three each). The animals were allowed free access to water and food. However, all the animals were deprived of food for 2 hr before and 4 hr after dosing. Each animal was observed each time for the first 5 min after loading for signs of regurgitation and then kept in individual polypropylene rat cage. Each animal was observed for every 15 min in the first 4 h after dosing, then every 30 min for the successive 6 h and then daily for the successive 48 h for the short-term outcome and the remaining 14 d for the long-term possible lethal outcome.

Experimental induction of diabetes

Streptozotocin (STZ) was freshly dissolved in (0.1M, pH 4.5) citrate buffer and maintained on ice prior to use. Non-insulin-dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of Streptozotocin (60 mg/kg b. w), all animals were given free access to food and water. Blood glucose levels were measured 2 d after STZ injection and used as parameters to obtain matching pairs of rats with diabetes of similar level of severity. Only rats with fasting blood glucose levels greater than 220 mg/dl were considered to be diabetic and were used in the experiment. The animals were randomly assigned to five different groups i.e. group I to V. Group I served as a control containing 6 normal rats [8].

Experimental design

Five groups of rats were used to study the effect of 50 % ethanolic extract of *M. hexandra.* Each group consisting of six rats.

Group I-Control rats received vehicle normal saline solution

Group II-Diabetic control rats received vehicle normal saline solution

Group III and IV-Diabetic rats treated with extract 250 & 500 mg/kg body weight respectively.

Group V-Diabetic rats treated with standard drug Glibenclamide 600 µg/kg body weight

Treatment of experimental animals with plant extracts was initiated 2 d post streptozotocin injection and was carried out once daily, by gavage, for 21 d. Food and water were made freely available. The blood glucose level was determined by glucometer (Sugar scan). The values of the sample treated were compared with that of the standard group which was treated with Glibenclamide. After the experimental regimen, the animals were sacrificed by cervical dislocation under mild ether anesthesia. The kidney and pancreas were exposed and perfused with cold saline phosphate buffer of pH 7.4 for histopathological examination. The collected blood samples were immediately centrifuged at 3000 rpm for 10 min. The serum separated was collected in fresh serum tubes and stored in the refrigerator (2-4˚C) after tightly capped. The total serum Cholesterol, Triglyceride, and lipid concentrations were determined using commercial kits by enzymatic photocolorimetry methods [9].

Histopathological examination

At the end of the treatment period the control, as well as treated rats, were sacrificed by using cervical dislocation and the organs viz. pancreas and kidney were dissected out and immediately fixed in bouin's fluid for 24 hr and washed in running tap water to remove the colour of bouin's fluid and dehydrated in alcohol in ascending and descending order, embedded in paraffin and cut at 5µm in a rotary microtome. These sections were then deparaffinized in xylene and stained with hematoxylin-eosin and mounted with canada balsam. The histopathological slides were examined, and photographs were captured with a digital stereomicroscope (Olympus, B061) [10].

Statistical analysis

The statistical analysis of all the pharmacological analysis was carried out using GraphPad Prism version 3.03 for windows. The values are represented as mean±SD. for six rats data were analyzed by ANOVA with post-hoc difference was analyzed using Newman-keuls method.

RESULTS

Acute toxicity study

In acute toxicity study, extract treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weights and food consumption when compared to the vehicle-treated group. Also, no gross pathological changes were seen. Thus, it was concluded that aqueous alcoholic extract of *M. hexandra* was safe at 2000 mg/kg. On the basis of toxicity study, two doses i.e. 250 and 500 mg/kg were selected for antidiabetic activity.

Antihyperglycemic activity

There was a significant elevation in serum glucose, total cholesterol, triglycerides and lipid in the diabetic control group animals. Table 1 shows the effect of the daily oral administration of 50 % ethanolic extract (250 mg/kg and 500 mg/kg) on the blood glucose level in 21 d. By the end of treatment, the blood glucose level reduced from 245.33 mg/dL to 124.33 mg/dL in rats treated at a dose of 250 mg/kg ($p<0.01$). For the oral administration of 500 mg/kg of the extract, the blood glucose level reduced from 248.16 mg/dL to 85.00 mg/dL at 21 d ($p<0.001$). For glibenclamide (600 µg/kg), the blood glucose level reduced from 241.16 mg/dL to 85.83 mg/dL ($p<0.001$). Table 2 shows the levels of biochemical parameters such as the levels of total cholesterol, triglycerides, and total lipids. MHE at the dose of 250 mg/kg & 500 mg/kg decreasing the cholesterol (82.59±5.06 & 76.11±5.53 mg/dl respectively as compared to diabetic control group (136.51±5.85 mg/dl) ($p<0.001$) and triglycerides level (96.20±5.01 & 82.88±3.72 mg/dl as compared to diabetic control group (154.81±3.83) ($p<0.001$). MHE also showed significantly ($p<0.001$) effect on HDL and LDL level. By contrast, the 50 % ethanolic extract of *M. hexandra* bark in the groups treated at a dose of 250 mg/kg or 500 mg/kg once daily for 21 d prevented the diabetic condition in a dose-related manner.

Table 1: Effect of 50 % ethanolic extract of *M. hexandra* on serum glucose level in STZ induced diabetic rats

Groups	Treatment/Dose	0 d (mg/dl)	After 7 d (mg/dl)	After 14 d (mg/dl)	After 21 d (mg/dl)
I	Normal control	76.16±4.74	76.83±4.17	76.33±5.02	74.50±3.09
II	Diabetic control	246.33±6.62****	234.33±10.99****	200.33±4.81****	235.66±4.95****
III	MHE (250 mg/kg)	245.33±4.60	230.33±7.49	185.66±5.93*	124.33±4.57**
IV	MHE (500 mg/kg)	248.16±4.29	219.16±8.27	184.16±10.12**	85.00±5.32***
V	Glibenclamide (600µg/kg)	241.16±8.83	210.66±10.59	180.83±8.98	85.83±8.93***

The value represents the means±S. D. for 6 rats per group. *p<0.05, **p<0.01 and ***p<0.001 compared to diabetic control group. ****p<0.001 as compared to the normal group.

Table 2: Effect of 50 % ethanolic extract of *M. hexandra* stems bark on TG, Cholesterol, HDL and LDL in STZ induced diabetic rats

Groups	Treatment/Dose	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
I	Normal control	71.03±2.92	74.69±4.72	39.45±3.97	20.16±2.52
II	Diabetic control	154.8±3.83****	136.51±5.85****	25.08±3.40****	33.31±3.98****
III	MHE (250 mg/kg)	96.2±5.01***	82.59±5.06***	35.47±4.93***	19.50±1.63***
IV	MHE (500 mg/kg)	82.88±3.72***	76.11±5.53***	45.33±4.71***	12.74±3.74***
V	Glibenclamide (600µg/kg)	75.35±3.27***	84.98±5.41***	34.35±5.47***	24.14±2.15***

The value represents the means±SD. for 6 rats per group. ***p<0.001 compared to diabetic control group. ****p<0.001 as compared to normal group.

Histopathological investigation

Pancreas

Microscopically examined pancreas section of the control group (1A) showed Islets are normal. The architecture is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

Microscopically examined pancreas section of the diabetic control group (1B) showed Islets with depletion of cells. The architecture is preserved. The acini lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

Microscopically examined pancreas section of 50 % ethanolic extract of the bark of *M. hexandra* treated group 250 mg/kg (1C) showed the architecture is partially effaced. The islets are normal. The acinar cells are normal. There is a mild and diffuse infiltrate of lymphocytes within the stroma.

Microscopically examined pancreas section of 50 % ethanolic extract of the bark of *M. hexandra* treated group 500 mg/kg (1D) showed the architecture is normal. The islets show depletion of the acinar cell. The acinar cells show moderate cytoplasm and round to oval nuclei. There is no evidence of inflammation.

The normal architecture was restored to the same as that of the standard drug (Glibenclamide) treated pancreas (1E).

Kidney

Microscopically examined kidney section of the control group (2A) showed the glomeruli appear and tubules are normal and lined by a single layer of cuboidal cells.

Microscopically examined kidney section of the diabetic control group (2B) revealed the glomeruli show normal mesangial hypercellularity and focal glomerulosclerosis. The stroma shows a diffuse infiltrate of lymphocytes.

Microscopically examined kidney section of 50 % ethanolic extract of the bark of *M. hexandra* at a dose of 250 and 500 mg/kg treated group (2C & 2D) illustrate that the glomeruli show mesangial hypercellularity. The tubules are normal. The stroma is normal.

The normal architecture was restored to the same as that of the standard drug (Glibenclamide) treated kidney (2E). which revealed that glomeruli appear normal. The tubules are normal and lined by a single layer of cuboidal cells. The stroma is normal. This is evident that 50 % ethanolic extract of the bark of *M. hexandra* possess good antidiabetic activity.

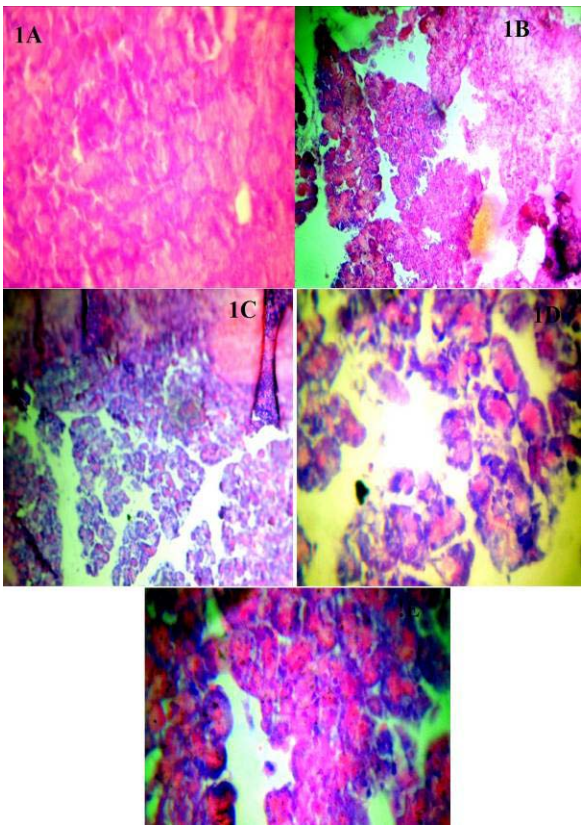

Fig. 1: Histopathology of pancreas

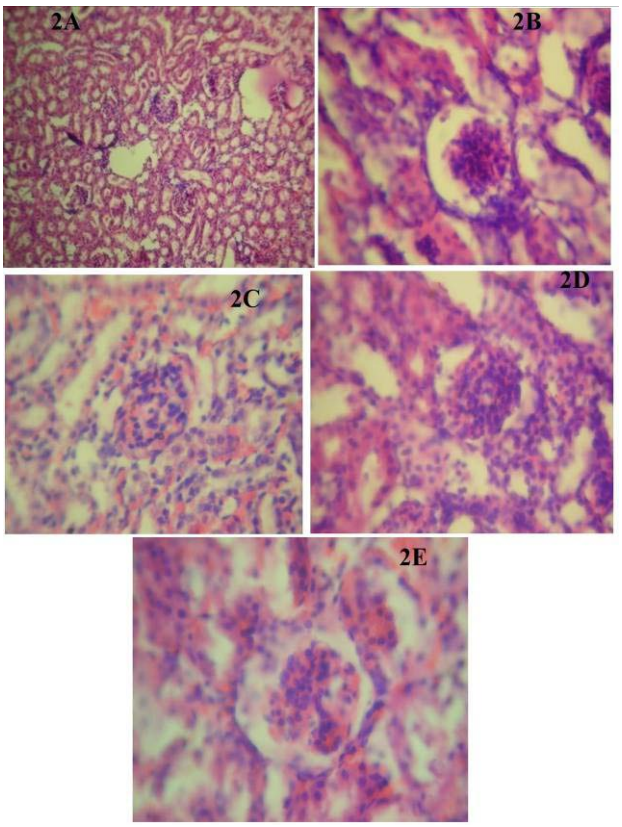

Fig. 2: Histopathology of kidney

DISCUSSION

Streptozotocin-induced diabetes in rats appears to be the most suitable animal model because it reflects the symptoms of diabetes in human. The elevated blood glucose levels in diabetes are thought to lead to cell death through oxidative stress induction that occurs as a common sequel of diabetes-induced modification of sugar moieties on proteins and lipids [11]. Hyperglycemia increases oxidative stress through the overproduction of reactive oxygen species, which results in an imbalance between free radicals and the antioxidant defense systems of the cells. In the present study, 50 % ethanolic extract of bark of *Manilkara hexandra* (MHE) at different doses (250 and 500 mg/kg body weight) showed a marked antihyperglycemic and hypolipidemic effects which could be attributed to the potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells or their radical scavenging activities. Diabetes is also known to be associated with an increase in the synthesis of cholesterol, which may be due to the increased activity of HMG-CoA reductase. A number of observations indicate that plasma high-density lipoprotein (HDL) cholesterol is low in untreated insulin-deficient diabetics, [12, 13] which was associated with a decline in HDL-turnover rate. Further, the HDL-cholesterol levels correlate with lipoprotein lipase (LPL) levels in IDDM patients [14]. Increased LDL-cholesterol may arise from glycosylation of the lysyl residues of apoprotein B as well as from decreasing affinity for the low-density lipoprotein (LDL) receptor and hence, decreased metabolism [15]. The ability of LDL-cholesterol to form lipid peroxides was found specifically responsible for the atherogenesis in diabetic patients.[16] Bruan and Severson (1992) have reported that deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of triglycerides in diabetes. The elevated serum phospholipid levels are a consequence of elevated lipoproteins [17]. Our investigations indicate the efficiency of the extract in the maintenance of blood glucose levels in streptozotocin-induced diabetic rats. Administration of aqueous ethanolic extract of *M. hexandra* to diabetic rats showed a significant reduction in the levels of blood glucose. The possible mechanism by which 50 % ethanolic extract brings about its hypoglycemic action in diabetic rats may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form due to the presence of flavonoids in the bark extract. The investigations also indicate that oral administration of plant extracts normalized these effects, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues. Thus, it may be concluded plant extracts may have a stimulatory effect on insulin. The restoration of phospholipids by plant extract may be controlled mobilization of serum triglycerides; controlling the tissue metabolism and improving the level of insulin secretion and action presumably mediate cholesterol and phospholipids. This is also evidenced by histopathological examination of isolated organs showing reduced the injuries induced by streptozotocin.

CONCLUSION

The present study provided evidence indicating that 50 % ethanolic extract of *M. hexandra* bark significantly reduces the levels of glucose in diabetic rats. In addition, treatment with the extract causes the recovery of certain altered biochemical parameters of diabetic animals. This being so, these results validates the traditional use of the plant in the treatment of diabetes.

ACKNOWLEDGEMENT

The authors sincerely thank Prof. (Dr.) Suvakanta Dash, Principal, Girijananda Chowdhury Institute of Pharmaceutical Science, Guwahati, for providing all the animal house facilities to carry out preclinical studies.

CONFLICTS OF INTERESTS

We declare that we have no conflict of interest.

REFERENCES

1. Joy KL, Kuttan R. Antidiabetic activity of Picorrhiza kurroa extract. J Ethnopharmacol 1999;167:143-8.
2. Rates SMK. Plants as a source of drugs. Toxicon 2001;39:603-13.
3. Khare CP. *Indian Medicinal Plants*, An Illustrated Dictionary, Springer-Verlag Berlin; 2007. p. 397-8.
4. Misra G, Mitra CR. *Mimusops hexandra*–III. Constituents of the root, leaves and mesocarp. Phytochem 1968;7:2173–276.
5. Eskander J, Haggag E, El–Gindi M, Mohamedy M. A novel saponin from *Manilkara hexandra* seeds and anti–inflammatory activity. Med Chem Res 2013;23:717–24.
6. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*, Nirali prakashan; 2008. p. 607-11.
7. OECD. Guidelines number 423 for the testing of chemicals revised draft guideline 423. (Acute oral toxicity). Paris: OECD; 2000.
8. Mishra SB, Vijayakumar M. Antihyperglycemic and antioxidant effect of *Saraca asoca* (Roxb. De Wilde) flowers in streptozotocin-nicotinamide-induced diabetic rats: a therapeutic study. J Bioanal Biomed 2014; S12:1-5.
9. Mishra SB**,** Verma A, Vijayakumar M. Preclinical evaluation of antihyperglycemic and antioxidant action of Nirmali (*Strychnos potatorum*) seeds in streptozotocin-nicotinamide-induced diabetic Wistar rats: a histopathological investigation. Biomarkers Genomic Med 2013;5:157-63.
10. Culling CFA. Handbook of histopathological and histochemical techniques. Butterworth and Co. (Publishers) Ltd. London; 1974. p. 194-6.
11. Donnini D, Zambito AM, Perella G. Glucose may induce cell death through a free radical-mediated mechanism. Biochem Biophys Res Commun 1996;219:412-7.
12. Glasgow AM, August GP, Hung W. Relationship between control and serum lipids in juvenile onset diabetes. Diabetes Care 1981;4:76–80.
13. Goodman MW, Michels LD, Keane WF. Intestinal and hepatic cholesterol synthesis in the alloxan diabetic rats. Proc Soc Exp Biol Med 1982;170:286–90.
14. Nikkila EA, Huttunen JK, Ehnholm C. Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. Diabetes 1977;26:11–21.
15. Golay A, Chen YD, Reaven GM. Effect of differences in glucose tolerance on insulin stability regulate carbohydrate and free fatty acid metabolism in obese individuals. J Clin Endocrin Metabol 1986;2:1081–8.
16. Kondo A, Muranaka Y, Ohta I, Notsu K, Manabe M, Kotani K, *et al*. Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. Clin Chem 2001;47:893–900.
17. Bruan JEA, Severson DL. Lipoprotein lipase release from cardiac myocytes is increased by decavanadate but not insulin. Am J Physiol 1992;262: E663–70.