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Effect of N-benzoyl-D-phenylalanine on streptozotocin-induced changes in the lipid and lipoprotein profile in rats

N. Ashokkumar, L. Pari, A. Manimekalai and K. Selvaraju

Abstract

The effect of N-benzoyl-D-phenylalanine (NBDP) and metformin combination treatment on circulatory lipids, lipoproteins and lipid peroxidation markers were studied in neonatal streptozotocin (nSTZ) non-insulin dependent diabetic rats. Non-insulin dependent diabetes mellitus (NIDDM) was induced by a single dose injection of streptozotocin (100 mg kg^{-1}, i.p.) to two-day-old rats. After 10–12 weeks, rats weighing above 150 g were selected for screening for the NIDDM model. The rats were checked for fasting blood glucose levels to confirm the status of NIDDM. NBDP (50,100 or 200 mg kg^{-1}) was administered orally for six weeks to the confirmed diabetic rats (to evaluate the effective dose). The levels of serum lipids and lipid peroxidation markers were significantly increased, whilst the activity of glucose-6-phosphate dehydrogenase was significantly decreased in nSTZ diabetic rats. NBDP and metformin were able to restore the altered serum lipids, lipoproteins, lipid peroxidation marker levels and glucose-6-phosphate dehydrogenase activity to almost control levels. The results showed the antihyperlipidaemic properties of NBDP and metformin in addition to its antidiabetic action. Combination treatment was more effective then either drug alone. The results indicated that the coadministration of NBDP with metformin to nSTZ diabetic rats normalized blood glucose and caused marked improvement in altered serum lipids, lipoproteins and lipid peroxidation markers during diabetes. The data indicated that NBDP represented an effective antihyperglycaemic and antihyperlipidaemic adjunct for the treatment of diabetes, and may be a potential source of new orally active agents for future therapy.

Introduction

Diabetes mellitus is a major source of morbidity in developed countries and, among its comorbid conditions, atherosclerosis is perhaps the most important. In spite of the availability of insulin, up to three-quarters of all deaths among diabetics can be directly attributed to coronary artery disease (Bierman 1992). A number of known factors for coronary artery disease, such as hypertension, obesity, and dyslipidaemia are more common in diabetics than in the general population. The number of patients with diabetes mellitus worldwide is increasing and it is estimated that there will be more than 220 million people with this disease by the year of 2010 (Zimmet 1999), and most of the people will have type 2 diabetes. Cardiovascular disease constitutes the main cause of morbidity and mortality in diabetes, especially in type 2 diabetes.

Oxidative stress is postulated to be increased in patients with diabetes mellitus. There are many ways by which hyperglycaemia may increase the generation of oxygen free radicals, such as glucose autoxidation (Diaz-Flores et al 2004), autoxidative glycosylation (Gillery 2001), activation of protein kinase C (Yakubu et al 2001), and increased polyol pathway metabolism (Bonnardel-Phu & Vicaut 2000). There is also evidence that elevations in glucose concentrations may depress natural antioxidant defenses such as glutathione (Yoshida et al 1995). The imbalance between the generation of oxygen free radicals and antioxidant defense system may increase the oxidative stress and lead to the damage of macromolecules, such as DNA, proteins and lipids.

A number of oral hypoglycaemic or antihyperglycaemic agents have been developed for the treatment of type 2 diabetes with differing mechanisms of action. The most recently

Department of Biochemistry, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu – 608 002, India

N. Ashokkumar, L. Pari

Department of Chemistry, Faculty of Science, Annamalai University, Annamalainagar, Tamil Nadu – 608 002, India

A. Manimekalai, K. Selvaraju

Correspondence: L. Pari, Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India.
E-mail: paribala@sancharnet.in

developed group of compounds, which are structurally related to sulfonylurea and biguanides, is the non-sulfonylurea, of which N-benzoyl-D-phenylalanine (NBDP) is the member currently in use (Lenzen & Peckmen 2001). NBDP acts directly on the pancreatic β-cell to stimulate insulin secretion that is rapid and has a short duration, and depends upon the ambient glucose level. Generally D-phenylalanine derivative controls hyperglycaemia, resulting in improved overall glycaemic control in patients with type 2 diabetes.

The biguanide metformin (1,1-dimethyl biguanide) is used as an orally active antihyperglycaemic drug in the treatment of human type 2 diabetes mellitus (Bailey et al 1996). The antihyperglycaemic effect of metformin may depend on reduced intestinal glucose absorption (Bailey 1995) and its main action seems to be due to improved insulin sensitivity in peripheral insulin target tissues and suppressed hepatic glucose output (Bailey et al 1996). Metformin improves glycaemic control as monotherapy in combination with other oral antidiabetic agents, such as sulfonylureas and thiazolidinediones (Rendell et al 2003).

In this study, we have evaluated the efficacy and safety of a combination of NBDP and metformin compared with either drug monotherapy in neonatal streptozotocin (nSTZ) diabetic rats. The study was designed to establish whether the combination of NBDP and metformin, with complementary pharmacological actions, would result in normalized blood glucose and cause a marked improvement in altered serum lipids, lipoproteins and lipid peroxidation markers during diabetes.

Materials and Methods

Drugs and chemicals

All the biochemicals and chemicals used in this experiment were purchased from Sigma Chemical Company Inc. (St Louis, MO). The chemicals were of analytical grade.

Synthesis of N-benzoyl-D-phenylalanine

N-benzoyl-D-phenylalanine was prepared by mixing equimolar amounts of D-phenylalanine with benzoyl chloride in the presence of sodium hydroxide solution and adopting the general procedure reported for benzoylation (Vogel 1987). The product was recrystallized twice from ethanol and the yield was noted as 60%. IR and ^{13}C NMR spectrum were recorded for the product N-benzoyl-D-phenylalanine (Figures 1 and 2).

In the ^{13}C NMR spectrum, two sets of signals were observed for benzoyl carbons and ring carbons indicating the presence of two isomers in solution. The assignment of

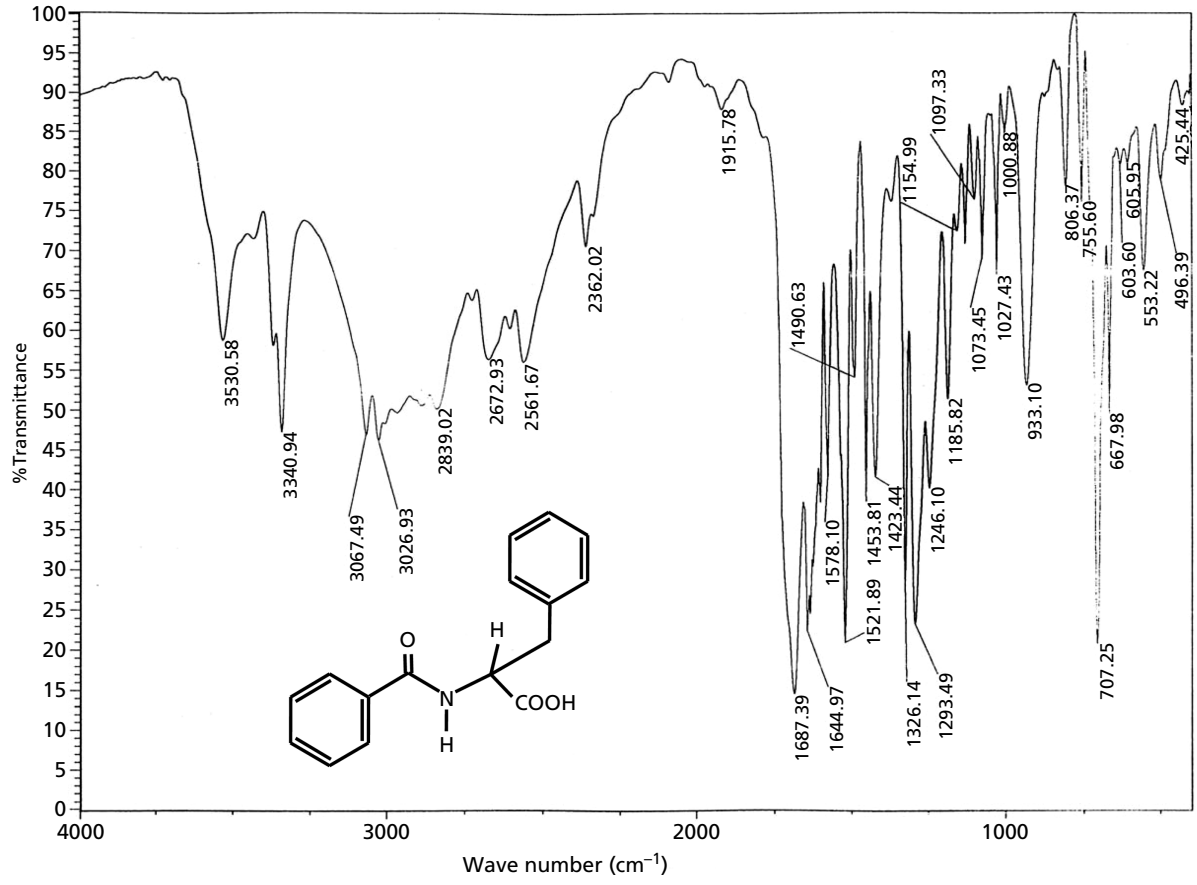

Figure 1 IR spectrum of N-benzoyl-D-phenylalanine.

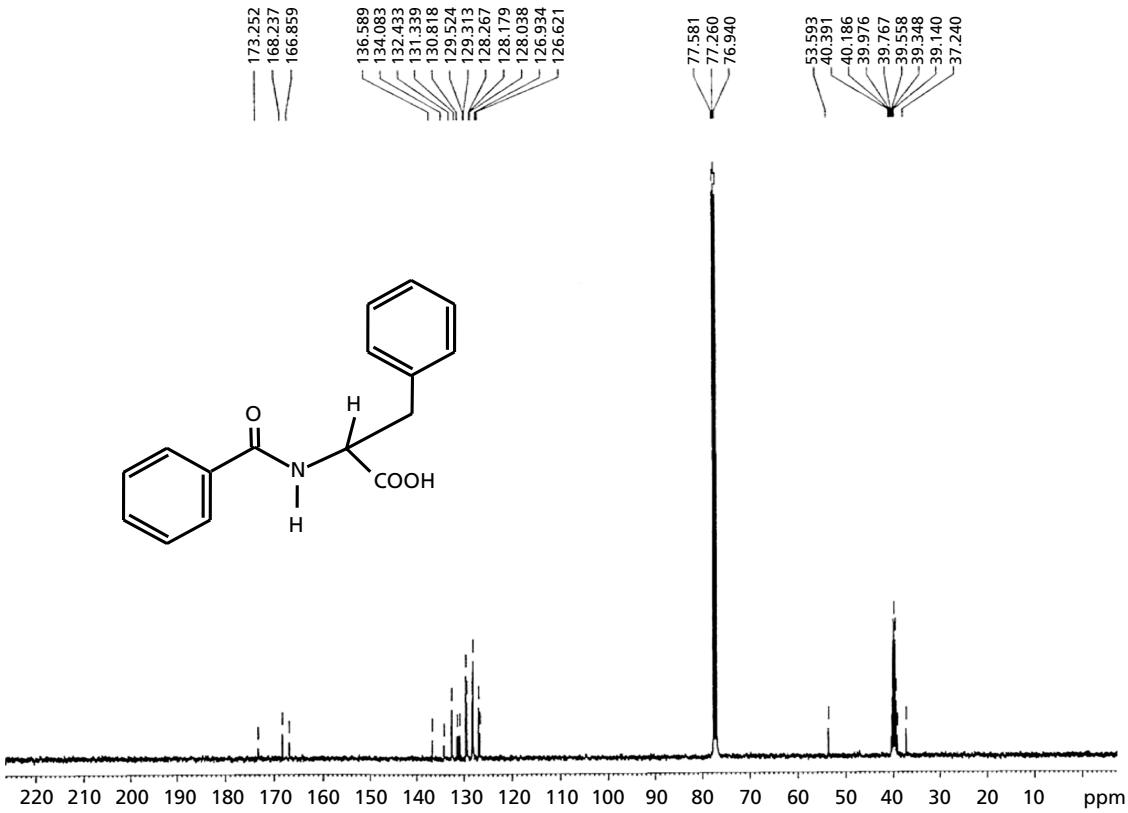

Figure 2 ^{13}C NMR spectrum of N-benzoyl-D-phenylalanine.

the signals in N-benzoyl-D-phenylalanine was based on comparison of the signals with those of D-phenylalanine. It has been reported that the benzoylation caused the resonance of the α-carbon to move upfield (Krishnapillay et al 2000). Based on this, assignment of the signals was made. Table 1 illustrates the assignments in N-benzoyl-D-phenylalanine.

Animals

Healthy albino Wistar strain rats, kept for breeding in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used in this study. The rats were fed a pellet diet (Hindustan Lever Limited, Mumbai, India) and water was freely available. The rats were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and the study approved by the ethical committee (Vide. No: 99, 2002), Annamalai University.

Table 1 IR and ^{13}C NMR spectral data of N-benzoyl-D-phenylalanine

IR (KBr pellet)	^{13}C NMR
γ_{OH} - 3530 cm^{-1}	C1 – 173.25
γ_{NH} - 3340 cm^{-1}	C2 – 53.59
γ_{CO} of COOH - 1687 cm^{-1}	C3 – 37.24
γ_{CO} of COC6H5 - 1644 cm^{-1}	C4 – 134.08; 132.43
Aromatic C-H in plane bending vibration - 707, 667 cm^{-1}	C5 – 131.34; 129.31
	C6 – 128.18; 128.04
	C7 – 126.62; 126.93
	Benzoyl carbon – 168.24; 166.86

Experimental induction of type 2 diabetes (NIDDM) in rats

The model was developed according to the description of Bonner Weir et al (1981). Wistar rats of either sex, aged 48 ± 2 h, were injected intraperitoneally with streptozotocin in citrate buffer (pH 4.5) at a dose of 100 mg kg^{-1}. After 10–12 weeks, only male rats weighing above 150 g were selected for screening in the NIDDM model.

Experimental procedure

A total of 42 rats (36 diabetic surviving rats, 6 control rats) were divided into seven groups of six rats each. Group 1 was the control vehicle-treated group. Group 2 were the diabetic control rats. Group 3 were the diabetic rats given NBDP 50 mg kg^{-1}/day in 1 mL 0.5% methylcellulose suspension (Klimes et al 1998) for six weeks. Group 4 were the diabetic rats given NBDP 100 mg kg^{-1}/day in 1 mL 0.5% methylcellulose suspension for six weeks. Group 5 were the diabetic rats given NBDP 200 mg kg^{-1}/day in 1 mL 0.5% methylcellulose suspension for six weeks. Group 6

were the diabetic rats given metformin 500 mg kg^{-1}/day (Soon & Tan 2000) in 1 mL saline for six weeks. Group 7 were the diabetic rats given NBDP (100 mg kg^{-1}/day in 1 mL 0.5% methylcellulose suspension) and metformin (500 mg kg^{-1}/day in 1 mL saline) for six weeks.

At the end of the experimental period, the rats were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose and glycosylated haemoglobin, and another tube without anticoagulant for serum separation. Plasma was separated for the assay of insulin. Liver was dissected out, washed in ice-cold saline, patted dry and weighed.

Analytical methods

Blood glucose level was estimated by the O-toluidine method (Sasaki et al 1972). Plasma insulin was assayed using an enzyme-linked immunosorbent assay (ELISA) kit (Boeheringer–Mannheim, Germany). Glycosylated haemoglobin was estimated according to the method of Sudhakar Nayak & Pattabiraman (1981) with modifications according to Bannon (1982). Glucose-6-phosphate dehydrogenase (G6PD) was determined by the method of Ellis & Kirkman (1961).

The total cholesterol was estimated by the method of Zlatkis et al (1953). Serum HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C) were estimated by the method of Burnstein et al (1970) and Friedward et al (1972). Serum VLDL cholesterol (VLDL-C) was calculated according to Friedward et al (1972). The triglycerides, free fatty acids and phospholipids were estimated by the method of Foster & Dunn (1973), Falholt et al (1973) and Zilversmit & Davis (1950), respectively.

Lipid peroxidation markers in plasma were estimated colorimetrically by thiobarbituric acid-reactive substances (TBARS) and hydroperoxides by the method of Niehius & Samuelsson (1968) and Jiang et al (1992), respectively. Reduced glutathione (GSH) was determined by the method of Ellman (1959).

Statistical analysis

All the data were expressed as mean ± s.d. of a number of experiments (n = 6). The statistical significance was evaluated by one-way analysis of variance using SPSS version 7.5 (SPSS, Cary, NC) and the individual comparisons were obtained by Duncan's multiple range test. Values were considered statistically significant when $P < 0.05$ (Duncan 1957). The power of the study was analysed by using NCCS and PASS 2004 (Cruncher Statistical Systems, Kaysville, UT).

Results

The levels of blood glucose and insulin in control and experimental animals are shown in Figures 3 and 4. The level of blood glucose was significantly increased whereas the plasma insulin was significantly decreased in nSTZ diabetic rats. The administration of NBDP significantly decreased the blood glucose levels and significantly increased the plasma insulin activity. NBDP at a dose of 100 mg kg^{-1} showed a highly significant effect when compared with 50 and 200 mg kg^{-1}. The effect of NBDP at 100 mg kg^{-1} was used for further biochemical analysis. Combined administration of NBDP and metformin was more effective than either drug alone. Based on blood glucose and plasma insulin values, the calculated power of the study was 1.

The change in the level of glycosylated haemoglobin and the activity of hepatic glucose-6-phosphate dehydrogenase in the different groups are shown in Figures 5 and 6, respectively. The level of glycosylated haemoglobin significantly increased whereas the activity of glucose-6-phosphate dehydrogenase was significantly decreased in nSTZ diabetic rats. Administration of NBDP plus metformin to diabetic

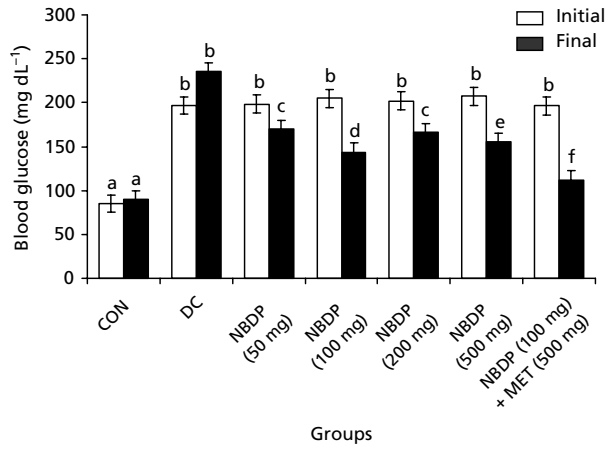

Figure 3 Effect of N-benzoyl-D-phenylalanine (NBDP) and metformin (MET) on the levels of blood glucose in control and experimental rats. CON, control; DC, diabetic control. Values are mean ± s.d. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test).

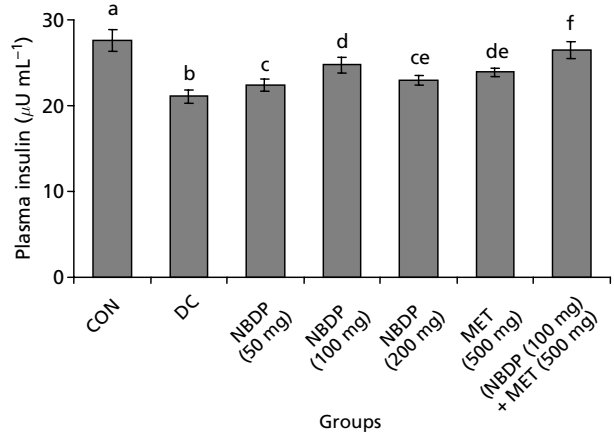

Figure 4 Effect of N-benzoyl-D-phenylalanine (NBDP) and metformin (MET) on the level of plasma insulin in control and experimental rats. CON, control; DC, diabetic control. Values are mean ± s.d. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test).

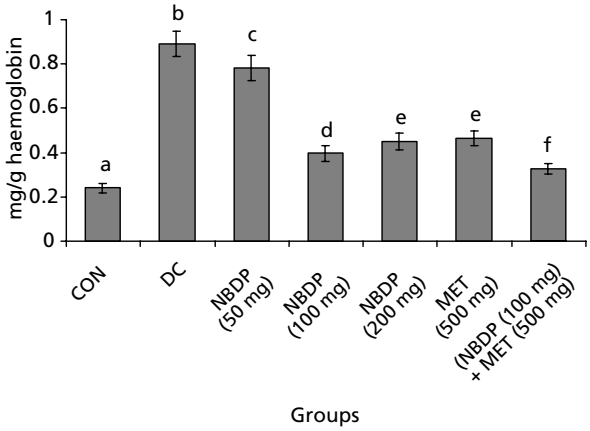

Figure 5 Changes in the level of glycosylated haemoglobin in control and experimental rats. CON, control; DC, diabetic control; NBDP, N-benzoyl-D-phenylalanine; MET, metformin. Values are mean ± s.d. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test).

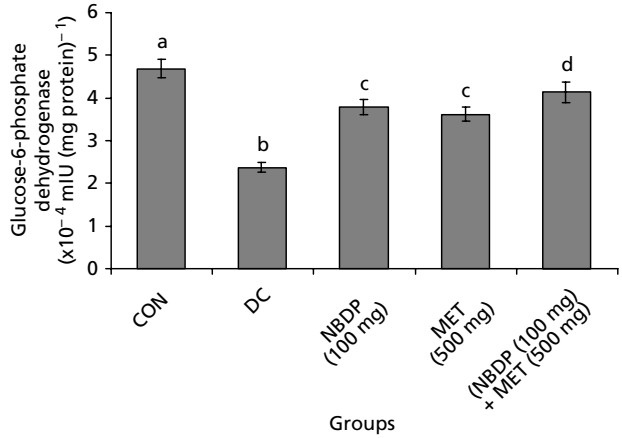

Figure 6 Changes in the activities of hepatic glucose-6-phosphate dehydrogenase in control and experimental rats. CON, control; DC, diabetic control; NBDP, N-benzoyl-D-phenylalanine; MET, metformin. Values are mean ± s.d. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test).

rats reversed the changes in the level of glycosylated haemoglobin and the activity of glucose-6-phosphate dehydrogenase to almost control levels. The effect of NBDP and metformin combination treatment was more prominent when compared with either drug alone.

Table 2 shows the levels of serum lipoproteins and total cholesterol. Table 3 shows the levels of the triglycerides, free fatty acids and phospholipids in control and experimental rats. The level of total cholesterol, LDL-C and VLDL-C, triglycerides, free fatty acids and phospholipids

Table 2 Effect of N-benzoyl-D-phenylalanine (NBDP) and metformin on serum total cholesterol and lipoproteins in control and experimental rats

Groups	Total cholesterol ($mg\,dL^{-1}$)	HDL cholesterol ($mg\,dL^{-1}$)	LDL cholesterol ($mg\,dL^{-1}$)	VLDL cholesterol ($mg\,dL^{-1}$)
Control	82.83 ± 5.14^{a}	56.58 ± 3.78^{a}	26.78 ± 1.61^{a}	13.46 ± 0.72^{a}
Diabetic control	161.66 ± 11.30^{b}	31.94 ± 1.99^{b}	79.26 ± 4.87^{b}	27.78 ± 2.01^{b}
NBDP ($100\,mg\,kg^{-1}$)	119.33 ± 6.23^{c}	44.43 ± 3.19^{c}	53.35 ± 3.99^{c}	20.53 ± 1.40^{c}
Metformin (500 mg/kg)	127.83 ± 6.74^{c}	42.55 ± 2.54^{c}	59.51 ± 4.30^{d}	16.48 ± 1.12^{d}
NBDP ($100\,mg\,kg^{-1}$) + metformin ($500\,mg\,kg^{-1}$)	105.50 ± 6.23^{d}	50.25 ± 3.90^{d}	43.80 ± 2.96^{e}	

Duncan's values are given as mean ± s.d. from six rats in each group; analysis of variance followed by Duncan's multiple range test. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan's procedure; range for the level 2.91, 3.06, 3.16, 3.22.

Table 3 Effect of N-benzoyl-D-phenylalanine (NBDP) and metformin on serum triglycerides, free fatty acids and phospholipids in control and experimental rats

Groups	Triglycerides ($mg\,dL^{-1}$)	Free fatty acids ($mg\,dL^{-1}$)	Phospholipids ($mg\,dL^{-1}$)
Control	84.50 ± 5.51^{a}	85.16 ± 5.78^{a}	98.83 ± 6.96^{a}
Diabetic control	153.66 ± 9.92^{b}	148.83 ± 11.29^{b}	169.50 ± 11.52^{b}
NBDP ($100\,mg\,kg^{-1}$)	121.33 ± 7.43^{c}	114.00 ± 8.20^{c}	131.83 ± 9.11^{cd}
Metformin ($500\,mg\,kg^{-1}$)	129.50 ± 8.99^{c}	122.13 ± 9.82^{c}	141.33 ± 8.82^{c}
NBDP ($100\,mg\,kg^{-1}$) + metformin ($500\,mg\,kg^{-1}$)	105.16 ± 6.09^{d}	98.45 ± 6.44^{d}	121.66 ± 8.80^{d}

Duncan's values are given as mean ± s.d. from six rats in each group; analysis of variance followed by Duncan's multiple range test. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan's procedure; range for the level 2.91, 3.06, 3.16, 3.22.

Table 4 Effect of N-benzoyl-D-phenylalanine (NBDP) and metformin on plasma TBARS, hydroperoxides and reduced glutathione (GSH) in control and experimental rats

Groups	TBARS (nmol mL^{-1})	Hydroperoxides ($\times 10^{-5}$ mM dL^{-1})	GSH (mg dL^{-1})
Control	1.63 ± 0.11^{a}	8.82 ± 0.54^{a}	24.66 ± 1.15^{a}
Diabetic control	3.24 ± 0.19^{b}	15.58 ± 1.11^{b}	15.01 ± 0.79^{b}
NBDP (100 mg kg^{-1})	2.16 ± 0.11^{c}	11.82 ± 0.60^{c}	18.33 ± 1.48^{c}
Metformin (500 mg kg^{-1})	2.23 ± 0.13^{c}	12.65 ± 0.61^{c}	20.07 ± 1.60^{d}
NBDP (100 mg kg^{-1}) + metformin (500 mg kg^{-1})	1.91 ± 0.12^{d}	10.53 ± 0.69^{d}	21.57 ± 1.27^{d}

Duncan's values are given as mean ± s.d. from six rats in each group; analysis of variance followed by Duncan's multiple range test. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (Duncan's multiple range test). Duncan's procedure; range for the level 2.91, 3.06, 3.16, 3.22.

were significantly increased, whereas the level of HDL-C was significantly decreased in nSTZ diabetic rats. Administration of NBDP and metformin to nSTZ diabetic rats significantly decreased the levels of lipids and increased the level of HDL-C to near normal level.

Lipid peroxidation markers and reduced glutathione are represented in Table 4. TBARS and hydroperoxides levels were significantly increased, whereas reduced glutathione levels were significantly decreased in plasma of nSTZ diabetic rats. Treatment with NBDP and metformin significantly increased the reduced glutathione levels with a significant decrease in the level of TBARS and hydroperoxides.

Discussion

The combined administration of NBDP and metformin to decrease the elevated blood glucose level to normal glycaemic level was an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. NBDP belongs to the group of hypoglycaemic agents that induce insulin secretion through the interaction with the sulfonylurea receptor in the plasma membrane of the pancreatic β-cell (Lenzen & Peckmen 2001). The possible mechanism by which NBDP promoted insulin secretion was by closure of K^{+}-ATP channels, membrane depolarization and stimulation of Ca^{2+} influx, an initial key step in insulin secretion. Metformin reduces the plasma glucose level during fasting by reducing rates of hepatic glucose production (Bailey et al 1996), glycogenolysis (Cusi et al 1996) and gluconeogenesis (Stumvoll et al 1995). Combination treatment significantly decreased blood glucose level and increased plasma insulin activity compared with either drug alone.

One possible source of oxygen free radicals in diabetes is autoxidation of glucose (Diaz-Flores et al 2004). Glucose oxidation generates oxygen free radicals and these may largely participate in the formation of glycated proteins, which are themselves a source of oxygen free radicals (Sakurai & Tsuchiya 1998). The glycation of haemoglobin was found to be significantly increased in diabetic animals and this increase was directly proportional to fasting blood glucose (Latha & Pari 2003). Administration of NBDP with metformin controlled the glycation of haemoglobin by its normoglycaemic activity and thus decreased the level of glycated haemoglobin in nSTZ diabetic rats.

Decreased activity of G6PD might have also slowed down the pentose phosphate pathway in the diabetic condition (Abdel-Rahim et al 1992). During diabetes, the hepatic lipogenesis is decreased and lipolysis is increased (West 1982). A sequential metabolic correlation existed between increased glycolysis, decreased gluconeogenesis, increased lipid biosynthesis in liver and normoglycaemia stimulated by NBDP along with metformin administration in nSTZ diabetic rats.

In this study, nSTZ diabetes mellitus characterized by hyperglycaemia caused a significant rise in serum lipids. This indicated that diabetes mellitus was accompanied by an increased risk of atherosclerosis. Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease (Pari & Latha 2002).

Diabetic dyslipidaemia is characterized by elevated levels of LDL-C, triglycerides and decreased HDL-C. Triglycerides and total cholesterol levels are proven predictors of cardiovascular diseases in diabetes (Gandhi 2001). Increased triglyceride and reduced HDL-C levels are the key characteristics of dyslipidaemia in type 2 diabetes (Gandhi 2001). Hypertriglyceridaemia in type 2 diabetes can result from an increased hepatic VLDL-C over-production and impaired catabolism of triglyceride-rich particles. The function of lipoprotein lipase (Lpl), a key enzyme in removal and degradation of triglycerides from the circulation, is attenuated by both insulin deprivation and insulin resistance. Dysfunction of Lpl contributes to hypertriglyceridaemia in the fasting and postprandial state. It has been postulated that high plasma triglyceride influences LDL size and density through a cycle of lipid exchange (Taskinen et al 1996). The altered composition of lipoproteins in diabetes is facilitated by the action of cholesterol ester transfer protein (Eisenberg 1985). Triglyceride-rich particles have a prolonged residence time within the circulation and are exposed to the action of this enzyme for a longer period of time, the consequence of which is the net transfer of triglyceride from VLDL-C to HDL-C and LDL-C, with cholesterol moving in the opposite direction

(McEneny et al 2000). The increase and fall in the individual lipoprotein levels is the reflection of the total serum cholesterol levels, that is, the level of HDL-C, LDL-C and VLDL-C increases or decreases with levels of total serum cholesterol, and it is their ratio that determines the pathophysiology of lipoprotein metabolism.

Excess of free fatty acids in serum produced by the nSTZ-induced diabetes promotes conversion of excess free fatty acids into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into blood in the form of lipoproteins (Bopanna et al 1997). The abnormal high concentration of serum lipids in the diabetic subject is due, mainly, to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolaemia and hypertriglyceridaemia have been reported to occur in nSTZ diabetic rats (Chakrabarti et al 2003) and the significant increase observed in our experiment was in accordance with these studies. The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots (Goodman & Gilman 1985). The antihyperlipidaemic effect of NBDP and metformin may be due to the down regulation of NADPH and NADH, a cofactor in fat metabolism. The levels of serum lipids and lipoproteins were brought to near normal levels by combined NBDP and metformin treatment compared with diabetic control rats.

Several studies have shown that increased lipid peroxidation in clinical and experimental diabetes (Sundaram et al 1996; Kakkar et al 1998) results in an increase in TBARS, an indirect evidence of intensified free radical production (Montilla et al 1998). Lipid peroxide-mediated damage has been observed in the development of type 1 and type 2 diabetes mellitus (Prince et al 1998). Chronic hyperglycaemia increases oxidative stress by the autoxidation of monosaccharides, which leads to production of superoxide and hydroxyl radicals. These radicals cause tissue damage by reacting with polyunsaturated fatty acids in membranes and increased lipid peroxidation (Das et al 2000). Reduced glutathione functions as a free radical scavenger and in the repair of radical-caused biological damage (Garg et al 1996). Chronic hyperglycaemia-induced toxicity decreased the level of GSH (Robertson et al 2003). Decreased activity of G6PD in diabetes results in reduced availability of NADPH and hence a decreased level of GSH. The combined NBDP and metformin treatment caused a significant decrease in TBARS and hydroperoxide levels, and significant increases in the level of GSH and the activity of G6PD in nSTZ diabetic rats when compared with diabetic control rats.

In conclusion, NBDP possessed an antihyperlipidaemic effect in addition to its antidiabetic activity, which might have exerted a beneficial action on insulin in nSTZ diabetic rats. The results indicated that hyperglycaemia coupled with hyperlipidaemia increased the risk of cardiovascular disease. The antidiabetic effect of NBDP with metformin could have possible therapeutic value. Due to the antidiabetic and antihyperlipidaemic effects of NBDP, further mechanistic studies are necessary to find the efficacy of NBDP in diabetic patients that would help achieve good glycaemic and metabolic control.

References

Abdel-Rahim, E. A., El-Saadany, S. S., Abo-Eytta, A. M., Wasif, M. M. (1992) The effect of sammo administration on some fundamental enzymes of pentose phosphate pathway and energy metabolites of alloxanized rats. *Nahrung* **36**: 8–14

Bailey, C. J. (1995) Metformin and intestinal glucose handling. *Diabetes Metab. Rev.* **11**: S23–S32

Bailey, C. J., Path, M. R. C., Turner, R. C. (1996) Metformin. *N. Engl. J. Med.* **334**: 574–579

Bannon, P. (1982) Effect of pH on the elimination of the labile fraction of glycosylated haemoglobin. *Clin. Chem.* **28**: 2183

Bierman, E. L. (1992) George Lyman Duff Memorial Lecture: atherogenesis in diabetes. *Arterioscler. Thromb.* **12**: 647–656

Bonnardel-Phu, E., Vicaut E. (2000) Reactive oxygen species and acute modulation of albumin microvascular leakage in the microcirculation of diabetic rats in vivo. *J. Vasc. Res.* **37**: 32–38

Bonner Weir, S., Trent, D. F., Honey, R. N., Weir, G. C. (1981) Response of neonatal rat islets to streptozotocin. Limited β-cell regeneration and hyperglycemia. *Diabetes* **30**: 64–69

Bopanna, K. N., Kannan, J., Sushma, G., Balaraman, R., Rathod, S. P. (1997) Antidiabetic and antihyperlipemic effects of neem seed kernel powder on alloxan diabetic rabbits. *Ind. J. Pharmacol.* **29**: 162–167

Burnstein, M., Scholnic, H. R., Mortn, R. (1970) Rapid method of isolation of lipoprotein from human serum by precipitation of polyanion. *J. Lipid. Res.* **11**: 583–587

Chakrabarti, S., Biswas, T. K., Rokeya, B., Ali, L., Mosihuzzaman, M., Nahar, N., Azad Khan, A. K., Mukherjee, B. (2003) Advanced studies on the hypoglycemic effect of Caesalpinia bonducella F. in type 1 and 2 diabetes in Long Evans rats. *J. Ethnopharmacol.* **84**: 41–46

Cusi, K., Consoli, A., DeFronzo, R. A. (1996) Metabolic effect of metformin on glucose and lactate metebolism in non–insulin dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* **81**: 4059–4067

Das, S., Vasisht, S., Snehalatha, M., Das, N., Srivastava, M. (2000) Correlation between total antioxidant status and lipid peroxidation in hypercholesterolemia. *Curr. Sci.* **78**: 486

Diaz-Flores, M., Baiza-Gutman, L. A., Ibanez-Hernandez, M. A., Pascoe-Lira, D., Guzman-Greenfel, A. M., Kumate-Rodriguez, J. (2004) Molecular aspects of chronic hyperglycemia-induced tissue damage. *Gac. Med. Mex.* **140**: 437–447

Duncan, B. D. (1957) Multiple range test for correlated and heteroscedastic means. *Biometrics* **13**: 359–364

Eisenberg, S. (1985) Preferential enrichment of large sized very low-density lipoprotein populations with transferred cholesterylesters. *J. Lipid. Res.* **26**: 487–494

Ellis, H. A., Kirkman, H. N. (1961) A colorimetric method for assay of erythrocyte glucose-6-phosphate dehydrogenase. *Proc. Soc. Exp. Biol. Med.* **106**: 607–609

Ellman, G. L. (1959) Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82**: 70–77

Falholt, K., Falholt, W., Lund, B. (1973) An easy colorimetric method for routine determination of free fatty acids in plasma. *Clin. Chim. Acta.* **46**: 105–111

Foster, J. B., Dunn, R. T. (1973) Stable reagents for determination of serum triglyceride by colorimetric condensation method. *Clin. Chim. Acta.* **19**: 338–340

Friedward, W. T., Levy, R. I., Fradrickson, D. S. (1972) Estimation of concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. *Clin. Chem.* **19**: 449–452

Gandhi, H. R. (2001) Diabetes and coronary artery disease: importance of risk factors. *Cardiol. Today* **1**: 31–34

Garg, M. C., Ojha, S., Bansal, D. D. (1996) Antioxidant status of streptozotocin diabetic rats. *Indian. J. Exp. Biol.* **34**: 264–266

Gillery, P. (2001) Advanced glycation end products (AGEs), free radicals and diabetes. *J. Soc. Biol.* **195**: 387–390

Goodman, L. S., Gilman, A. (1985) *The pharmacological basis of therapeutics*. 7th edn, MacMillan, NewYork, pp 1490–1510

Jiang, Z. Y., Hunt, J. V., Wolft, S. D. (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. *Anal. Biochem.* **202**: 384–389

Kakkar, R., Mantha, S. V., Radhi, J., Prasad, K., Kalra, J. (1998) Increased oxidative stress in rat liver and pancreas during progression of streptozotocin induced diabetes. *J. Clin. Sci.* **94**: 623–632

Klimes, I., Sebokova, E., Gasperikova, D., Mitkova, A., Kuklova, S., Bohov, J., Stanek, J. (1998) Search for extra pancreatic effects of new oral hypoglycemic agent A-1466: 1. oral glucose tolerance tests in normal and hereditary insulin resistant rats. *Endocrine Regul.* **32**: 115–123

Krishnapillay, M., Krishnakumar, R., Natarajan, A., Jayaraman, G. (2000) Conformational analysis of some heterocyclic systems: part II – Conformational analysis of substituted N-benzoylpiperidin-4-ones. *Indian J. Chem.* **39**: 419–425

Latha, M., Pari, L. (2003) Antihyperglycaemic effect of *Cassia auriculata* in experimental diabetes and its effects on key metabolic enzymes involved in carbohydrate metabolism. *Clin. Exp. Pharmacol. Physiol.* **30**: 38–43

Lenzen, S., Peckmen, T. (2001) Effect of tolbutamide and N-benzoyl-D-phenylalanine (NBDP) on the regulation of (Ca^{2+})i oscillations in mouse pancreatic islets. *Biochem. Pharmacol.* **62**: 923–928

McEneny, J., O'Kane, M. J., Moles, K. W., McMaster, C., McMaster, D., Mercer, C., Trimble, E. R., Young, I. S. (2000) Very low density lipoprotein subfractions in Type II diabetes mellitus; alterations in composition and susceptibility to oxidation. *Diabetologia* **43**: 485–493

Montilla, P. L., Vargas, J. F., Tunez, I. F., Munoz de Agueda, M. C., Valdelvira, M. E., Cabrera, E. S. (1998) Oxidative stress in diabetic rats induced by streptozotocin: preventive effects of melatonin. *J. Pineal. Res.* **25**: 94–100

Niehius, W. G., Samuelsson, D. (1968) Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem.* **6**: 126–130

Pari, L., Latha, M. (2002) Effect of Cassia auriculata flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. *Singapore Med. J.* **43**: 617–621

Prince, P. S. M., Menon, V. P., Pari, L. (1998) Hypoglycemic activity of *Syzigium cumini* seeds: effect on lipid peroxidation in alloxan diabetic rats. *J. Ethnopharmacol.* **61**: 1–7

Rendell, M. S., Glazer, N. B., Zhan, Ye. (2003) Combination therapy with pioglitazone plus metformin or sulfonylurea in patients with type 2 diabetes influence of prior antidiabetic drug regimen. *J. Diabetes Complicat.* **17**: 211–217

Robertson, R. P., Harmon, J., Tran, P. O., Tanaka, Y., Takahashi, H. (2003) Glucose toxicity in beta–cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* **52**: 581–587

Sakurai, T., Tsuchiya, S. (1998) Superoxide production from nonenzymatically glycated proteins. *FEBS Lett.* **236**: 406–410

Sasaki, T., Matsy, S., Sonae, A. (1972) Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. *Rinsh. Kagaku.* **1**: 346–353

Soon, Y. Y., Tan, B. K. H. (2000) Evaluation of the hypoglycemic and antioxidant activities of *Morida officinalis* in streptozotocin induced diabetes rats. *Singapore Med. J.* **43**: 77–85

Stumvoll, M., Nurjhan, N., Periello, G., Dailey, G., Gerich, J. E. (1995) Metabolic effects of metformin in non-insulin dependent diabetes mellitus. *N. Engl. J. Med.* **333**: 550–554

Sudhakar Nayak, S., Pattabiraman, T. N. (1981) A new colorimetric method for the estimation of glycosylated hemoglobin. *Clin. Chim. Acta.* **109**: 267–274

Sundaram, R. K., Bhaskar, A., Vijayalingam, S., Viswanatthan, M., Moha, R., Shanmugasundaram, K. R. (1996) Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clin. Sci.* **90**: 255–260

Taskinen, M. R., Lahdenpera, S., Syvanne, M. (1996) New insights into lipid metabolism in non-insulin-dependent diabetes mellitus. *Ann. Med.* **28**: 335–340

Vogel, A. I. (1987) *Elementary practical organic chemistry, Part 1*. 2nd edn, CBS publishers and distributors, New Delhi, p. 270

West, K. M. (1982) Hyperglycemia as a cause of long-term complications. In: Keen H, Jarret J. (eds) *Complications of diabetes*, 2nd edn. Edward Arnold, London, pp 3–18

Yakubu, M. A., Sofola, O. A., Igbo, I., Oyekan. A. O. (2001) Link between free radicals and protein kinase C in glucose-induced alteration of vascular dilation. *Circ. Res.* **88**: 14–22

Yoshida, K., Hirokawa, J., Tagami, S., Kawakami, Y., Urata, Y., Kondo, I. (1995) Weakened cellular scavenging activity against oxidative stress in diabetes mellitus: regulation of glutathione synthesis and efflux. *Diabetologia* **88**: 201–210

Zilversmit, B. B., Davis, A. K. (1950) Micro determination of plasma phospholipids by TCA precipitation. *J. Lab. Clin. Med.* **35**: 155–160

Zimmet, P. Z. (1999) Diabetes epidemiology as a tool to trigger diabetes research and care. *Diabetologia* **42**: 499–518

Zlatkis, A., Zak, B., Boyle, G. J. (1953) A method for the determination of serum cholesterol. *J. Clin. Med.* **41**: 486–492