

ORIGINAL CONTRIBUTION

Antihyperlipidemic and Antidiabetic Effects of Umbelliferone in Streptozotocin Diabetic Rats

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The aim of the study was to evaluate blood glucose and lipid lowering effects of Umbelliferone (UMB) in streptozotocin (STZ) diabetic rats. Male albino Wistar rats (180 to 200 g) were induced diabetes by administration of STZ (40 mg/kg) intraperitoneally. Normal and diabetic rats were treated with UMB in 10 percent dimethyl sulfoxide (DMSO) for 45 days. Diabetic rats had increased plasma glucose and decreased insulin, total proteins (TP), and albumin in addition to decreased food intake and body weight. Elevation in total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), triglycerides (TG), free fatty acids (FFA), and phospholipids (PL), and reduction in high density lipoprotein cholesterol (HDL-C) in the plasma were observed. Liver and kidney tissues of diabetic rats had elevation in the levels of TC, TG, FFA, and PL. Treatment with UMB decreased plasma glucose and increased insulin, TP, and albumin apart from food intake and body weight. In UMB-treated diabetic rats, plasma and tissue TC, TG, PL and FFA, and plasma LDL-C, VLDL-C, and HDL-C reversed to near normal. Thus, reduction of blood glucose and lipid profiles indicates that UMB has antidiabetic and antihyperlipidemic effects in diabetic rats.

INTRODUCTION

Diabetes mellitus is a syndrome that is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism [1]. The association of hyperglycemia and altering of lipid parameters present a major risk of cardiovascular diseases in diabetic patients [2, 3]. The lowering of lipid concentration through dietary or drugs therapy seems to be associated with a decrease in the risk of

vascular disease [4]. Since currently available hypolipidemic agents lack desired properties of an ideal drug, researchers are involved to find out an effective, safe, and less expensive drug.

Coumarin, a phenolic compound present in human dietary fruits and vegetables, is known to have antioxidant potential like vitamin E (a-tocopherol) and have lipid lowering potential [5]. Umbelliferone (UMB)[†] (7-hydroxycoumarin), a deriva-

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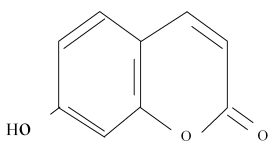
[†]Abbreviations: DMRT, Duncan's Multiple Range Test; DMSO, dimethyl sulfoxide; EDTA, ethylenediamine tetra acetic acid; FFA, free fatty acids; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; PL, phospholipids; STZ, streptozotocin; TC, total cholesterol; TG, triglycerides; TP, total proteins; UMB, umbelliferone; VLDL-C, very low density lipoprotein cholesterol.

tive of coumarin, is a benzopyrone in nature and is present in the edible fruits, golden apple (*Aegle marmelos Correa*) [6], and bitter orange (*Citrus aurantium*) [7]. The parent compound coumarin has been reported to reduce blood glucose level [8]. We have reported that UMB has antioxidant activity [9], but no detailed study has been carried out on the effect of UMB on blood glucose, plasma insulin, and protein and lipid profiles in streptozotocin (STZ)-diabetic rats. Hence, the present study was designed to investigate the effect of UMB on lipid profiles in plasma and tissues such as liver and kidney, and protein profiles in the plasma of STZ-diabetic rats. The structure of UMB is depicted in Figure 1.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain with a body weight ranging from 180 to 200 g, were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and were maintained in an air conditioned room ($25 \pm 1^\circ\text{C}$) with a 12-hour light/12-hour dark cycle. Standard pellets (purchased from Pranav Agro Industries, Ltd., Pune, India) and water was provided *ad libitum*. Studies were carried out in accordance with Indian National Law on Animal Care and Use, and the study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg. No: 160/1999/CPCSEA



UMB (7-hydroxycoumarin)

Figure 1. Structure of UMB.

[Committee for the Purpose of Control and Supervision of Experiments on Animals]), Annamalai University, Annamalainagar, Tamilnadu, India.

Chemicals

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, United States. UMB was procured from Carl Roth GmbH & Co, Germany. All the other chemicals were of analytical grade obtained from E. Merck, Germany, and HIMEDIA, India.

Experimental induction of diabetes

The animals were rendered diabetic by a single intraperitoneal injection of STZ (40 mg/kg b-wt) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. STZ-injected animals were given 20 percent glucose solution for 24 hours to prevent initial drug-induced hypoglycemic mortality. STZ-injected animals exhibited massive glycosuria (determined by Benedict's qualitative test), and diabetes in STZ rats was confirmed by measuring fasting blood glucose concentration by glucose oxidase method, 96 hours after injection with STZ. The animals with blood glucose above 235 mg/dl were considered to be diabetic and used for the experiment.

Experimental design

The animals were randomly divided into five groups of six animals each as given below. The UMB and glibenclamide were administered intraperitoneally once a day using vehicle solution (10 percent DMSO).

Group I: Normal control (10 percent DMSO)

Group II: Normal + UMB (30 mg/kg/b-wt in 10 percent DMSO)

Group III: Diabetic control (10 percent DMSO)

Group IV: Diabetic + UMB (30 mg/kg/b-wt in 10 percent DMSO)

Table 1. Effect of UMB on blood glucose and plasma insulin in diabetic rats.

Group	Blood glucose (mg/dl)		Insulin (μ U/ml)
	0 day	45th day	
Normal control	79.60 \pm 5.25	82.44 \pm 2.68 ^b	18.04 \pm 0.77 ^{a,b}
Normal + UMB (30 mg/kg/b-wt)	82.14 \pm 3.19	74.39 \pm 4.17 ^a	18.73 \pm 0.84 ^a
Diabetic control	240.47 \pm 5.82	289.28 \pm 3.18 ^d	5.38 \pm 0.37 ^c
Diabetic + UMB (30 mg/kg/b-wt)	244.63 \pm 6.29	114.28 \pm 5.71 ^c	17.11 \pm 0.66 ^b
Diabetic + glibenclamide (600 μ g/kg/b-wt)	242.85 \pm 5.04	107.23 \pm 7.23 ^c	17.49 \pm 0.60 ^b

Values are given as means \pm SD from six rats in each group. Values not sharing a common superscript differ significantly at $p < .05$ (Duncan's Multiple Range Test).

Group V: Diabetic + glibenclamide (600 μ g/kg b-wt in 10 percent DMSO)

After 45 days of treatment, the 12 hour-fasted animals were anesthetized between 8:00 a.m. and 9:00 a.m., using Ketamine (24 mg/kg b-wt) (intramuscular injection) and sacrificed by decapitation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of blood glucose and in tubes with ethylenediamine tetra acetic acid (EDTA) for the estimation of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA), phospholipids (PL), high density lipoprotein (HDL-C), and protein profiles such as total proteins (TP), albumin, and globulin

(A/G) ratio. Tissues such as liver and kidney were collected for the estimation of TC, TG, FFA, and PL.

Biochemical determinations

Blood glucose was estimated by the method of Trinder using reagent kit [10]. The insulin in the rat plasma was measured by method of Burgi et al [11]. Plasma and tissue lipids were extracted by the methods of Folch et al [12]. Plasma and tissue TC, TG, FFA, and PL were estimated by the methods of Siedel et al [13], Foster and Dunn [14], Falholt et al [15], and Zilversmit and Davis [16], respectively. Plasma HDL-C was estimated by the method of Warnick et al [17]. LDL-C and

Table 2. Effect of UMB on body weight and food intake in diabetic rats.

Group	Body weight (g)		Average food intake (g/day)
	0 day	45th day	
Normal control	181.33 \pm 4.22	198.83 \pm 6.88 ^b	15.35 \pm 0.93 ^{a,b}
Normal + UMB (30 mg/kg/b-wt)	179.42 \pm 4.71	196.16 \pm 5.07 ^b	16.15 \pm 1.25 ^a
Diabetic control	180.08 \pm 5.84	150.50 \pm 4.92 ^a	12.25 \pm 1.01 ^c
Diabetic + UMB (30 mg/kg/b-wt)	178.33 \pm 5.00	197.57 \pm 5.84 ^b	14.10 \pm 1.13 ^b
Diabetic + glibenclamide (600 μ g/kg/b-wt)	183.00 \pm 4.69	210.00 \pm 6.00 ^c	14.71 \pm 1.05 ^b

Values are given as means \pm SD from six rats in each group. Values not sharing a common superscript differ significantly at $p < .05$ (Duncan's Multiple Range Test).

Table 3. Effect of UMB TC, TG, LDL-C, VLDL-C, and HDL-C in the plasma of diabetic rats.

Group	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	HDL-C (mg/dl)
Normal control	84.33 ± 1.50 ^b	62.1 ± 5.66 ^a	21.14 ± 2.10 ^b	12.42 ± 1.13 ^b	50.76 ± 1.75 ^b
Normal + UMB (30 mg/kg/b-wt)	79.00 ± 2.09 ^a	55.8 ± 5.57 ^a	18.38 ± 1.42 ^a	11.16 ± 1.11 ^a	53.51 ± 1.05 ^a
Diabetic control	136.00 ± 2.82 ^e	178.2 ± 4.82 ^d	78.24 ± 4.06 ^e	35.64 ± 1.96 ^e	28.85 ± 1.25 ^e
Diabetic + UMB (30 mg/kg/b-wt)	96.33 ± 3.10 ^d	78.3 ± 7.23 ^c	36.01 ± 2.74 ^d	14.22 ± 1.26 ^c	46.06 ± 2.62 ^d
Diabetic + glibenclamide (600 µg/kg/b-wt)	91.00 ± 2.09 ^c	71.1 ± 6.31 ^b	27.16 ± 2.40 ^c	15.66 ± 1.44 ^d	48.18 ± 3.56 ^c

Values are given as means ± SD from six rats in each group. Values not sharing a common superscript differ significantly at $p < .05$ (Duncan's Multiple Range Test).

VLDL-C were calculated by Friedwald's formula [18]. Plasma TP and albumin were estimated by the methods of Gornall et al [19] and Corcoran and Durnan [20], respectively.

Statistical analysis

Values are given as means ± SD for six rats in each group. Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using SPSS-10. The limit of statistical significance was set at $p < .05$.

RESULTS

The levels of plasma insulin and blood glucose in diabetic rats are given in the Table 1. Diabetic rats exhibited

decreased level of plasma insulin and an elevated level of blood glucose as compared with normal control rats. Treatment with UMB and glibenclamide showed the reversal of blood glucose and plasma insulin to near normal levels. The effect of UMB on body weight and food intake is given in the Table 2. Decreased body weight and food intake were observed in diabetic rats as compared with normal control rats, and treatment with UMB had increased body weight and food intake to near normalcy.

Table 3 shows the levels of TC, TG, LDL-C, VLDL-C, and HDL-C in the plasma of diabetic rats. The diabetic rats had elevated levels of plasma TC, TG, LDL-C, and VLDL-C and decreased level of HDL-C as compared with normal control rats. Diabetic rats treated with UMB and

Table 4. Effect of UMB on FFA and PL in the plasma of diabetic rats.

Group	FFA (mg/dl)	PL (mg/dl)
Normal control	81.33 ± 6.02 ^{a,b}	90.93 ± 7.18 ^b
Normal + UMB (30 mg/kg/b-wt)	74.66 ± 4.13 ^a	84.48 ± 3.14 ^a
Diabetic control	156.00 ± 4.38 ^d	143.73 ± 7.20 ^d
Diabetic + UMB (30 mg/kg/b-wt)	96.00 ± 7.15 ^c	106.18 ± 4.81 ^c
Diabetic + glibenclamide (600 µg/kg/b-wt)	88.00 ± 6.82 ^b	100.60 ± 4.37 ^c

Values are given as means ± SD from six rats in each group. Values not sharing a common superscript differ significantly at $p < .05$ (Duncan's Multiple Range Test).

Table 5. Effect of UMB on TC, TG, FFA, and PL in the liver of diabetic rats.

Group	TC (mg/100 g tissue)	TG (mg/100 g tissue)	FFA (mg/100 g tissue)	PL (mg/100 g tissue)
Normal control	341.33 ± 7.29 ^a	315.00 ± 8.04 ^a	790.40 ± 14.45 ^{a,b}	1936.00 ± 39.35 ^b
Normal + UMB (30 mg/kg/b-wt)	332.00 ± 4.38 ^a	307.50 ± 6.77 ^a	784.00 ± 12.08 ^a	1899.33 ± 33.12 ^b
Diabetic control	648.00 ± 3.64 ^d	730.50 ± 10.52 ^c	1382.53 ± 17.17 ^d	3740.00 ± 39.35 ^a
Diabetic + UMB (30 mg/kg/b-wt)	368.00 ± 11.49 ^c	334.50 ± 6.54 ^b	819.20 ± 15.67 ^c	1796.66 ± 33.12 ^d
Diabetic + glibenclamide (600 µg/kg/b-wt)	354.40 ± 6.76 ^b	327.00 ± 4.64 ^b	807.04 ± 10.70 ^{b,c}	1848.00 ± 39.35 ^c

Values are given as means ± SD from six rats in each group. Values not sharing a common superscript differ significantly at $p < .05$ (Duncan's Multiple Range Test).

glibenclamide reversed serum lipid profiles to near normal levels.

Table 4 represents the levels of FFA and PL in the plasma of diabetic rats. The diabetic rats had elevated levels of plasma FFA and PL as compared with normal control rats. Diabetic rats treated with UMB and glibenclamide reversed plasma lipid profiles to near normal levels.

The levels of TC, TG, FFA, and PL in liver and kidney of diabetic rats are given in Tables 5 and 6. The diabetic rats had elevated levels of tissue TC, TG, FFA, and PL when compared with normal control rats. Diabetic rats treated with UMB and glibenclamide reversed tissue lipid profiles to near normal levels.

The levels of TP, albumin, globulin, and albumin/globulin ratio in the plasma of diabetic rats are presented in the Table 7. The diabetic rats had decreased levels of plasma total proteins, albumin, globulins and albumin/globulin ratio when compared with normal control rats. After treatment with UMB and glibenclamide, TP, albumin, globulins, and albumin/globulin ratio were brought back to near normal levels.

DISCUSSION

In type 2 diabetes, insulin secretion is defective and insufficient to compensate for insulin resistance which may improve

Table 6. Effect of UMB on TC, TG, FFA, and PL in the kidneys of diabetic rats.

Group	TC (mg/100 g tissue)	TG (mg/100 g tissue)	FFA (mg/100 g tissue)	PL (mg/100 g tissue)
Normal control	377.33 ± 9.35 ^b	265.50 ± 9.43 ^{a,b}	374.40 ± 20.13 ^a	2024.00 ± 43.15 ^a
Normal + UMB (30 mg/kg/b-wt)	364.00 ± 8.39 ^a	259.50 ± 6.77 ^a	364.80 ± 17.17 ^a	1980.00 ± 39.35 ^a
Diabetic control	678.13 ± 3.84 ^d	006.39 ± 8.04 ^d	919.68 ± 12.56 ^d	2912.00 ± 62.32 ^d
Diabetic + UMB (30 mg/kg/b-wt)	414.66 ± 11.77 ^c	282.00 ± 7.34 ^c	426.00 ± 15.67 ^c	2148.66 ± 51.43 ^c
Diabetic + glibenclamide (600 µg/kg/b-wt)	387.46 ± 7.53 ^b	273.00 ± 6.28 ^{b,c}	393.60 ± 8.67 ^b	2090.00 ± 46.14 ^b

Values are given as means ± SD from six rats in each group. Values not sharing a common superscript differ significantly at $p < .05$ (Duncan's Multiple Range Test).

Table 7. Effect of UMB on TP, albumin, globulin, and A/G in the plasma of diabetic rats.

Group	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Normal control	7.08 ± .38 ^b	3.85 ± .27 ^b	3.16 ± .29 ^b	1.21 ± .07 ^b
Normal + UMB (30 mg/kg/b-wt)	8.28 ± .42 ^a	4.37 ± .31 ^a	3.86 ± .25 ^a	1.48 ± .08 ^a
Diabetic control	4.33 ± .37 ^d	1.93 ± .18 ^d	2.35 ± .18 ^d	0.82 ± .06 ^d
Diabetic + UMB (30 mg/kg/b-wt)	6.18 ± .45 ^c	3.21 ± .28 ^c	2.86 ± .21 ^c	1.12 ± .10 ^c
Diabetic + glibenclamide (600 µg/kg/b-wt)	6.50 ± .49 ^c	3.50 ± 21 ^{b,c}	3.01 ± .27 ^{b,c}	1.16 ± .09 ^c

Values are given as means ± SD from six rats in each group. Values not sharing a common superscript differ significantly at $p < .05$ (Duncan's Multiple Range Test).

with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal [21]. UMB as a pharmacological agent may improve the condition. Further, as UMB is having an antihyperlipidemic effect it may also decrease insulin resistance and improve the condition. It is now well established that STZ selectively destroys the pancreatic cells and produces hyperglycemia [22], which is evidenced by the decreased level of plasma insulin. In a previous report, coumarin has been reported to reduce blood glucose level [8]. Coumarin may be a prodrug, and 7-hydroxycoumarin is the pharmacologically active agent [23]. Treatment with UMB and glibenclamide showed the reversal of blood glucose to near normal levels which is supported by the elevated level of plasma insulin. STZ-induced diabetes is characterized by severe loss in body weight [24], and the loss may be due to degradation of structural proteins since structural proteins are known to contribute to the body weight [25]. In our study, weight loss and decreased food intake were observed, and treatment with UMB reversed the weight loss and food intake, which may be due to increased secretion of insulin by UMB.

The levels of serum lipids are elevated in diabetes mellitus, and such an elevation represents a risk factor for coronary

heart disease [26]. Lowering of serum and tissue lipids through diet or drug seems to be associated with a decrease in the risk of vascular disease [27]. The abnormal high concentration of serum lipids in diabetic subjects is mainly due to increase in the mobilization of free fatty acids from fat deposits [28] since insulin is required for the inhibition of hormone-sensitive lipase. On the other hand, glucagon and other hormones enhance lipolysis. The marked hyperlipidemia that characterizes the diabetic state may, therefore, be regarded as a consequence of uninhibited actions of lipolytic hormones on the fat deposits [29]. Diabetic rats treated with UMB and glibenclamide brought TC and TG back to near normal levels, which could be due to an increase in insulin secretion, which, in turn, inhibits hormone sensitive lipase and increases the utilization of glucose and thereby decreasing the mobilization of free fatty acids from the fat depots. The decreased level of FFA is also associated with decreased actions of lipolytic hormones, which, in turn, decreased the activity of hormone sensitive lipases on fat deposits.

High levels of TC and, more importantly, LDL-C are major coronary risk factors [30], and low plasma levels of HDL-C is a relevant cardiovascular risk factor [31]. In diabetic rats, the rise in TC and TG

is associated with the increase in LDL-C and VLDL-C and decrease in HDL-C. In our study, the diabetic rats treated with UMB showed an elevation in HDL-C and reduction in LDL-C and VLDL-C as evidenced by decreased levels of TC and TG. Thus, UMB could alleviate the risk of cardiovascular diseases.

Phospholipids are vital components of biomembranes and play an important role in the transport of triglycerides [32]. In STZ-diabetic rats, the elevated level of phospholipids may be due to the elevated levels of FFA [33] and TC, which can promote the synthesis of phospholipids [34]. In UMB-treated diabetic rats, the decreased level of phospholipids may be due to decreased levels of TC and FFA.

Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation [35]. Previous reports show that protein synthesis is decreased in all tissues due to decreased production of ATP and absolute or relative deficiency of insulin [36], which may be responsible for the decreased level of plasma proteins in diabetic rats. In our study, the elevated level of plasma total proteins, albumin, and globulins may be related with increased levels of plasma insulin in diabetic rats treated with UMB and glibenclamide. Rasch and Mogensen [37] have reported that the plasma A/G ratio was lower in diabetic animals. Increased protein catabolism in diabetes might have induced a direct adverse effect on the synthesis and secretion of albumin. Diabetic rats treated with UMB and glibenclamide also brought A/G ratios back to near normal level.

CONCLUSION

Thus, our findings demonstrate that UMB has an antidiabetic effect, which is evidenced by decreased blood glucose, elevated plasma insulin and protein profile, and hypolipidemic effect, which is

evidenced by the decreased levels of TC, TG, LDL-C, VLDL-C, FFA, and PL, and elevated levels of HDL-C in diabetic rats. Since UMB is a natural product, combination with reduced dosage of already existing antidiabetic drug may prevent side-effects. This requires further investigation.

REFERENCES

1. Bennett PH. Definition, diagnosis and classification of diabetes mellitus and impaired glucose tolerance. In: Kahn CR and Weir GC, eds. *Jaslin's Diabetes Mellitus*, 13th ed. Philadelphia: Lea and Febiger; 1994, pp. 193-200.
2. Jensen T, Stender S, and Deckert T. Abnormalities in plasma concentrations of lipoproteins and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased urinary albumin excretion. *Diabetologia* 1988;31:142-5.
3. Motta M, Giugno I, Bosco C, Pistone G, Ruello P, Maugeri D, and Malaguarera M. Serum lipoprotein changes in acute myocardial infarction. *Panminerva Med* 2001;43:77-80.
4. Betteridge J. Lipid disorders in diabetes mellitus. In: Pickup JC and Williams G, eds. *Textbook of Diabetes*, 2nd ed. London: Blackwell Science; 1997, pp 1-55.
5. Madhavan GR, Balraju V, Mallesham B, Chakrabarti R, and Lohray VB. Novel coumarin derivatives of heterocyclic compounds as lipid-lowering agents. *Bioorg Med Chem Lett* 2003;13:2547-51.
6. Parmar C and Kaushal MK. *Aegle marmelos*. In: *Wild Fruits*. New Delhi, India: Kalyani Publishers; 1982, pp 1-5
7. Wu FJ and Sheu SJ. Analysis and processing of Chinese herbal drugs: the study of *Fructus Aurantii Immaturus* (Chin.). *Chin Pharm J*. 1992;44:257-63.
8. Marles RJ and Farnsworth N. Antidiabetic plants and their active constituents: an update. *Prof J Bot Med* 1996;3:85-135.
9. Ramesh B and Pugalendi KV. Impact of umbelliferone on erythrocyte redox status in STZ-diabetic rats. *Yale J Biol Med* 2005;78:133-40.
10. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969;6:24.
11. Burgi W, Briner M, Franken N, and Kessler ACH. One step sandwich enzyme immunoassay for insulin using monoclonal antibodies. *Clin Biochem* 1988;21:311-4.
12. Folch J, Lees M, and Solane SGH. A simple method for isolation and purification of

- total lipids from animal tissues. *J Biol Chem* 1957;26:497-509.
13. Siedel J, Hagele EO, Ziegenhorn J, and Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. *Clin Chem* 1983;20:1075.
 14. Foster LB and Dunn RT. Stable reagents for determination of serum triglycerides by colorimetric hantzsch condensation method. *Clin Chem* 1973;19:338-40.
 15. Falholt K, Falholt W, and Lund B. An easy colorimetric method for routine determination of free fatty acids in plasma. *Clin Chim Acta* 1973;46:105-11.
 16. Zilversmit DB and Davis AK. Microdetermination of phospholipids by TCA precipitation. *J Lab Clin Med* 1950;35:155-9.
 17. Warnick GR, Nguyen T and Alberts AA. Comparison of improved precipitation methods for quantification of high-density lipoprotein cholesterol. *Clin Chem* 1985; 31:217.
 18. Friedwald WT, Levy RJ, and Fredrickson DS. Estimation of LDL-C in the plasma without the use of preparative ultracentrifuge. *Clin Chem* 1972;18:449.
 19. Gornall AG, Bardawill CJ, and David MM. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 1949; 177:751.
 20. Corcoran RM and Durnan SM. Albumin determination by a modified bromocresol green method. *Clin Chem* 1977;23:765.
 21. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2005;28:S37-S42.
 22. Gilman AG, Rall TW, Nies AS, and Tayer P, eds. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed. New York: McGraw-Hill; 1990, pp. 1317-22.
 23. Ritschel WA, Grummich KW, Kaul S, and Hardt TJ. Biopharmaceutical parameters of coumarin. and. 7-hydroxycoumarin *Pharm Ind* 1981 43:271.
 24. Chen V and Ianuzzo CD. Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. *Can J Physiol Pharmacol* 1982;60:1251-6.
 25. Rajkumar L and Govindarajulu P. Increased degradation of dermal collagen in diabetic rats. *Ind J Exp Biol* 1991;29: 1081-3.
 26. Davidson MB. *Diabetes Mellitus, Diagnosis and Treatment*. Hoboken, New Jersey: John Wiley and Sons; 1981, p. 109.
 27. Al-Shamaony L, Al-Khazrajoi SM, and Twaij HAA. Hypoglycemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some serum parameters in diabetic animals. *J Ethnopharmacol* 1994; 43:167-71.
 28. Rhoads GG, Gulbrandse CL, and Kagan A. Serum lipoproteins and coronary artery diseases in a population study of Hawaii, a Japanese man. *N Engl J Med* 1976; 294:293-8.
 29. Goodman LS and Gilman A. *The Pharmacological Basis of Therapeutics*, 7th ed. New York: MacMillan; 1985, p. 490.
 30. National Cholesterol Education Program. Second report of the expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. *Circulation*. 1994;89:1329.
 31. Gordon D and Rifkind B. Current concepts: High density lipoproteins: the clinical implications of recent studies. *N Engl J Med* 1989;321:1311-5.
 32. Draznin B and Eckel RH. Diabetes and atherosclerosis. *Mol Basis Clin Aspects* 1993;12:203.
 33. Frayn KN. Insulin resistance and lipid metabolism. *Curr Opin Lipidol*. 1993;4: 197-204.
 34. Marsc D, Knowled PF, and Rattle HWF. *Magnetic Resonance of Biomolecules*. New York: John Wiley and Sons; 1996: 237.
 35. Murray RR, Granner DK, Mayes PA, and Rodwell VW. *Harper's Biochemistry. Gluconeogenesis and the Control of Blood Glucose*, 26th edition. Stamford, Connecticut: Appleton and Lange; 2003, pp. 153-62.
 36. Chatterjee MN and Rana S. Text book of medical biochemistry. Metabolism of carbohydrate. New Delhi, India: Jaypee Brothers Medical Publishers ; 1994, p. 421.
 37. Rasch R and Mogensen CE. Urinary excretion of albumin and TP in normal and streptozotocin diabetic rats. *Acta Endocrinol* 1980; 95: 376-81.