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) intraperitonially. 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-
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 ± 1°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 [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 intraperitonial 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 intraperitonially 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)

![Figure 1. Structure of UMB.](image-url)
Table 1. Effect of UMB on blood glucose and plasma insulin in diabetic rats.

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

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).

Group V: Diabetic + glibenclamide (600 µg/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 ± 4.22   | 198.83 ± 6.88b              | 15.35 ± 0.93a,b  |
| Normal + UMB (30 mg/kg/b-wt) | 179.42 ± 4.71   | 196.16 ± 5.07b              | 16.15 ± 1.25a    |
| Diabetic control           | 180.08 ± 5.84   | 150.50 ± 4.92a              | 12.25 ± 1.01c    |
| Diabetic + UMB (30 mg/kg/b-wt) | 178.33 ± 5.00   | 197.57 ± 5.84b              | 14.10 ± 1.13b    |
| Diabetic + glibenclamide (600 µg/kg/b-wt) | 183.00 ± 4.69   | 210.00 ± 6.00c              | 14.71 ± 1.05b    |

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

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

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 Durman [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 shown 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
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.

DISCUSSION

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

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        | 315.00 ± 8.04        | 790.40 ± 14.45        | 1936.00 ± 39.35      |
| Normal + UMB (30 mg/kg/b-wt)| 332.00 ± 4.38        | 307.50 ± 6.77        | 784.00 ± 12.08        | 1899.33 ± 33.12      |
| Diabetic control            | 648.00 ± 3.64        | 730.50 ± 10.52       | 1382.53 ± 17.17       | 3740.00 ± 39.35      |
| Diabetic + UMB (30 mg/kg/b-wt) | 368.00 ± 11.49    | 334.50 ± 6.54        | 819.20 ± 15.67        | 1796.66 ± 33.12      |
| Diabetic + glibenclamide (600 µg/kg/b-wt) | 354.40 ± 6.76 | 327.00 ± 4.64        | 807.04 ± 10.70        | 1848.00 ± 39.35      |

Values are given as means ± SD from six rats in each group. Values not sharing a common superscript differ significantly at p < 0.05 (Duncan’s Multiple Range Test).

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        | 265.50 ± 9.43        | 374.40 ± 20.13        | 2024.00 ± 43.15      |
| Normal + UMB (30 mg/kg/b-wt)| 364.00 ± 8.39        | 259.50 ± 6.77        | 364.80 ± 17.17        | 1980.00 ± 39.35      |
| Diabetic control            | 678.13 ± 3.84        | 006.39 ± 8.04        | 919.68 ± 12.56        | 2912.00 ± 62.32      |
| Diabetic + UMB (30 mg/kg/b-wt) | 414.66 ± 11.77     | 282.00 ± 7.34        | 426.00 ± 15.67        | 2148.66 ± 51.43      |
| Diabetic + glibenclamide (600 µg/kg/b-wt) | 387.46 ± 7.53 | 273.00 ± 6.28        | 393.60 ± 8.67         | 2090.00 ± 46.14      |

Values are given as means ± SD from six rats in each group. Values not sharing a common superscript differ significantly at p < 0.05 (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. 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

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

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.
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. In UMB-treated rats, the decreased level of plasma proteins may be due to decreased levels of TC and FFA.

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.

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