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Protective effect of Umbelliferone on membranous fatty acid composition in streptozotocin-induced diabetic rats

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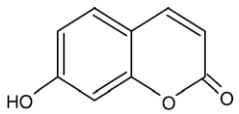
Abstract

Umbelliferone (UMB), a natural antioxidant, is benzopyrone in nature, and it is present in the fruits of golden apple and bitter orange. Earlier we evaluated and reported the effect of Umbelliferone on antidiabetic, antioxidant and antihyperlipidemic properties, and this study was designed to evaluate the effect of Umbelliferone on membrane fatty acid composition and histopathology of liver and kidney of control and streptozotocin (STZ) diabetic rats. Male albino Wistar rats (180–200 g) were made diabetic by an intraperitonial administration of STZ (40 mg/kg). The control and diabetic rats were treated with Umbelliferone and glibenclamide dissolved in 10% dimethyl sulfoxide for 45 days. Diabetic rats had decreased insulin and increased glucose, and increased levels of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes. The levels of palmitic, stearic and oleic acids increased and the levels of linolenic and arachidonic acids decreased in diabetic rats as compared with control rats. Thus, the saturated fatty acids and monounsaturated fatty acids increased and the polyunsaturated fatty acids decreased in diabetic rats. Diabetic rats had decreased liver weight and increased activities of alanine transaminase and aspartate transaminase; increased kidney weight and urine albumin, and decreased levels of urea, uric acid and creatinine in the urine. Histopathological studies of liver and kidney in diabetic rats showed fatty changes surrounding portal triad; enlargement of lining cells of tubules, fatty infiltration, large area of hemorrhage and lymphocyte infiltration. Treatment with Umbelliferone and glibenclamide reversed these changes to near normalcy. Our results showed that Umbelliferone has a protective effect on membrane fatty acid composition of liver and kidney as supported by antioxidant and antihyperlipidemic effects of Umbelliferone reported earlier as evidenced by improved histopathological changes, hepatic and nephritic markers, indicating recovery from the risk of diabetic complications.

Keywords: Diabetes mellitus; Streptozotocin; Umbelliferone; Fatty acid composition

1. Introduction

Fatty acids carry out many functions that are necessary for normal physiological health. Saturated fatty acids are non-essential fatty acids and are harmful if ingested excessively in food. They favour excess weight, insulin resistance (Folsom et al., 1996), increased LDL-cholesterol and are atherogenic. On the contrary, non-essential monounsaturated fatty acids, and namely its main component, oleic acid, have a beneficial effect on cholesterol metabolism and a protective role against cardiovascular diseases (De Lacruz et al., 2000). Polyunsaturated fatty acids are designated as "essential" for good health as their metabolic precursors cannot be synthesized in the body and must be ingested by food intake (Roche, 1999). Polyunsaturated fatty acids have important effects on the structure and physical properties of localized membrane domains. They modulate enzyme activities, carriers and membrane receptors (LDL receptors, insulin, antibodies neurotransmitters, drugs receptors, etc.). Polyunsaturated fatty acids are involved in eicosanoid (prostaglandins, prostacyclins, thromboxanes, leukotrienes) production, signal



Umbelliferone ($C_9H_6O_3$)

Fig. 1. Structure of Umbelliferone.

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Table 1
Effect of Umbelliferone on glucose, body weight and insulin in control and diabetic rats

Name of the group	Glucose (mg/dL)		Body weight (g)		Insulin (μU/mL)
	0 day	45th day	0 day	45th day	
Normal control	79.60±5.25	82.44±2.68^{b}	181.33±4.22	198.83±6.88^{b}	18.04±0.77^{ab}
Normal+Umbelliferone (30 mg/kg/day)	82.14±3.19	74.39±4.17^{a}	179.42±4.71	196.16±5.07^{b}	18.73±0.84^{a}
Diabetic control	240.47±5.82	289.28±3.18^{d}	180.08±5.84	150.50±4.92^{a}	5.38±0.37^{c}
Diabetic+Umbelliferone (30 mg/kg/day)	244.63±6.29	114.28±5.71^{c}	178.33±5.00	197.57±5.84^{b}	17.11±0.66^{b}
Diabetic+glibenclamide (600 μg/kg/day)	242.85±5.04	107.23±7.23^{c}	183.00±4.69	210.00±6.00^{c}	17.49±0.60^{b}

Values are given as means±S.D. from six rats in each group.
Values not sharing a common superscript vertically differ significantly at $p<0.05$.
All five groups were compared with each other.

transduction, and the activation of nuclear transcription factors (Spector, 1999). Parent essential fatty acids are linoleic acid (18:2, n-6) and α-linolenic acid (18:3, n-3) (Connor, 1999). After their absorption, they are metabolised by chain elongation and desaturation to long-chain polyunsaturated fatty acids containing 20 or more carbon atoms of the n-6 family (arachidonic acid 20:4, n-6) and n-3 family (eicosapentaenoic acid 20:5, n-3 and docosahexaenoic acid 22:6, n-3).

Plant derived phenolic coumarins might play a role as dietary antioxidants because of their consumption in the human diet especially in fruits and vegetables (Hoult and Paya, 1996). Umbelliferone (7-hydroxycoumarin), a natural antioxidant, is benzopyrone in nature, and it is present in the edible fruits such as golden apple (*Aegle marmelos* Correa) (Parmar and Kaushal, 1982) and bitter orange (*Citrus aurantium*) (Wu and Sheu, 1992). The parent compound coumarin has been reported to reduce plasma glucose (Marles and Farnsworth, 1996). Recently we have reported the effect of Umbelliferone on glycemic control and lipids (Ramesh and Pugalendi, 2006a, 2005a) and antioxidants (Ramesh and Pugalendi, 2005b,c, 2006b), glycoprotein components (Ramesh and Pugalendi, 2006c), and hepatic marker enzymes (Ramesh and Pugalendi, 2006d) in streptozotocin-diabetic rats. In the present study, we have analyzed the effect of Umbelliferone on fatty acid composition and histopathological studies of liver and kidney as no detailed study has been carried out on these aspects so far. The structure of Umbelliferone is given in Fig. 1.

2. Materials and methods

2.1. Animals

Male albino rats of Wistar strain with body weight ranging from 180 to 200 g (9 weeks old), were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and maintained in an air conditioned room (25±1 °C) with a 12 h light:12 h dark cycle. Standard pellet diet and water were provided ad libitum. Studies were carried out in accordance with Indian National Law on Animal Care and Use, and ethical clearance was provided by The Committee for the Purpose of Control and Supervision of Experiments on Animals of Rajah Muthiah Medical College and Hospital (Reg. No.: 160/1999/CPCSEA), Annamalai University, Annamalainagar, Tamil Nadu, India.

2.2. Chemicals

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. Umbelliferone was procured from Carl Roth GmbH & Co, Germany. All other chemicals used were of analytical grade obtained from E. Merck or HIMEDIA, Mumbai, India.

2.3. Induction of diabetes

The animals were made diabetic by a single intraperitonial injection of streptozotocin (40 mg/kg) in a freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. Streptozotocin-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. Streptozotocin-injected animals exhibited glycosuria (determined by Benedict's qualitative test, Qualigen Diagnostics, Mumbai), and these animals were tested for hyperglycemia by measuring fasting plasma glucose (by glucose oxidase method, Qualigen Diagnostics, Mumbai), 96 h after the injection of streptozotocin. The animals with the blood glucose

Table 2
Effect of Umbelliferone lipid peroxidation markers in the plasma of control and diabetic rats

Name of the group	Thiobarbituric acid reactive substances (mmol/dL)	Lipid hydroperoxides (mmol/dL)	Conjugated dienes (ratio of absorbance at 240 and 214 nm)
Normal control	0.200±0.03^{b}	9.51±0.92^{b}	0.66±0.02^{b}
Normal+Umbelliferone (30 mg/kg/day)	0.173±0.04^{a}	8.02±0.82^{a}	0.50±0.03^{a}
Diabetic control	0.337±0.02^{d}	24.15±1.8^{d}	0.93±0.05^{e}
Diabetic+Umbelliferone (30 mg/kg/day)	0.227±0.01^{c}	11.60±0.97^{c}	0.74±0.06^{d}
Diabetic+glibenclamide (600 μg/kg/day)	0.212±0.03^{bc}	10.41±1.34^{bc}	0.69±0.04^{c}

Values are given as means±S.D. from six rats in each group.
Values not sharing a common superscript vertically differ significantly at $p<0.05$.
All five groups were compared with each other.

Table 3
Effect of Umbelliferone on fatty acid composition in the liver of control and diabetic rats

Name of the group	C16:0	C18:0	C18:1	C18:3	C20:4
	Palmitic acid	Stearic acid	Oleic acid	Linolenic acid	Arachidonic acid
	(Percentage of fatty acids)				
Normal control	20.07±1.17[a]	11.58±0.73[ab]	8.68±0.52[a]	6.66±0.33[a]	21.62±1.15[a]
Normal+Umbelliferone (30 mg/kg/day)	19.71±1.00[a]	11.02±0.65[a]	8.29±0.58[a]	7.15±0.41[a]	22.19±1.26[a]
Diabetic control	26.60±1.09[c]	18.14±0.67[d]	12.54±0.71[c]	2.70±0.12[c]	14.39±0.70[c]
Diabetic+Umbelliferone (30 mg/kg/day)	22.50±1.36[b]	12.52±0.88[c]	9.61±0.45[b]	5.73±0.57[b]	19.14±1.53[b]
Diabetic+glibenclamide (600 μg/kg/day)	21.95±1.45[b]	12.19±0.56[bc]	9.02±0.71[ab]	6.05±0.53[b]	19.71±1.77[b]

Values are given as means±S.D. from six rats in each group.
Values not sharing a common superscript vertically differ significantly at $p<0.05$.
All five groups were compared with each other.

Table 4
Effect of Umbelliferone on fatty acid composition in the kidney of control and diabetic rats

Name of the group	C16:0	C18:0	C18:1	C18:3	C20:4
	Palmitic acid	Stearic acid	Oleic acid	Linolenic acid	Arachidonic acid
	(Percentage of fatty acids)				
Normal control	22.59±1.43[ab]	13.60±0.65[ab]	5.47±0.46[ab]	6.57±0.52[ab]	11.60±1.04[ab]
Normal+Umbelliferone (30 mg/kg/day)	21.69±1.57[a]	13.08±0.91[a]	4.89±0.32[a]	6.91±0.44[a]	12.34±1.18[a]
Diabetic control	31.19±1.57[c]	20.98±1.44[d]	10.82±1.13[d]	1.24±0.06[d]	5.80±0.88[c]
Diabetic+Umbelliferone (30 mg/kg/day)	24.21±1.30[b]	15.75±0.97[c]	6.78±0.73[c]	5.26±0.23[c]	10.43±0.61[b]
Diabetic+glibenclamide(600 μg/kg/day)	23.79±1.04[b]	14.63±1.62[bc]	6.24±0.74[bc]	6.15±0.73[b]	10.72±1.08[b]

Values are given as means±S.D. from six rats in each group.
Values not sharing a common superscript vertically differ significantly at $p<0.05$.
All five groups were compared with each other.

levels more than 11.11 mmol/L were considered diabetic and used for the experiment.

2.4. Experimental design

The animals were randomly divided into 5 groups of six animals each and treated as given below. Dimethyl sulphoxide was used as a vehicle solution for the intraperitonial administration of Umbelliferone and glibenclamide. Rats occasionally showed minimal changes with dimethyl sulphoxide at 9 mL/kg (1.8 mL/200 g) (Noel et al., 1975) and in our study, at 0.1 mL/200 g/day, no negative effects were found in the hepatic markers analyzed. (Ramesh and Pugalendi, 2006c).

Group I: Normal control received 10% dimethyl sulphoxide
Group II: Normal+Umbelliferone (30 mg/kg/day) in 10% dimethyl sulphoxide
Group III: Diabetic control received 10% Dimethyl sulphoxide
Group IV: Diabetic+Umbelliferone (30 mg/kg/day) in 10% dimethyl sulphoxide
Group V: Diabetic+glibenclamide (600 μg/kg/day) in 10% dimethyl sulphoxide

After 45 days of treatment, the 12 h fasted animals were anaesthetized by giving an intramuscular injection of ketamine (24 mg/kg), and sacrificed by decapitation between 8 a.m and 9 a.m. Liver and kidney were collected in a solvent mixture (chloroform and methanol, 1:1) and further processed for the analysis of fatty acid composition, and in 10% formalin for histopathological studies.

Table 5
Effect of Umbelliferone on liver weight and serum hepatic marker enzymes in control and diabetic rats

Name of the group	Liver weight (g)	Alanine transaminase (IU/L)	Aspartate transaminase (IU/L)
Normal control	6.53±0.08[b]	24.43±2.04[b]	74.53±5.06[ab]
Normal+Umbelliferone (30 mg/kg/day)	6.66±0.05[a]	20.04±2.66[a]	69.52±4.83[a]
Diabetic control	4.33±0.09[e]	58.91±4.43[d]	117.58±6.20[d]
Diabetic+Umbelliferone (30 mg/kg/day)	5.95±0.10[d]	29.90±2.16[c]	81.86±5.47[c]
Diabetic+glibenclamide (600 μg/kg/day)	6.23±0.08[c]	28.28±2.42[c]	79.20±6.31[bc]

Values are given as means±S.D. from six rats in each group.
Values not sharing a common superscript vertically differ significantly at $p<0.05$.
All five groups were compared with each other.
IU-μmol of pyruvate liberated per hour.

2.5. Biochemical determinations

Plasma glucose was measured by glucose oxidase method (Trinder, 1969). Plasma insulin was assayed with an ELISA kit by the method of Burgi et al. (1988). Tissue lipids were extracted by the method of Folch et al. (1957). The estimation of TBARS, HP and CD was done by the methods of Nichans and Samuelson (1968), Jiang et al. (1992) and Klein (1979), respectively. Fatty acid methyl esters were extracted by the method of Morrison and

Table 6
Effect of Umbelliferone on urine volume, and the levels of urea, uric acid, creatinine and albumin in the urine of control and diabetic rats

Name of the group	Relative kidney weight (g)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	Albumin (μg/day)
Normal control	1.208±0.07^{b}	141.21±10.79^{ab}	7.42±0.59^{ab}	2.79±0.28^{ab}	142.50±12.5^{a}
Normal+Umbelliferone (30 mg/kg/day)	1.116±0.05^{a}	147.85±9.48^{a}	7.72±0.81^{a}	3.02±0.32^{a}	133.75±11.03^{a}
Diabetic control	1.558±0.09^{d}	112.36±6.03^{c}	5.71±0.64^{c}	1.78±0.24^{d}	303.75±34.45^{c}
Diabetic+Umbelliferone (30 mg/kg/day)	1.325±0.06^{c}	132.50±8.17^{b}	6.958±0.43^{b}	2.45±0.19^{c}	171.29±13.29^{b}
Diabetic+glibenclamide (600 μg/kg/day)	1.266±0.08^{bc}	135.21±10.48^{b}	7.02±0.30^{ab}	2.53±0.31^{bc}	166.29±16.09^{b}

Values are given as means±S.D. from six rats in each group.
Values not sharing a common superscript vertically differ significantly at $p<0.05$.
All five groups were compared with each other.

Smith (1964) and fatty acid composition was determined by Gas Liquid Chromatography. The activities of serum AST and ALT, and level of total proteins were measured by using commercially available kits (Boehringer Mannheim, Mannheim, Germany). Urea in the plasma and urine was estimated by using the diagnostic kit based on the method of Fawcett and Scott (1960). Uric acid in the plasma and urine was estimated by using the diagnostic kit based on the enzymic method described by Caraway (1955). Creatinine in the serum and urine was estimated using the diagnostic kit based on the methods of Teitz (1987) using Jaffe's (1886) color reaction. Albumin in urine was measured by the method of Reinhold (1953). Histopathological studies of liver and kidney were done by the methods of Pearse (1981).

2.6. Statistical analysis

All quantitative measurements were expressed as means±S.D. The data were analyzed using one way analysis of variance on Statistical Package for Social Sciences, Personal Computer, and the group means were compared by Duncan's Multiple Range Test. The results were considered statistically significant if p values were less than 0.05.

3. Results

Table 1 shows the levels of glucose and insulin in the plasma and body weight in control and diabetic rats. Diabetic rats had an elevated level of plasma glucose and decreased body weight and level of plasma insulin as compared with control rats, and treatment with Umbelliferone (30 mg/kg/day) and glibenclamide (600 μg/kg/day) reversed glucose, body weight and insulin to near normalcy.

The levels of TBARS, HP and CD in the plasma of diabetic and control rats are presented in the Table 2. Diabetic rats had elevated levels of TBARS, HP and CD in the plasma when compared with normal control rats. Treatment with Umbelliferone and glibenclamide showed reversal of these parameters to near normalcy.

Fatty acid composition of liver and kidney of control and diabetic rats is presented in Tables 3 and 4. The levels of palmitic, stearic and oleic acids increased and the levels of linolenic and arachidonic acids decreased in diabetic rats. Thus, the saturated fatty acids and monounsaturated fatty acids increased and the polyunsaturated fatty acids decreased in diabetic rats, and treatment with Umbelliferone and glibenclamide reversed fatty acid changes to near normalcy.

Liver weight and the activities of ALT and AST in the control and diabetic rats are given in the Table 5. Diabetic rats had decreased liver weight and increased activities of ALT and AST in the plasma and treatment with Umbelliferone and glibenclamide reversed these changes to near normalcy. Table 6 shows the levels of urea, uric acid, creatinine and albumin in the urine of control and diabetic rats. Diabetic rats show decreased levels of urea, uric acid, creatinine and presence of albumin, and treatment with Umbelliferone and glibenclamide has reversed these parameters to near normalcy.

In our study, histopathological examination of diabetic pancreas (Figs. 2–6) showed shrinkage of islet cells and growth of adipose tissue in the pancreas. Treatment with Umbelliferone and glibenclamide reduced the changes in the pancreas, which supports the biochemical analysis.

Histopathological changes of liver and kidney in control and diabetic rats are shown in Figs. 7–16. Diabetic rats showed fatty changes surrounding portal triad in the liver; enlargement of

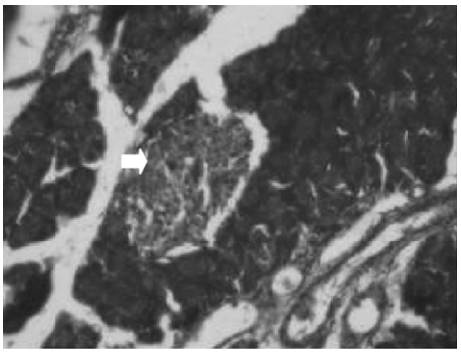

Fig. 2. Normal rat pancreas H&E×20 showing normal islet cells [⇨].

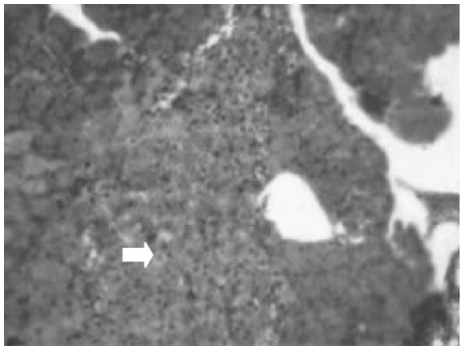

Fig. 3. Normal+Umbelliferone (30 mg) treated rat pancreas H&E×20 showing normal islet cells [⇨].

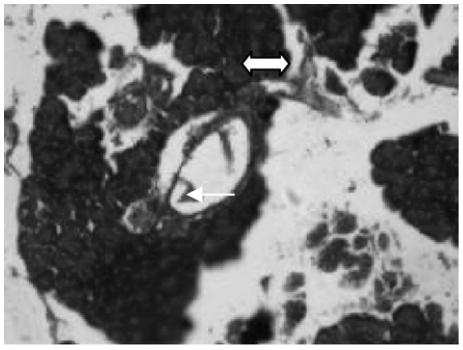

Fig. 4. Diabetic control rats H&E×20 showing growth of adipose tissue [⟺] and shrinkage of islets [→].

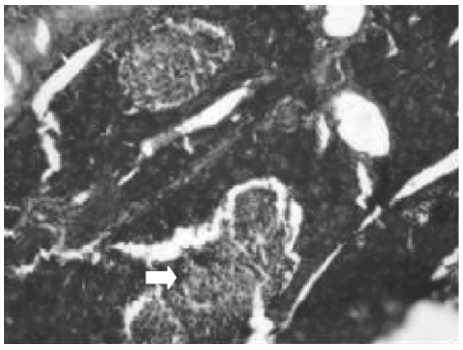

Fig. 5. Diabetic+Umbelliferone (30 mg) treated rat pancreas H&E×20 showing reduction in adipose tissue and pancreatic islets within normal limit [⇨].

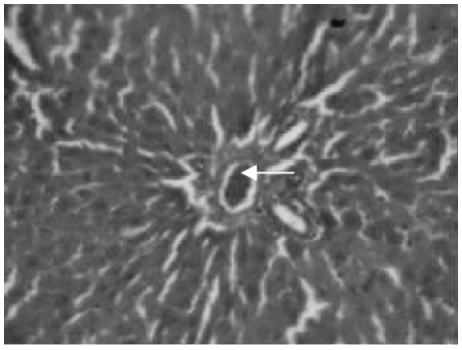

Fig. 7. Normal rat liver H&E×20 showing normal hepatocytes [→].

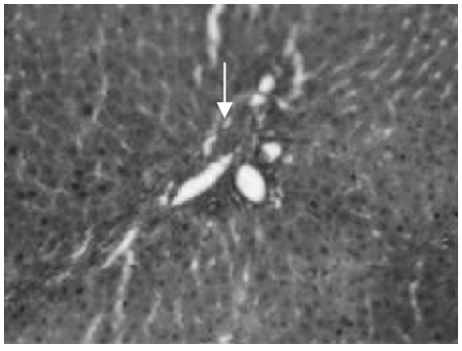

Fig. 8. Normal+Umbelliferone (30 mg) treated rat liver H&E×20 showing normal hepatocytes [→].

lining cells of tubules, fatty infiltration, large area of hemorrhage and lymphocyte infiltration in the kidney, and treatment with Umbelliferone and glibenclamide reversed these changes to near normalcy.

4. Discussion

It is now well established that streptozotocin selectively destroys the pancreatic cells and produces hyperglycemia (Gilman et al., 1990), which is evidenced by the decreased level of plasma insulin. In the previous report, coumarin has been reported to reduce blood glucose level (Marles and Farnsworth, 1996). Coumarin may be a prodrug and 7-hydroxycoumarin is the pharmacologically active agent. Treatment with Umbelliferone and glibenclamide showed the reversal of blood glucose to near normal level which is supported by the elevated level of plasma insulin. The elevated insulin in Umbelliferone treatment could be due to increased secretion by regenerated β-cells which is supported by histopathology of pancreas. Streptozotocin-induced diabetes is characterized by severe loss in body weight, and the loss may be due to degradation of structural proteins since structural proteins are known to contribute to the body weight. In our study, weight loss was observed, and treatment with Umbelliferone reversed the weight loss, which may be due to increased secretion of insulin by Umbelliferone.

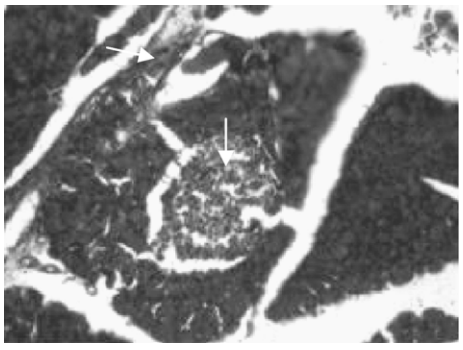

Fig. 6. Diabetic+glibenclamide (600 μg) treated rat pancrease H&E×20 showing reduction in adipose tissue and pancreatic islets within normal limit [⇨].

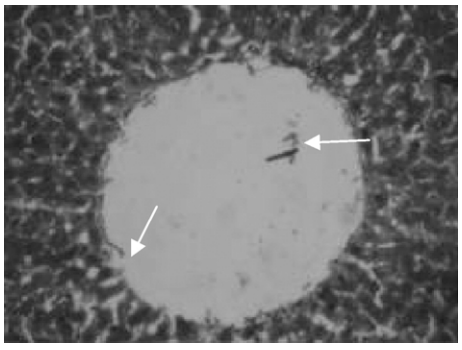

Fig. 9. Diabetic control rats H&E×20 showing fatty change surrounding portal triad [→].

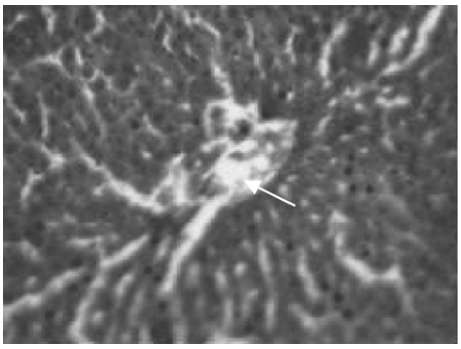

Fig. 10. Diabetic + Umbelliferone (30 mg) treated rat liver H&E × 20 showing no fatty change [→].

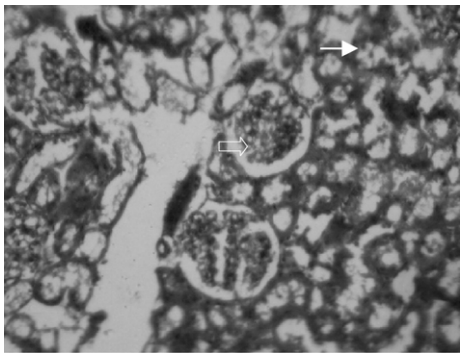

Fig. 12. Normal rat kidney H&E × 10 showing normal glomerulus (⇨) and tubular cells (→).

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Insulin regulates blood glucose homeostasis and the activities of Δ6 and Δ9 desaturases during normoglycemic condition, and fails to regulate during hyperglycemic conditions.

Liver is the main effector organ for maintaining plasma glucose levels within narrow limits. The increase of free radical mediated toxicity is well documented in clinical diabetes (Nourooz-Zedeh et al., 1997) and STZ-diabetic rats (Wohaieb and Godin, 1987). Hyperglycemia can generate a redox imbalance inside the cells, especially in the liver (Gallou et al., 1993). Free radicals result in the consumption of antioxidant defenses which may lead to disruption of cellular functions and oxidative damage to membranes and enhance susceptibility to lipid peroxidation (Vallabhji et al., 2001). It was reported that the lipid peroxidation markers (TBARS, HP and CD) are elevated in diabetic rats (Zhang and Tan, 2000) and treatment with Umbelliferone had reversed these parameters to near normalcy which could be due to improved antioxidant status (Ramesh and Pugalendi, 2006b).

Fatty acid composition is changed in humans (Ruiz-Gutierrez et al., 1993) and animals with diabetes (Holman et al., 1983). Diabetes inhibits delta-6-desaturase (Friedmann et al., 1966), which converts linoleic acid into gamma linolenic acid, the precursor of arachidonic acid, and ultimately, several vasoactive prostanoids. In experimental and clinical diabetes, gamma linolenic acid production is reduced. Consequently, the levels of dihomo gamma linolenic acid, which is a product of gamma linolenic acid elongation, and arachidonic acid also are reduced (Jamal, 1990).

Synthesis of unsaturated fatty acid takes place in the microsomes of the liver. Δ6 desaturase in the liver, a key enzyme of fatty acid desaturation (Brenner, 1977b), has been shown to be suppressed in diabetes mellitus, and rapidly restored with insulin treatment. The suppression of this enzyme is responsible for the altered fatty acid composition in diabetes mellitus (Faas and Carter, 1980; Holman et al., 1983), which resembles the present study. Treatment with Umbelliferone and glibenclamide brought back linolenic and arachidonic acids to near normalcy which could be due to elevated level of insulin as reported earlier by us (Ramesh and Pugalendi, 2005a) which restores Δ6 desaturase activities in the liver of diabetic rats.

Previous studies show that the fatty acid composition of various tissues is altered in both experimental and human diabetes (Faas and Carter, 1980; Eck et al., 1979). Diabetic rats had increased levels of palmitic, stearic and oleic acids and decreased levels of linolenic acid and arachidonic acid in the kidney which could be due to decreased Δ9 desaturation of palmitic and stearic acids (Mercuri et al., 1967) as well as the Δ6 desaturation of oleic, linoleic and α-linolenic acids and Δ6 desaturase activity (Castuma et al., 1972), which resemble the present work. Treatment with Umbelliferone and glibenclamide reversed the levels of both saturated and unsaturated fatty acids to near normalcy, which might be associated with increased insulin secretion which regulates the activities of Δ9 and Δ6 desaturases.

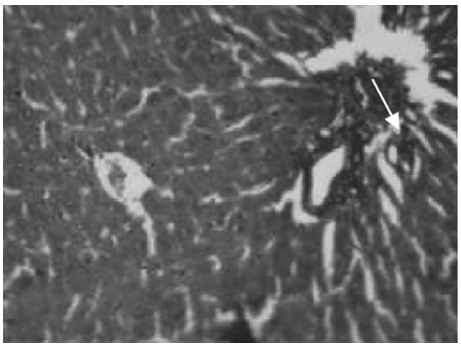

Fig. 11. Diabetic + glibenclamide (600 μg) treated rat liver H&E × 20 showing no fatty change [→].

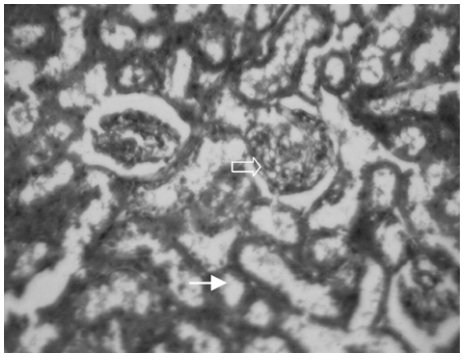

Fig. 13. Normal + Umbelliferone (30 mg) treated rat kidney H&E × 10 showing normal glomerulus (⇨) and tubular cells (→).

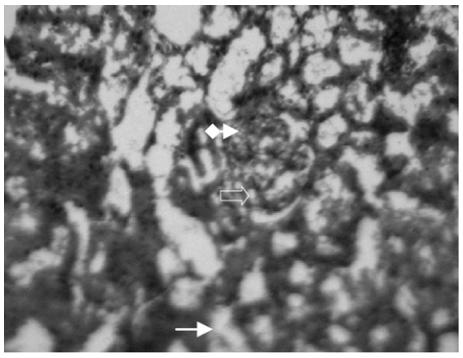

Fig. 14. Diabetic rat kidney H&E × 10 showing enlargement of glomerulus (⇨), mesangial tuff (◆▶), tubular cells (—►), fatty infiltration, large area of hemorrhage and lymphocyte infiltration.

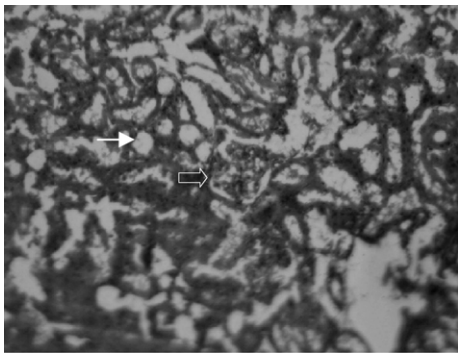

Fig. 16. Diabetic + glibenclamide (600 μg) treated rat kidney H&E × 10 showing glomerulus (⇨) and tubular cells (—►) with normal and mild lymphocyte infiltration.

A marked decrease in liver weight observed in diabetic rats could be due to an increased breakdown of glycogen and protein degradation, and increased gluconeogenesis. Treatment with Umbelliferone has elevated liver weight, glycogen content and plasma proteins which can be due to increased plasma insulin level (Ramesh and Pugalendi, 2006a). Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation. Further antioxidant property of Umbelliferone may also contribute by reducing tissue damage. Enzymes directly associated with the conversion of amino acids to keto acids are ALT and AST. ALT and AST activities are used as the indicators of hepatocyte damage (Whitehead et al, 1999). Diabetic rats have increased activities of these enzymes which may be due to hepatic damage and treatment with Umbelliferone has decreased the activities of these enzymes, by its antioxidant properties (Ramesh and Pugalendi, 2006d).

Histopathological study of liver showed fatty changes surrounding portal triad in the liver of diabetic rats and treatment with Umbelliferone and glibenclamide recovered from membrane damage by decreasing lipid peroxidation and improving antioxidants' status which was reported earlier by us (Ramesh and Pugalendi, 2006b), restoring fatty acid composition, and also supported by regulated glycoprotein components (Ramesh and Pugalendi, 2006c) and reversal of serum hepatic marker enzymes (Ramesh and Pugalendi, 2006d).

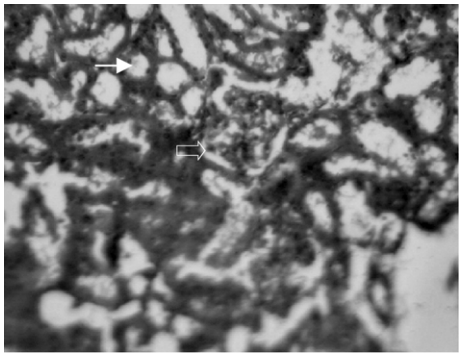

Fig. 15. Diabetic + umbelliferone (30 mg) treated rat kidney H&E × 10 showing glomerulus (⇨) and tubular cells (—►) with normal and mild hemorrhage.

The kidneys of rats with streptozotocin-induced diabetes become enlarged. Histopathological studies of kidney showed enlargement of lining cells of tubules, fatty infiltration, large area of hemorrhage and lymphocyte infiltration in the diabetic rats, which may be associated with membrane damage caused by hyperglycemia mediated oxidative stress and altered fatty acid composition, and treatment with Umbelliferone and glibenclamide reversed these changes to near normalcy, which could be associated with decreased membrane damage as evidenced by improved antioxidants' status (Ramesh and Pugalendi, 2006b), reversed fatty acid changes as evidenced by improved insulin level (Ramesh and Pugalendi, 2005a), and also supported by regulated glycoprotein components (Ramesh and Pugalendi, 2006c).

The classic symptoms of diabetes mellitus include polydipsia, polyuria and weight loss (American Diabetes Association, 2005). The clinical manifestation of diabetic nephropathy is the development of microalbuminuria. Gomes et al. (1997) observed that untreated diabetic animals developed albuminuria which may be due to leakage of albumin by damaged glomerular membrane. Treatment with Umbelliferone reduced urine albumin to near normal level which reflects the recovery of kidney from the damage by hyperglycemia induced oxidative stress.

Urea is the major nitrogen containing metabolic product of protein metabolism; uric acid is the major product of purine nucleotides, adenosine and guanosine; creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate (Burtis and Ashwood, 1996). The diabetic rats had decreased levels of urine urea, uric acid and creatinine, which are considered as significant markers of renal function (Almdal and Vilstrup, 1988), and this is in agreement with the present result. Treatment with Umbelliferone reversed these parameters to near normal level which could be due to decreased metabolic disturbances of other pathways such as protein and nucleic acid metabolisms as Umbelliferone improved glycemic control.

5. Conclusion

Our results show that Umbelliferone treatment reversed both saturated fatty acids and polyunsaturated fatty acids to near

normalcy. Manipulation of lipid sources might decidedly be beneficial in diabetes, characterized by an insulin deficiency, because it is known that the binding and affinity of the hormone to the receptor and the functional coupling of the occupied receptor via G-proteins to effector enzymes depends on membrane lipid composition and fluidity (Stubbs and Smith, 1984). Thus, Umbelliferone has reversed membrane fatty acid composition of liver and kidney, also supported by antioxidant and antihyperlipidemic effects of Umbelliferone as reported earlier (Ramesh and Pugalendi, 2005b,c, 2006b) as evidenced by improved histopathology of liver and kidney, which indicate that Umbelliferone has minimized the risk of diabetic complications.

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