Effect of Glymin, a Polyherbal Formulation on Lipid Profile and Histopathological Examination in Streptozotocin-Induced Diabetic Rats

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ABSTRACT
Diabetes Mellitus is a chronic widely spread human disease. Streptozotocin-induced diabetes in rats had been shown to be associated with functional and morphological changes in liver and kidney. This study was undertaken to investigate the effect of glymin on lipid profile and histopathological study of liver and kidney in streptozotocin-induced diabetic rats. Lipid profiles were investigated in serum and tissues (liver and kidney) of normal and streptozotocin-induced diabetic rats treated with glymin (50 and 100 mg/kg/day) for the period of 35 days. Diabetic rats exhibited an increase in the levels of total cholesterol, triglycerides, free fatty acids, phospholipids and low density lipoprotein cholesterol (LDL-cholesterol), and a decrease in the level of high density lipoprotein cholesterol (HDL-cholesterol). The administration of glymin shows decreased levels of total cholesterol, triglycerides, free fatty acids, phospholipids and LDL-cholesterol, and an increased level of HDL-cholesterol. The findings of this study indicate that the administration of glymin resulted in a better lipid profile which helps to recover the pathological effects of diabetes on liver and kidney of streptozotocin-induced diabetic rats.

Keywords: Diabetes mellitus, Streptozotocin, Glymin, Histopathology, Lipid profile.

INTRODUCTION
Diabetes mellitus (DM) is one of the most severe, incurable metabolic disorders characterized by hyperglycemia as a result of a relative, or an absolute, lack of insulin, or the action of insulin on its target tissue or both. [1] Besides hyperglycemia, several other symptoms, including hyperlipidemia, are involved in the development of microvascular complication of diabetes, which are the major causes of morbidity and death. [2] Hyperlipidemia is a complication associated with diabetes mellitus [3] due to qualitative and quantitative abnormalities in lipoproteins. Chronic hyperglycemia in diabetes leads to over production of free radicals and these contribute to the development of diabetic nephropathy. [4] Atherosclerosis and coronary heart disease are the major health problems. [5] A number of epidemiological investigations have shown a clear association between dietary saturated fat and atherosclerosis. [6] Moreover, many studies have shown that elevated total or low density lipoprotein (LDL) cholesterol in the blood are powerful risk factors for coronary heart disease [5], whereas high HDL-cholesterol: LDL-cholesterol ratio may protect against coronary heart disease. [7] Plant drugs [8] and herbal formulation [9-11] are frequently considered to be less toxic and freer from side effects than synthetic one. Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicine are important. [12] The attributed antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence treatment with herbal drugs has an effect on protecting β-cells and smoothing out fluctuation in glucose levels. [13-14] In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than individual. Various herbal formulations such as diamed [15], coagent db [16] and hyponidd [17], are well known for their antidiabetic effects. The present study was performed to assess the antidiabetic effects of glymin on streptozotocin-induced diabetic rats, and the possible morphologic changes in the liver and kidney.

MATERIALS AND METHODS
Experimental Animals
Male albino wistar rats (150-170g) obtained from Venkateswar Enterprises, Bangalore were used in this study.
They were housed in polypropylene cages (47×34×20 cm) lined with husk, renewed every 24 hours under a 12:12 hours light/dark cycle at around 22°C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolisable energy of 3, 600 kcal. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

**Chemicals**

Streptozotocin (STZ) was purchased from Himedia Laboratories Private Limited, Mumbai, India. Glymin was purchased from Venkateswara Medical Store, Salem, India. Chloroform, heptane, methanol, triethanolamine were purchased from Sigma Chemical Company, St. Louis, MO, USA. Triglycerides (TG) and HDL cholesterol kits were purchased from Agappe Diagnostics, Kerala, India. All other chemicals used in the study were of analytical grade.

**Induction of Experimental Diabetes**

Streptozotocin was used to induce diabetes mellitus in normoglycemic male albino wistar rats. A freshly prepared solution of streptozotocin (STZ) (45 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg body weight in overnight fasted rats. After 48 hours of STZ administration, rats with moderate diabetes having glycosuria and hyperglycemia were used in this study.

**Experimental Design**

In the experiment, a total of 30 rats (18 diabetic surviving rats, 12 control rats) were used in the study. The rats were divided into 5 groups of 6 rats in each group.

- **Group 1**: Normal control rats
- **Group 2**: Normal rats treated with Glymin (100 mg/kg)
- **Group 3**: Diabetic control rats (45 mg/kg)
- **Group 4**: Diabetic + Glymin (50 mg/kg)
- **Group 5**: Diabetic + Glymin (100 mg/kg)

Glymin was dissolved in 0.2% Dimethyl sulfoxide (DMSO) and administrated to rats orally using an intragastric tube daily for a period of 35 days. At the end of the treatment period, all rats were anaesthetized with pentobarbital sodium (35 mg/kg) and sacrificed by cervical decapitation. The blood collected by using potassium oxalate and sodium fluoride as anticoagulant for estimation of fasting blood glucose. The liver and kidney were dissected out, washed in ice-cold saline, and patted dry and weighed. Plasma and serum were separated by centrifugation. The liver and kidney were weighed and 10% tissue homogenate was used for various biochemical parameters.

**Biochemical Estimations**

Lipids were extracted from serum and tissues by the method of Folch et al. using chloroform: methanol mixture (2:1 v/v). Total cholesterol (TC) was estimated by the method of Zlatkis et al. Free fatty acids (FFA) levels were estimated by the method of Falholt et al. Phospholipids (PL) levels were estimated by the method of Zilversmit and Davis. HDL levels were estimated by Assmann method and triglycerides (TG) levels were estimated by Schettler and Nussel method using a commercial kit from Agappe Diagnostics.

Cholesterol in the lipoprotein fractions was also determined by the method of Zlatkis et al. as described earlier. HDL-cholesterol was estimated in the supernatant obtained after precipitation with phosphotungstic acid/MgCl₂. LDL-cholesterol and VLDL-cholesterol were calculated as follows:

- **LDL-C = Total cholesterol - (HDL-C + VLDL-C)**
- **VLDL-C = TGs/5**

**Histopathology**

Tissues (liver and kidney) obtained from all experimental groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissue was processed by embedding in paraffin. Then, the tissue was sectioned and stained with hematoxylin and eosin (H&E) and examined under high power microscope (400X) and photomicrographs were taken.

**Statistical analysis**

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) using Statistical Package for the Social Sciences (SPSS) software package version 9.05. *P* values <0.05 were considered significant.

**RESULTS**

**Effect of glymin on lipid profiles in serum and tissues**

The levels of lipid parameters such as total cholesterol, TG, HDL, LDL and VLDL in serum and tissues of normal and STZ-induced diabetic rats are shown in Fig. 1 (a, b, c) & 2 (a, b). The levels of total cholesterol, TG, LDL and VLDL levels were increased significantly and decrease in the levels of LDL. Oral administration of glymin (50 and 100 mg/kg/day) for a period of 35 days significantly decreases the level of total cholesterol, TG, LDL- cholesterol and VLDL and increases the levels of HDL in STZ- induced diabetic rats. The levels of FFA and PL in serum and tissues of STZ-induced diabetic and control rats are given in Fig. 3 (a, b, c). STZ-induced diabetic rats had elevated levels of FFA and PL in serum, liver and kidney. Diabetic rats treated with glymin reversed these lipid profiles to near normal levels.

**Effect of glymin on histopathology of diabetic rat tissues (liver and kidney)**

The effects of glymin on histopathology of diabetic rat liver are shown in Fig. 4 (a-e). Normal control rats showed normal hepatic structure (Fig. 4a). Normal rats treated with glymin (100 mg/kg) showed the normal architecture of the hepatic tissue (Fig. 4b). STZ-induced diabetic control rats showed severe necrosis of the hepatocytes, surrounded the portal area with oedema and inflammatory cells (Fig. 4c). Rats treated with glymin (50 mg/kg) showed moderate separation of the muscle fibers in hepatocytes and accumulation of inflammatory cells with mild oedema (Fig. 4d). Rats treated with glymin (100 mg/kg) showed normal appearance with few inflammatory cells (Fig. 4e). The effects of glymin on histopathology of diabetic rat kidney are shown in Fig. 5 (a-e). Normal control rats showed normal architecture of the mesangial cells and glomeruli (Fig. 5a). Normal rats treated with glymin (100 mg/kg) showed normal architecture of the renal tissue (Fig. 5b). STZ-induced diabetic control rats showed severe epithelial atrophy and mild sclerotic changes in glomeruli and
Fig. 1 (a, b, c): Effect of glymin on the levels of cholesterol and triglycerides in serum, liver and kidney in normal and STZ-induced diabetic rats. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P < 0.05, DMRT).
Fig. 2 (a, b): Effect of glymin on the levels of serum low-density lipoproteins (LDL), high-density lipoproteins (HDL) and very low-density lipoproteins (VLDL) in normal and STZ-induced diabetic rats. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P < 0.05, DMRT).

Fig. 3 (a, b, c): Effect of glymin on the levels of free fatty acids and phospholipids in serum, liver and kidney in normal and STZ-induced diabetic rats. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P < 0.05, DMRT).
moderate congestion of capillaries (Fig. 5c). Rats treated with glymin (50 mg/kg) showed mild atrophy and sclerotic changes with inflammatory cells (Fig. 5d). Rats treated with glymin (100 mg/kg) showed mild glomerular changes and congestion and oedema with inflammatory cells (Fig. 5e). For all the parameters studied, glymin at a dose 100 mg/kg showed better effect than 50 mg/kg. Glymin treatment to normal rats (100 mg/kg) didn’t show any significant effect.

4a. Normal control rats showed normal hepatic structure

4b. Normal rats treated with glymin (100 mg/kg) showed the normal architecture of the hepatic tissue.

4c. STZ-induced diabetic control rats showed severe necrosis of the hepatocytes, surrounded the portal area with oedema and inflammatory cells.

4d. Rats treated with glymin (50 mg/kg) showed moderate separation of the muscle fibres in hepatocytes and accumulation of inflammatory cells with mild oedema.

4e. Rats treated with glymin (100 mg/kg) showed near normal appearance with few inflammatory cells.

Fig. 4 (a-e): Histopathological examination in liver of normal and STZ-induced diabetic rats

5a. Normal control rats showed normal architecture of the mesangial cells and glomeruli.

5b. Normal rats treated with glymin (100 mg/kg) showed normal architecture of the renal tissue.

5c. STZ-induced diabetic control rats showed severe epithelial atrophy and mild sclerotic changes in glomeruli and moderate congestion of capillaries.
microviscosity and survival. PL are important for the maintenance of cellular integrity, Phospholipids are vital components of biomembrane. These liver by the reverse cholesterol transport pathway. the transport of cholesterol from the peripheral blood to the disease. It is well known that HDL plays an important role in High levels of TC are major risk factors for cardiovascular [24] increased plasma TG levels observed in obese adolescents. Resistance to the action of insulin on lipoprotein lipase in [25] increased levels of plasma TG levels observed in obese adolescents. High levels of TC are major risk factors for cardiovascular disease. It is well known that HDL plays an important role in the transport of cholesterol from the peripheral blood to the liver by the reverse cholesterol transport pathway. The plasma levels of TC and TG increases, contributing to secondary complications of diabetes. Phospholipids are vital components of biomembrane. These PL are important for the maintenance of cellular integrity, microviscosity and survival. Increased levels of PL and FFA were observed in STZ-induced diabetic rats may be due to membrane damage caused by increased lipid peroxidation. The abnormal high concentrations of serum lipids in diabetic animals are due to an increase in the mobilization of FFA from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase. Excess fatty acids in the serum of diabetic rats are converted into PL and cholesterol in the liver. Diabetic rats treated with glymin reversed the levels of TC, TG, FFA and PL to near normal levels. Lipoprotein abnormalities play an important role in the causation of diabetic atherosclerosis. Dyslipidaemia causes morbidity and mortality in patients with type 2 diabetic mellitus and the most common pattern in type 2 diabetic patients is elevated TG and LDL, and decreased HDL cholesterol concentrations. The modifications of LDL lipoprotein increase atherogenicity and available data suggest that LDL is more atherogenic in individuals with type 2 diabetes mellitus. In the present study, diabetic rats exhibited a significant elevation of LDL-C and VLDL-C, while HDL-C was decreased. Glymin administration resulted in lowering the plasma levels of LDL-C and VLCL-C with elevation of HDL-C level.

Histopathology of liver in control rats showed normal hepatic structure. Diabetic control liver showed severe necrosis of the hepatocytes surrounded the portal area with oedema and inflammatory cells. Diabetic rats treated with glymin showed near normal appearance with few inflammatory cells. Normal rats treated with glymin showed normal architecture of the hepatic tissue. Histopathology of kidney in control rats revealed normal architecture of the mesangial cells and glomeruli. Diabetic control rats showed severe epithelial atrophy and mild sclerotic changes in glomeruli and moderate congestion of capillaries. Diabetic rats treated with glymin showed mild glomerular changes and congestion and oedema with inflammatory cells. Normal rats treated with glymin showed normal architecture of the renal tissue. This could be due to free radical scavenging, antioxidant and membrane stabilizing properties of glymin.

The liver and kidney exhibits numerous morphological and functional alterations during diabetes. Since both diabetes and hyperlipidemia are considered to be major risk factors for the premature atherosclerosis and essentially all the cholesterol in atherosclerotic plaques is derived from that of circulatory cholesterol. The antihyperlipidemic effect of glymin in particular could be considered as of possible therapeutic value.

ACKNOWLEDGEMENT
The authors gratefully acknowledge Dr. N. Pazhanivel, Associate Professor, Department of Veterinary Pathology, Madras Veterinary College, Chennai for his valuable help to carryout histopathological studies.

REFERENCES
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