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RESEARCH ARTICLE

EFFECT OF SINAPIC ACID ON MEMBRANE BOUND ENZYMES AND LIPID PROFILE IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETES IN WISTAR RATS

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ABSTRACT

The aim of the study was to evaluate membrane bound enzymes and lipid lowering effects of sinapic acid in normal and streptozotocin induced diabetic rats. Diabetes was induced in female wistar rats by a single intraperitoneal administration of streptozotocin (45 mg/ kg BW). Rats were divided into six groups: normal (untreated), normal + sinapic acid (15 mg/kg), normal + sinapic acid (30 mg/kg), diabetic control, diabetic + sinapic acid (15 mg/kg) and diabetic + sinapic acid (30 mg/kg). Diabetic rats exhibited increased level of plasma glucose and decreased levels of liver and muscle glycogen and membrane bound enzymes such as Na^+/K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase. STZ also caused significant elevation in total cholesterol, triglycerides, low density lipoprotein cholesterol and very low density lipoprotein cholesterol with consequent reduction in high density lipoprotein cholesterol in serum. Treatment with sinapic acid for a period of 35 days restored all these parameters to near normal. The results of the present study revealed that sinapic acid possesses a potential lipid lowering effect in streptozotocin -induced diabetic rats.

INTRODUCTION

Diabetes mellitus currently is a major health problem for the people of the world and is a chronic metabolic disorder/ syndrome resulting from a variable interaction of hereditary and environmental factors and is characterized by abnormal insulin secretion or insulin receptor or post receptor events affecting metabolism involving carbohydrates, proteins and fats in addition to damaging liver, kidney and β-cells of pancreas (Baynes, 1991). The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al*., 2004). In diabetes, there is inability to store fat and protein along with breakdown of existing fat and protein stores. Streptozotocin-induced diabetic rats showed significant increases in the levels of cholesterol, phospholipid, triglycerides and free fatty acid (Ravi

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et al., 2005; El-Agouza *et al.,* 2000). Oxidative stress induced by high glucose concentration plays a central role in the complications of diabetes (Medvedeva *et al.,* 2002). During diabetes, a profound alteration in the concentration and composition of lipids occurs. Liver and kidney are important for glucose and lipid homeostasis, they participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. Thus it is expected to have changes in liver and kidney during diabetes (Seifter and England, 1982).

The ubiquitous cellular enzyme Na^+/K^+-adenosine triphosphatase (ATPase) is responsible for the maintenance of intracellular sodium and potassium concentrations (McDonough *et al.,* 1990; Sweadner, 1989). The function of this enzyme is to transport three ions of sodium from the intracellular space to the extracellular environment and in return, to allow two ions of potassium to enter the cell. The high-affinity Ca^{2+}-ATPase is the major active calcium transport protein responsible for the maintenance of normal intracellular calcium levels in a variety of cell types. Maintenance of the cation gradient by high affinity Ca^{2+}-ATPase is of fundamental importance in the control of hydration, volume, nutrient uptake and fluidity of cells and is also essential for the contractility and excitability of muscles (Golfman *et al.,* 1996). Low-affinity Ca^{2+}-ATPase is considered to be responsible for the shape and deformability of the erythrocyte membranes (Levy *et al., 1*994).

It is well known that in diabetes oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defences catalase, superoxide dismutase and glutathione peroxidase (Oberley, 1988). In addition, there is a relationship between diabetes and impairment of lipid metabolism; high density lipoprotein (HDL) protects low density lipoprotein (LDL) oxidation, and this protection is impaired in diabetic cases (Sharpe *et al.*, 1998). Management of diabetes mellitus without any side effects is still a challenge to the medical system (Kameswara Rao and Appa Rao, 2001). Medicinal plants are a good source of natural antioxidants believed to exert their effects by reducing the formation of the final active metabolite of drug reactive molecular species to prevent their reaching a target site (Kaleem *et al.,* 2006).

Flavonoids are organic pigments occurring in plants which give plants a red, violet or blue colour. Flavonoids have a particularly broad spectrum of efficacy. Flavonoids inhibit the growth of bacteria and viruses, protect the cells against the damages of free radicals, protect against cancers and heart attacks, have a repressive effect against inflammations and influence blood coagulation (Spencer and Jeremy, 2008). Sinapinic acid or sinapic acid (Sinapine - Origin: L. Sinapi, sinapis, mustard, Gr., cf. F. Sinapine.), is a small naturally occurring carboxylic acid. It is a member of the phenylpropanoid family. Sinapic acid is a cinnamic acid derivative which possesses 4-hydroxy-3, 5-dimethoxy cinnamic acid is one of the phenolic acids widely distributed in edible plants such as cereals, nuts, oil seeds and berries (Shahidi and Naczk, 2004). Sinapic acid is a major free phenolic acid in rapseed meal, with the majority found in the esterified form of sinapine (Krygier *et al.,* 1982). Thus, our present study was designed to evaluate the effect of sinapic acid on glucose, glycogen, membrane bound enzymes and lipid profile in normal and STZ- induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals

Female albino wistar rats (150-200 g) obtained from Venkateswara Enterprises, Bangalore were used in this study. They were housed in polypropylene cages (47 x 34 x 20 cm) lined with husk. It was renewed every 24 hours under a 12:12 hour light: dark cycle at around 22º C and had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Limited., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 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 metabolizable energy of 3600 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.

Drug and Chemicals

Streptozotocin (STZ) was purchased from Himedia Laboratories Private Limited, Mumbai. Sinapic acid was purchased from Sigma- Aldrich, St. Louis, USA. Glucose, total cholesterol, triglycerides and HDL kits were purchased from Agappe diagnostics, Kerala, India. All other chemicals used in the study were of analytical grade.

Experimental Induction of Diabetes

STZ was used for the induction of diabetes mellitus in normoglycemic female albino wistar rats. Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared STZ (45 mg/ kg body weight) in citrate buffer (pH 4.5) in a volume of 1 ml/ kg (Siddiqui *et al.,* 1987). STZ injected animals were given 10% glucose solution for 5 days to prevent initial drug induced hyperglycemic mortality. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, 48 hours after injection with STZ. Albino rats with a blood glucose level above 240 mg/ dl were considered to be diabetic and were used in the experiment.

Experimental Design

In the experiment, a total of 36 rats (18 diabetic surviving rats and 18 control rats) were used. The rats were divided into 6 groups of 6 rats in each group.

Group 1: Normal control rats
Group 2: Control rats administrated orally with Sinapic acid (15 mg/kg)
Group 3: Control rats administrated orally with Sinapic acid (30 mg/kg)
Group 4: Diabetic control rats
Group 5: Diabetic rats treated orally with Sinapic acid (15 mg/kg)
Group 6: Diabetic rats treated orally with Sinapic acid (30 mg/kg)

Sinapic acid was dissolved in 0.2% dimethyl sulfoxide (DMSO) and administrated to rats orally using an intragastric tube daily for a period of 35 days.

Sample Collection

After 35 days of treatment, the animals were fasted for 12 hours and then sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifugation. The liver and kidney were carefully removed, weighed and washed in ice-cold saline to remove the blood. The liver, kidney and muscle were sliced into pieces and homogenized in an appropriate buffer (pH 7.0). The homogenates were centrifuged at 3000 rpm for 10 min at 0^{o} C in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Biochemical Estimations

Biochemical parameter such as plasma glucose was estimated by the method of Trinder **(**1969**).** Glycogen content in liver and muscle was estimated by the method of Wieland (1963). The activity of Na^{+}/K^{+}-ATPase was assayed according to the procedure of Bonting (1970). The activity of Ca^{2+}-ATPase was assayed according to the method of Hjerten and Pan (1983). The activity of Mg^{2+}-ATPase was assayed by the method of Ohnishi *et al.,* (1982).

Statistical Analysis

Results were expressed as mean ± SD for six rats in each experimental group. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 9.05 software. The data were analyzed using one-way analysis of variance (ANOVA) and group means were compared with Duncan's Multiple Range Test (DMRT). P-values < 0.05 were considered as significant.

RESULTS

Effect of sinapic acid on plasma glucose

The effect of sinapic acid on plasma glucose level of normal and STZ-induced diabetic rats are depicted in Figure 1. The diabetic rats exhibited a

significant (P<0.05) increase in plasma glucose when compared with normal rats. Oral administration of sinapic acid significantly (P<0.05) reduced the level of plasma glucose in STZ-induced diabetic rats as compared to diabetic controls.

Effect of sinapic acid on liver and muscle glycogen

The effect of sinapic acid on glycogen content in liver and muscle of normal and experimental rats are presented in Figure 2. STZ - induced diabetic

Table 1. Effect of Sinapic acid on membrane bound enzymes in tissues of normal and STZ-induced diabetic rats

Groups	Na^+/K^+-ATPase (Units*/mg protein)		$Ca2^+$-ATPase (Units*/mg protein)		$Mg2^+$-ATPase (Units*/mg protein)	
	Liver	Kidney	Liver	*Kidney*	Liver	*Kidney*
Normal control	0.88 ± 0.07[a]	0.71± 0.14[a]	0.85 ± 0.25[a]	0.84 ± 0.08[a]	0.69 ± 0.14[a]	0.75 ± 0.09[a]
Normal + Sinapic acid (15mg/kg)	0.86 ± 0.07[a]	0.72± 0.15[a]	0.83 ± 0.23[a]	0.92 ± 0.07[a]	0.64 ± 0.15[a]	0.73 ± 0.05[a]
Normal + Sinapic acid (30mg/kg)	0.85 ± 0.07[a]	0.70±0.16[a]	0.82 ± 0.24[a]	0.85 ± 0.04[a]	0.65 ± 0.13[a]	0.72 ± 0.05[a]
Diabetic control	0.37 ± 0.14[b]	0.31± 0.08[b]	0.45 ± 0.10[b]	0.33 ± 0.14[b]	0.26 ± 0.05[b]	0.44 ± 0.17[b]
Diabetic + Sinapic acid (15mg/kg)	0.53 ± 0.18[c]	0.56± 0.04[c]	0.66 ± 0.15[c]	0.45 ± 0.17[c]	0.40 ± 0.06[c]	0.55 ± 0.15 [c]
Diabetic + Sinapic acid (30mg/kg)	0.66 ± 0.16[d]	0.63± 0.03[d]	0.72 ± 0.17[d]	0.56 ± 0.19[d]	0.51 ± 0.06[d]	0.65 ± 0.16 [d]

* Activity expressed as units: µmoles of phosphorus liberated/min/mg protein

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 2. Effect of Sinapic acid on serum lipid profile of normal and STZ-induced diabetic rats

Groups	Total cholesterol (mg/dl)	TG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
Normal control	53.92 ± 1.74[a]	97.32 ± 2.00 [a]	12.21 ± 1.5[a]	22.25 ± 0.08[a]	19.46 ± 1.15[a]
Normal + Sinapic acid (15mg/kg)	53.78 ± 1.73[a]	97.21 ± 2.01 [a]	11.97 ± 1.56[a]	22.37 ± 0.09[a]	19.44 ± 1.20[a]
Normal + Sinapic acid (30mg/kg)	53.70 ± 1.71[a]	97.31 ± 2.04[a]	11.57 ± 1.53[a]	22.67 ± 0.05[a]	19.46 ± 1.18[a]
Diabetic control	132.39 ± 1.09[b]	146.49 ± 3.74[b]	82.46 ± 1.10[b]	20.63 ± 0.15[b]	29.30 ± 2.64[b]
Diabetic + Sinapic acid (15mg/kg)	124.38 ± 1.12[c]	93.60 ± 2.29[c]	68.37 ± 1.08[c]	37.24 ± 0.13[c]	18.72 ± 2.51[c]
Diabetic + Sinapic acid (30mg/kg)	122.28 ± 1.18[d]	89.65 ± 2.34[d]	64.88 ± 1.09[d]	39.43 ± 0.14[d]	17.93 ± 2.49[d]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

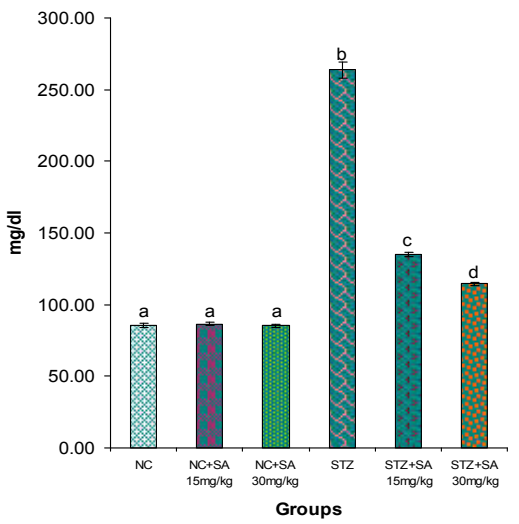

Fig 1. Effect of Sinapic acid on plasma glucose level in normal and STZ-induced diabetic rats

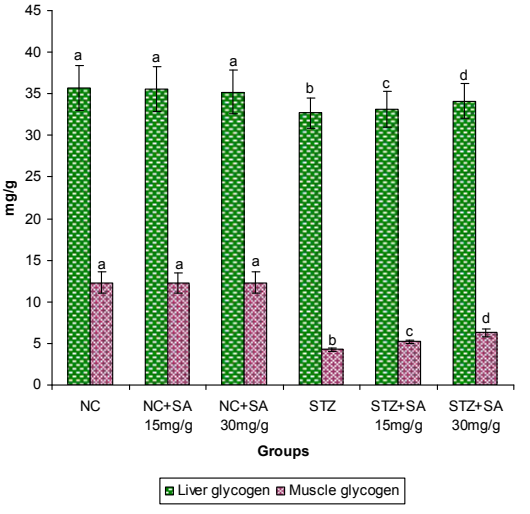

Fig 2 In x-axis, instead of mg/kg, it has been mentioned as mg/g. NC , NC +SA I5 mg/kg, NC+SA 30 mg/kg, STZ, STZ+ SA I5 mg/kg, STZ+ SA 30mg/kg

rats showed a significant (P<0.05) reduction in liver and muscle glycogen content when compared to normal control. Treatment with sinapic acid significantly (P<0.05) increased the concentration of liver and muscle glycogen in STZ - induced diabetic rats when compared with diabetic control rats.

Effect of sinapic acid on membrane bound enzymes in tissues

The activities of membrane bound enzymes such as Na^+/K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase in the liver and kidney of control and experimental rats are shown in Table 1. A significant (P<0.05) reduction in the activity of Na^+/K^+ - ATPase, Ca^{2+} - ATPase and Mg^{2+} ATPase were observed in the liver and kidney of diabetic rat as compared to normal rats. Treatment with sinapic acid restored the activities of Na^+/K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase to near normal in the liver and kidney of diabetic rats.

Effect of sinapic acid on serum lipid profile

The levels of lipid parameters such as total cholesterol, triglycerides, LDL- cholesterol, HDL-cholesterol and VLDL-cholesterol in normal and STZ-induced diabetic rats are presented in Table 2. STZ-induced diabetic rats showed a significant (P<0.05) elevation in the levels of total cholesterol, triglycerides, LDL- cholesterol and VLDL-cholesterol and decrease in the level of HDL-cholesterol when compared to normal rats. Oral administration of sinapic acid in diabetic rats significantly (P<0.05) reduced the levels of total cholesterol, triglycerides, LDL- cholesterol and VLDL-cholesterol and elevated the HDL-cholesterol level as compared to diabetic control rats.

DISCUSSION

Diabetes mellitus is one of the most common chronic diseases and is associated with hyperlipidemia and co-morbidities such as obesity and hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes (Bierman *et al.,* 1975). In the present study, an increase in the plasma glucose level in diabetic rats confirmed the induction of diabetes by STZ. For diabetic models, STZ was used to induce diabetic conditions in rats. Elsner *et al.* (2000) suggested that STZ induces hyperglycaemia by damaging DNA in the nuclei of pancreatic β-cells through alkylation, leading to an increase in poly (ADP-ribose) synthase, which results in a drastic decrease in nicotinamide adenine dinucleotide (NAD) concentrations of the β-cells, then a decrease in the number of β-cells and death of the cells. All these changes may induce dysfunction of the pancreas in insulin secretion. The fundamental mechanism underlying hyperglycemia in diabetes involves the overproduction of glucose by excessive hepatic glycogenolysis and gluconeogenesis and decreased utilization by the tissues (Yamamoto *et al.,* 1981). We have observed a significant decrease in plasma glucose in sinapic acid treated diabetic rats, when compared with diabetic control rats. This could be due to the regeneration of existing pancreatic beta cells and enhanced transport of glucose to the peripheral tissues by sinapic acid.

Glycogen is the primary intracellular storage form of glucose and its level in various tissues are a direct indication of insulin activity as insulin promotes the deposition of intracellular glycogen by stimulating glycogen synthase and inhibiting glycogen phosphorylase activity. The decreased hepatic glycogen content in STZ-induced diabetes could be due to the marked decrease in insulin level, since STZ causes selective destruction of β-cells and the liver also depends on insulin for the entry of glucose (Whitton and Hems, 1975). Thus, assessment of glycogen level serves as a marker for the evaluation of antidiabetic activity. Moreover, glycogen synthase and glycogen phosphorylase are the two key regulatory enzymes that catalyze glycogenesis and glycogenolysis, respectively. Ferrannini *et al.* (1990) reported that liver glycogen content was reduced significantly in the STZ-induced diabetic rats and the same was also observed in our study. Further, the decreased glycogen content in diabetic condition might be due to the increased activity of glycogen phosphorylase and decreased activity of glycogen synthase (Roesler and Khandelwal, 1986). The oral administration of sinapic acid to STZ-induced diabetic rats significantly increased the glycogen content by stimulating the glycogen synthase

activity and inhibiting the glycogen phosphorylase activity in the liver and muscle by stimulating the remnant β-cells to release insulin.

Membrane bound phosphatases are ubiquitous enzymes essential for the maintenance of electrolyte balance and fundamentally involved in the maintenance of ion gradients that drive the co-transport of amino acids and sugars, regulate cell volume and contribute to part of the membrane potential (Sweeney and Klip, 1998). ATPases are intimately associated with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium (Mourelle and Franco, 1991). Alterations of these ATPases are thought to be linked to several complications of diabetes (Shahid and Mahboob, 2003). Na^+/K^+-ATPase, a membrane-linked enzyme that catalyzes the hydrolysis of ATP and couples it to the transport of Na^+ and K^+ across the cell membrane thereby generating the transmembranous Na^+/K^+ gradient. This pump is essential for the regulation of cell volume, uptake of nutrients, regulation of neurotransmitters release and contractibility and excitability properties of nerve tissue (Hernandez, 1992). The diabetic rats in the present study showed decreased level of Na^+/K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase in tissues, which is in accordance with the report of Kjeldsen *et al*. (1987). This might be associated with the deficiency of insulin as insulin administration partially restored Na^+/K^+ ATPase activity (Gupta *et al.,* 1996). The oxidative damage of tissue lipids and proteins might have caused the inactivation of Na^+/K^+-ATPase, which is rich in thiol groups and oxidation of thiol groups has been reported to inhibit enzyme activity (Trinder, 1969). This may be due to insulin secretory effect along with decreasing peroxidative damage to membrane lipids (Ramesh and Pugalendi, 2006).

Decreased activity of membrane Ca^{2+}-ATPase activity may be responsible for increase in intracellular calcium and consequently, for elevated vascular resistance which is frequently associated with hypertension (Zemel *et al.,* 1990). The decrease in Ca^{2+}-ATPase activity in the diabetic state may be due to altered membrane properties (Winegrad, 1987). It is reported that glycosylation of membrane proteins in diabetes significantly inhibits Ca^{2+}-ATPase activity (Ramanadevi *et al.,* 1997). Insulin directly regulates the membrane bound ($Ca^{2+,}$ Mg^{2+})-ATPase (Hope-Gill and Nanda, 1979). In our study, diabetic rats showed decreased activity of low affinity Ca^{2+}-ATPase. This could be due to insulin deficiency as insulin is the regulator of the enzyme.

Mg^{2+}-ATPase activity is involved in energy requiring process in the cell membrane and its activity is sensitive to membrane lipid peroxidation. In general, lipid peroxidation and glycosylation of membrane proteins influences the functions of different ATPases in diabetic condition. Ecto ATPases (extracellular ATPases) are cell surface ATPases that hydrolyze extracellular ATPs. E-type ATPase activity was first designated as Mg^{2+}-ATPase (Sabbadini and Dahms, 1989). which might be associated with insulin secretory effect. Treatment with sinapic acid reversed diabetes- induced alterations in the activities of Na^+/K^+ -ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase. The free radical scavenging property of sinapic acid could be responsible for the maintenance of the functional activity of membranes.

Hyperlipidaemia, a metabolic abnormality is frequently associated with diabetes mellitus. Its prevalence is variable, depending on the type and severity of diabetes, glycaemic control, nutritional status, age and other factors. The most characteristic lipid abnormality in diabetics is hypertriglyceridaemia, with or without associated increase in plasma cholesterol (Goldberg, 1981; Taskinen, 1990). In the present study, significantly increased levels of serum total cholesterol, triglycerides, LDL and VLDL- cholesterol but markedly decreased levels of serum HDL-cholesterol were observed in STZ-induced diabetic rats. These results are in agreement with the report of Bolkent *et al. (*2004); Ravi *et al.* (2005); Sing *et al.* (2005); Rajasekaran *et al.* (2006). The abnormal high concentrations of serum lipids in diabetic animals are due to an increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone-sensitive lipase (Puspharaj *et al.,* 2000). Excess fatty acids in a

serum of diabetic rats are converted into phospholipids and cholesterol in the liver. These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the form of lipoproteins (Bopanna *et al.,* 1997). The present study showed that sinapic acid had favourably modified serum lipid profile in rats with significant decrease in total cholesterol, triglycerides, LDL and VLDL-cholesterol and increase in HDL-cholesterol levels. This effect may be due to the antilipidemic action of sinapic acid.

CONCLUSION

Thus, our findings demonstrate that sinapic acid has an antidiabetic effect, which is evidenced by decreased plasma glucose, elevated glycogen and membrane bound enzymes (Na^+/K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase) and hypolipidemic effect, which is evidenced by the decreased levels of total cholesterol, triglycerides, LDL and VLDL-cholesterol and elevated levels of HDL-cholesterol in diabetic rats.

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