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The Protective Effect of a Novel Coumarinic Derivative on Streptozotocin Induced Diabetic Rats

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Abstract

Introduction: Diabetes mellitus (DM) affects more than 415 million persons worldwide. It is one of the most important causative factors of mortality in the developing countries. It was established that *Streptozotocin* (STZ) is a chemical compound that induces diabetic complications in rats.

Objective: This study aimed to examine the ability of novel coumarine-caffeic acid derivative: Ethyl-3-(3, 4-dihydroxyphenyl) acrylamido--5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1, 8-dihydropyrano [3, 2-f] chromene-2-carboxylate (Compound I) to reduce the diabetic complications induced by STZ.

Method: Diabetes was induced in the rats by the injection with STZ (65 mg/Kg b.w). Diabetes was confirmed then we allowed 7 days to stabilize blood glucose level in these rats. Compound I (25 and 50 mg/kg b.w daily for 4 weeks) was injected into diabetic rats.

Results and Discussion: The treated diabetic rats with Compound I had significantly reduced the high blood glucose level, Homeostatic index of insulin resistance (HOMA IR), Triglycerides (TG), Total-Cholesterol (TC), Low Density Lipoprotein-Cholesterol (LDL-c), Very Low Density Lipoprotein-Cholesterol (VLDL-c) and atherogenic index. The treatment with Compound I also resulted in the improvement of the insulin and insulin resistance and significantly increased serum High Density Lipoprotein Cholesterol (HDL-c), Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) as well as decreased TBARS in the liver of diabetic rats.

Conclusion: Compound I showed protective effects against hyperglycemia. It decreases the blood glucose level and

improves the lipid profile by scavenging of free radicals and reducing the risk of diabetic complications.

Introduction

Coumarines and their derivatives are very important heterocyclic compounds with diverse biological functions, for example many of these compounds have proven to be active as antimicrobial, anti-inflammatory, and antitumor agents and in inhibition of hepatitis C virus [1–3].

In connection to our programme work of semi-synthetic coumarines of biological importance using the natural heterocyclic compound 6-hydroxy-7-methoxy-4 methyl coumarin isolated from aerial parts of the Egyptian medicinal plant *Ammimajus L.*

Diabetes Mellitus (DM) is a wide range of metabolic disorders characterized by hyperglycemia resulting from insulin deficiency or insulin resistance or both. Hyperglycaemic complications involve damage to the small vessels such as in neuropathy, nephropathy, and retinopathy, and also large blood vessels as in cardiovascular diseases [6].

STZ [2-deoxy-2- (3-methyl-3-nitrosoureido)-D-glucopyranose] was used as intracellular nitric oxide (NO) donor leading to generation of reactive oxygen species (ROS). NO plus ROS caused DNA fragmentation and was used to induce diabetic complication in experimental animals [7,8].

The present investigation was undertaken to assess the effect of compound I: Ethyl-3-(3, 4-dihydroxyphenyl) acrylamido--5-methoxy-1-(4-methoxyphenyl)-10-methyl-8-oxo-1, 8-dihydropyrano [3,2-f] chromene-2-carboxylateon blood glucose, plasma insulin, lipid profile and enzymatic antioxidant in STZ-induced diabetic rats.

Experimental

Animals

Adult male albino rats weighing 150–200 g were purchased from the Faculty of Veterinary Medicine, Cairo University. The animal use protocol had been approved by the Institutional Animals Ethics Committee (IAEC) of Tanta University.

Induction of diabetes in experimental animals

STZ was dissolved in citrate buffer 0.1 M, pH 4.5 at a dose of 65 mg/kg body weight and then injected to rats to induce diabetes [9]. Diabetes was verified by measurement of blood glucose levels [10].

Groups classification

The rats were divided into three major groups, Group I: the control groups-twenty four rats- divided into three sub-groups eight rats per each, Group 1a: Normal control rats received oral dose 5 ml/kg b.wt of propylene glycol, Group 1b: Normal control rats were treated with compound I (25 mg/kg b.wt.) and Group 1c: Normal control rats were

treated with compound I (50 mg/kg). Group II, Diabetic group -eight rats- (STZ-induced diabetic rats, was given STZ and propylene glycol, orally (5 ml/kgb.wt), and Group III Diabetic treated groups–sixteen rats- contain 2 sub-groups eight rats per each, Group IIIa: STZ-induced diabetic rats were treated with compound I (25 mg/kg b.wt) and Group IIIb: STZ- induced diabetic rats were treated with compound I (50 mg/kg b.wt).

Compound I was suspended in propylene glycol and given orally to animals for 28 days. The fasting blood glucose levels were measured at 0, 7, 14, 21 and 28 day. Animals were sacrificed and the blood was collected. The liver was also isolated and washed in saline.

Biochemical assays

Blood samples were centrifuged, and the plasma was used for the determination of the glucose [11]. Homeostatic index of insulin resistance (HOMA-IR), calculated by glucose (mM) X insulin (μU/ml)/22.5 [12]. Triglycerides [13], total cholesterol, HDL-cholesterol [14], LDL-cholesterol [15], LDL-cholesterol=Total cholesterol– Triacylglycerols/5–HDL-cholesterol. VLDL-cholesterol concentration, VLDL-cholesterol=triacylglycerols/5) and the atherogenic index log (TG/HDL-C)] was also calculated [16].

Part from the liver was homogenized with phosphate buffer saline and used for the estimation of GSH [17], GPx [18], Catalase (CAT) [19], SOD [20] and protein content [21] TBARS [22].

Statistical analysis

All data were expressed as mean ± SE. and SPSS 15.0. Statistical analysis was performed using one-way analysis of variance for the comparisons of means of the different groups. Diabetic control rats were compared with normal control rats while compound I treated diabetic rats were compared with diabetic control. The results are considered to be significant when P<0.05. All of the figures were performed using origin program version 8.

Results

Acute toxicity study

The dose of compound I that killed half of the mice (LD_{50}) was 230 mg/100g b.w. (Data not shown).

Effect of compound I on blood glucose, insulin and HOMA-IR levels

Administration of STZ to the rats significantly (P<0.01) increased blood glucose, HOMA-IR and decreased insulin levels in the diabetic rats when it was compared to normal control rats. In diabetic rats, the treatment with compound I at both 25 and 50 mg/kg body weight significantly (P<0.01) decreased blood glucose and HOMAIR level as well as the level of plasma insulin was significantly increased (P<0.01) in diabetic treated rats compared to diabetic control rats (Table 1).

	Control groups			STZ-diabetic group	STZ-diabetic/ treated groups	
	group 1a	group 1b	group 1c	group 2	group 3a	group 3b
Glucose (mM)	6.12 ± 0. 33	5.45 ± 0.31	4.84 ± 0.35	20.66 ± 1.78**	9.55 ± 1.2**	7.51 ± 0.6**
Insulin(μU/ml)	3.35 ± 0.13	3.4 ± 0.21	3.8 5 ± 0.18	1.55 ± 0.08**	2.06 ± 0.09**	2.51 ± 0.07**
(HOMA-IR)	0.911± 0.08	0.823 ± 0.06	0.828 ± 0.03	1.423 ± 0.09**	0.874 ± 0.05*	0.83 ± 0.06**

Values are given as mean ± SE for groups of eight animals each. Values are statistically significant at *P<0.05, **P<0.01. Diabetic control rats were compared with normal control rats.

Table 1: Effect of compound I on blood glucose, insulin and HOMA-IR levels

Effect of compound I on lipid profiles

In diabetic rats, TC, TG, LDL, VLDL and atherogenic index levels were increased and HDL level was decreased significantly (P<0.01) compared to normal control rats. In diabetic rats, administration of compound I at 25 and 50 mg/kg dose showed significant (P<0.01) decrease in elevated TC, TG, LDL, VLDL and atherogenic index levels compared to diabetic control rats. Also, a significantly (P<0.01) increased level of HDL was observed in diabetic rats treated with both doses of compound I compared to diabetic control rats (Table 2).

Antioxidant activity of compound I

Figure 1a, 1b, 1c, 1d, and 1e showed the antioxidant activity of compound I in liver in diabetic rats. STZ significantly (P<0.01) decreased levels of GSH, GPx, CAT, SOD and increased MDA compared to normal control rats. These altered above antioxidant levels were increased significantly (P<0.01) after the administration of compound I at 25 and 50 mg/kg dose compared to diabetic control rats.

	Control groups			STZ-diabetic	STZ-diabetic/ treated groups	
	Group 1a	Group 1b	Group 1c	Group 2	Group 3a	Group 3b
TG (mg/dl)	85.1 ± 2.5	77.3 ± 2.91	76.66 ± 2.79	170.11 ± 5.8**	122.51 ± 4.1**	95.11 ± 3.62**
TG (mg/dl)	105.21 ± 3.56	98.18 ± 4.11	96.41 ± 4.12	151.55 ± 5.0**	127.50 ± 4.1**	98.67 ± 3.4**
HDL-C(mg/dl)	40.1 ± 1.45	38.12 ± 1.7	39.5 ± 1.33	22.42 ± 1.4**	26.34 ± 1.5**	30.2± 1.4*

LDL-C(mg/dl)	50.5 ± 1.96	45.12 ± 1.72	43.00 ± 1.61	90.51 ± 2.3**	79.43 ± 2.1**	53.33 ± 2.31*
VLDL-C(mg/dl)	15.81 ± 1.2	15.15 ±1.10	15.11 ± 1.3	38.62 ± 1**	21.73 ±1.4*	15.17 ± 1.2*
Atherogenic	0.419 ± 0.05	0.410 ± 0.061	0.2387 ± 0.04	0.820 ± 0.08**	0.68 ± 0.041*	0.51 ± 0.031**

Index Values are given as mean ± SE for groups of eight animals each. Values are statistically significant at *$P<0.05$, **$P<0.01$. Diabetic control rats were compared with normal control rats.

Table 2: Effect of compound I on plasma triglyceride (TG), total Cholesterol (TC), HDL-cholesterol (HDL-C), LDL- cholesterol (LDLC), vLDL-cholesterol (vLDLC) and atherogenic index in control and experimental groups of rats.
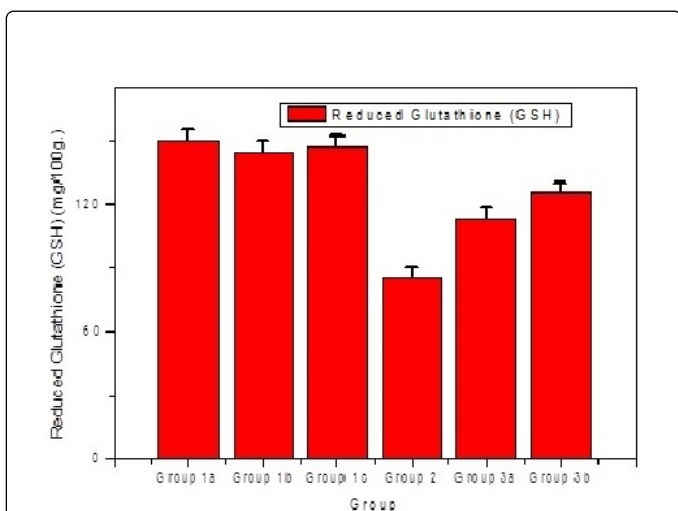

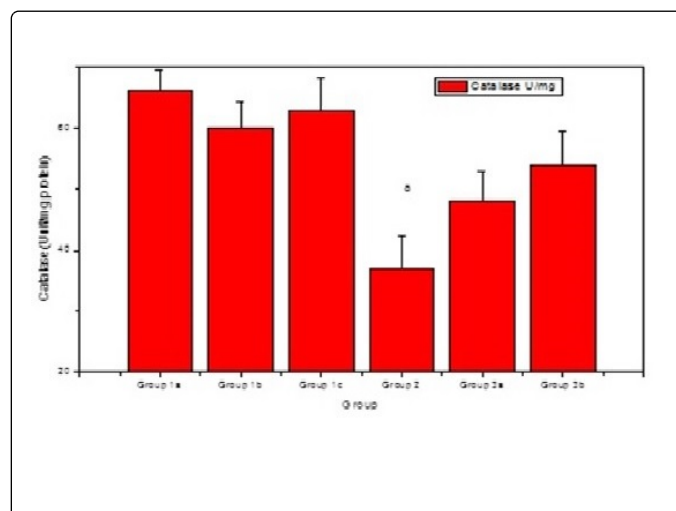**Figure 1a**: Effect of compound I administration on hepatic GSH GPx.

Figure 1c: Effect of compound I administration on hepatic GSH SOD.
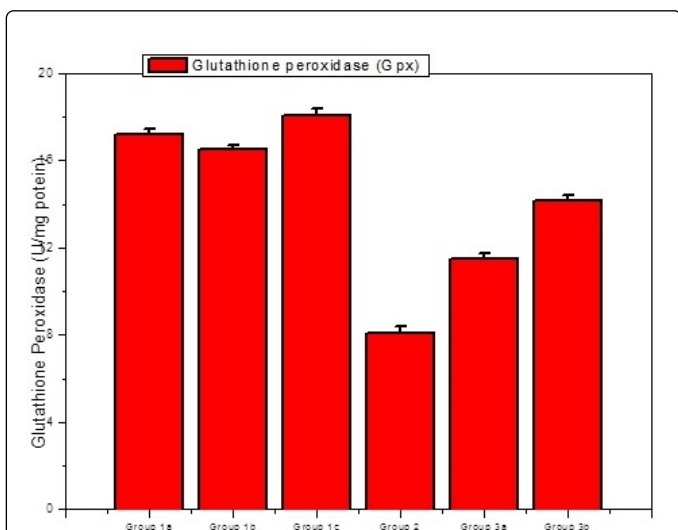

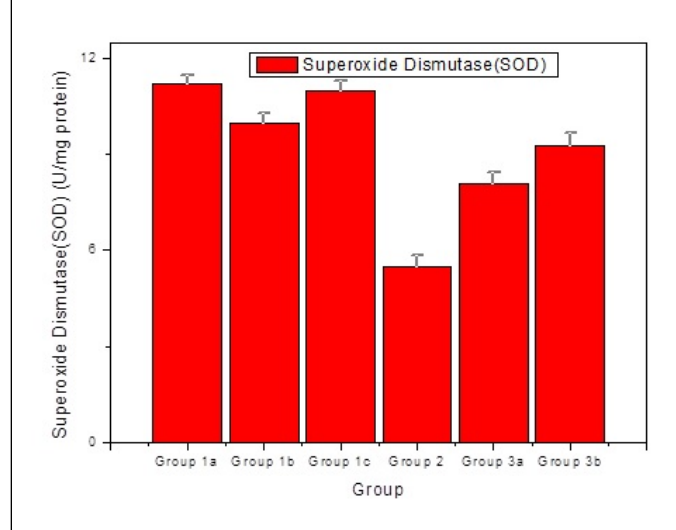**Figure 1b:** Effect of compound I administration on hepatic GSH Catalase.

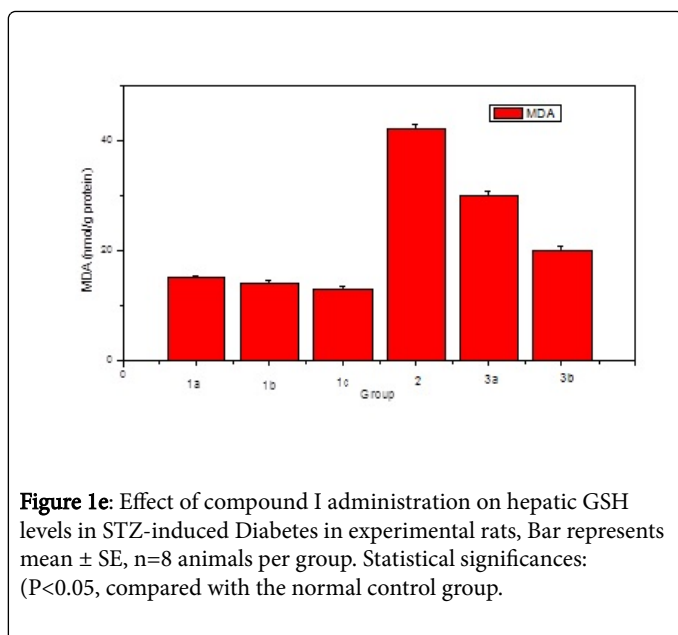**Figure 1d:** Effect of compound I administration on hepatic GSH and Malondialdhyde.

Figure 1e: Effect of compound I administration on hepatic GSH levels in STZ-induced Diabetes in experimental rats, Bar represents mean ± SE, n=8 animals per group. Statistical significances: (P<0.05, compared with the normal control group.

Discussion

This study aimed to evaluate the protective effect of compound I against STZ induced diabetic complications in rats. In acute toxicity study, the dose of compound I that killed half of the mice (LD_{50}) was 230 mg/100g b.w. The toxicity studies showed the low toxicity of compound I on normal rats (Data not shown).

STZ induced diabetes in experimental animals either by preventing glucose oxidation or reduction of insulin biosynthesis and secretion. The toxicity of STZ is due to DNA alkylation at 6 O-position of guanine which results in fragmentation of DNA and defects of the beta cells. Also, STZ act as an intracellular nitric oxide (NO) donor and generates reactive oxygen species (ROS). The action of both NO and ROS may also contribute to DNA fragmentation and other damages caused by STZ [23]. In our study, elevated blood glucose level and decreased insulin level were observed in STZ-induced diabetic rats and it may be due to above mechanism of STZ (Table 2).

In diabetic rats, treatment of compound I increased the insulin compared to diabetic control rats. The hypoglycemic activity of compound I may be due to its protective action against STZ-mediated damage to the pancreatic β-cells and also possibly because of regeneration of damaged beta cell or increased insulin release or secretion [24].

Abnormal plasma lipids and lipoproteins pattern in diabetes mellitus may lead to the development of coronary artery disease in diabetic patients [25].

Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated cardiovascular risk in diabetes mellitus [26]. Thus, the altered lipid and lipoprotein pattern observed in diabetic rats could be due to defect in insulin secretion and/or action. Accumulation of cholesterol and phospholipids in liver due to elevated plasma free fatty acids has been reported in diabetic rats. Hyperglycemia is an important contributor for the cardiovascular diseases (CVD) risk [27].

The prevalence of CVD is 2-8 folds higher in diabetic person compared to non-diabetic person. The accelerated coronary heart disease (CHD) has emerged as a leading cause of morbidity and mortality in diabetic patients in the worldwide. The vascular diseases occurred in diabetes due to disturbance in lipoprotein metabolism which causes acceleration of atherosclerosis [28].

In the present study, increases in plasma TC, TG, LDL, VLDL and atherogenic index and reduced level of HDL levels were observed in STZ- induced diabetic rats. The increased in HDL-cholesterol is a desirable feature. In addition, the reductions in TC, TG and LDL-cholesterol could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics [29]. This would definitely reduce the incidence of coronary events being the major cause of morbidity and deaths in diabetes subjects [30-32].

In our study, it has been proposed that compound I acted in a similar way by increasing insulin production in STZ-induced diabetic rats and lowering TG level by activation of the enzyme lipoprotein lipase, because insulin activates lipoprotein lipase [33].

Several studies have demonstrated the involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes [33]. It has been reported that in diabetes mellitus, oxygen free radicals are generated by stimulating H_2O_2 *in vitro* as well as in vivo and in pancreatic β-cells [34]. The increased lipid peroxidation in the diabetic animals may be due to the increase in the concentration of TBARS in the liver of diabetic rats.

In our study, decreased levels of hepatic GSH, GPx, SOD and CAT as well as increased level of TBARS were observed in liver tissues of STZ-induced diabetic rats compared to normal control rats. The reduction of above enzymes directly reflects the oxidative stress in diabetic rats and these enzyme level changes may be due to generation of free radicals by auto-oxidation of glucose, glycosylation in hyperglycemic condition as well as STZ mediated generation of ROS by its NO donor property to the intracellular molecules. In the present study, increased GSH, GPx, SOD, CAT levels and decreased TBARS (Figure 1a-1e) were noticed in diabetic rats after the administration of both doses of compound I at 25 and 50 mg/kg in liver. The above action represents the antioxidant property of compound I in diabetic condition and hence, compound I possesses a potential to reduce or prevent the diabetic complications by quenching of free radicals like all phenolic compounds.

Acknowledgements

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