

ANTIDIABETIC ACTIVITY OF CAFFEIC ACID AND 18 β -GLYCYRRHETINIC ACID AND ITS RELATIONSHIP WITH THE ANTIOXIDANT PROPERTY

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ABSTRACT

Objective: This study was performed to investigate the antidiabetic effect of caffeic acid and 18 β -glycyrrhethinic acid against diabetic rats.

Methods: In this experiment, the animals were divided into five groups. Group I: Normal rats. Group II: Diabetic control rats. Group III: Diabetic rats treated with 18 β -glycyrrhethinic. Group IV: Diabetic rats treated with caffeic acid. Group V: Diabetic rats treated with 18 β -glycyrrhethinic and caffeic acid. Fasting blood glucose, insulin, glutathione reductase (GR), glutathione peroxidase (GPx), total antioxidant (TAO), catalase (CAT), and superoxide dismutase (SOD) and malondialdehyde (MDA) were analyzed.

Results: Fasting blood glucose and MDA were significantly increased, whereas insulin, GR, GPx, TAO, CAT, and SOD were decreased significantly in diabetic rats. Though the diabetic rats treated with caffeic acid and 18 β -glycyrrhethinic acid individual exerts beneficial effects in all the biochemical parameters in diabetic rats. The combined treatment with caffeic acid and 18 β -glycyrrhethinic acid normalized all the above-mentioned biochemical parameters in diabetic rats.

Conclusion: Our findings demonstrated that 18 β -glycyrrhethinic acid and caffeic acid either used individually or in combination to diabetic rats has an antidiabetic effect and a good antioxidant property. From the results, the combined dose of 18 β -glycyrrhethinic acid and caffeic acid to diabetic rats showed the promising effect and antioxidant property compared to individual treatments.

Keywords: Diabetes, Caffeic acid, 18 β -glycyrrhethinic acid, Streptozotocin, Antioxidant.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that constitutes a major public health problem throughout the world. Current estimates indicate that approximately 4% of the global population suffers from DM, a percentage which is expected to reach 5.4% in 2025 [1]. Type 2 diabetes is the most common form of the disease and usually involves insulin resistance and β -cell dysfunction [2]. Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue [3].

Oxidative stress may occur as a consequence of abnormalities in glucose and lipid metabolism, which favor hyperglycemia and dyslipidemia [4]. The products of an oxidative stress could play an important role in diabetic complications which involve micro and macroangiopathic processes through lipid peroxidation (LPO) (low density lipoprotein oxidation) and the production of advanced glycosylation end-products, which are responsible for producing fragmentation, cross-linking and damage of basic structures, carbohydrates, lipids, proteins, and DNA [5].

Several therapeutic strategies are currently available for the treatment of this chronic metabolic disorder [6]. Complementary and alternative medicine applications have attracted special attention in recent research for they offer new promising opportunities for the development of efficient, side effect-free, and lower cost alternatives to existing synthetic hypoglycemic agents [7].

Phenolic acids are abundant in food and account for about a third of the phenolic compounds in our diet [8]. Caffeic acid (3,4-dihydroxycinnamic acid) is widely distributed in plants and their products such as fruits, vegetables, coffee, tea, wine, grains and Chinese medicinal herbs [9,10]. Caffeic acid has exhibited pharmacological antimutagenic activities [11], antioxidant [12,13], antiviral [14], anticancer [15], anti-inflammatory properties [16], and antidiabetic effect [11,17-19].

Licorice (*Glycyrrhiza glabra* Linn.) is one of the most widely used herbs from the ancient medical history of ayurveda, both as a medicine and also as a flavoring herb [20]. Glycyrrhizin (glycyrrhizic acid; glycyrrhizinate) constitutes 10-25% of licorice root extract and is considered the primary active ingredient [21]. After oral administration or intravenous injection, glycyrrhizin has been shown to be hydrolyzed by the glucuronidase in intestinal bacteria to its active principal aglycone, 18 β -glycyrrhethinic acid, which is then absorbed into the blood [22]. Glycyrrhizin and 18 β -glycyrrhethinic acid have been shown to possess several beneficial pharmacological activities, which include anti-inflammatory [23], antiviral activity [24,25], anti-tumorigenic, anti-ulcerative, and anti-hepatotoxic activity *in vitro* and *in vivo* [26]. Clinical trials clearly show that glycyrrhethinic acid has a good effect on all types of dermatitis [27], purulent scar disease [28]. The antidiabetic effect of glycyrrhizin in genetically diabetic KK-A γ mice has been reported [29]. Recent studies indicate that glycyrrhethinic acid enhanced glucose-stimulated insulin secretion and induced mRNA expression of insulin receptor substrate-2, pancreas duodenum homeobox-1, and glucokinase (GK) [30] and the antidiabetic and hypolipidemic effect of 18 β -glycyrrhethinic acid in streptozotocin (STZ)-diabetic rats [31,32].

In this study, we have reported the *in vivo* antidiabetic and antioxidant effect of caffeic acid and 18 β -glycyrrhethinic acid in diabetic rats.

METHODS

Chemicals

STZ, nicotinamide (NIC), caffeic acid, 18 β -glycyrrhethinic acid, and other chemicals were purchased from Sigma-Aldrich, Germany.

Animals

Adult male albino rats weighting 70-100 g body weight were housed at the Experimental Animal House of the Faculty of Science, Zagazig University. The animals were maintained in a controlled environment

of temperature, humidity, and light. They were fed on a commercial standard diet and tap water *ad libitum*.

Induction of Type 2 diabetes in rats

Type 2 diabetes was induced in rats by a single intraperitoneal (i.p.) injection of STZ (65 mg/kg, STZ) in overnight fasting rats or mice followed by the i.p. administration of NIC (110 mg/kg, NIC) after 15 minutes.

STZ was dissolved in citrate buffer (pH 4.5), and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture, and serum samples were analyzed for blood glucose [33].

Animals showing fasting blood glucose higher than 280 mg/dl were considered as diabetic and used for the further study.

Experimental design

In this experiment, a total of 50 rats (40 diabetic surviving rats, 10 normal rats) were used. The animals were divided into five groups of 10 each.

Group I: Normal rats; received a saline solution.

Group II: Diabetic control rats ([65 mg/kg, STZ]) followed by the i.p. administration of NIC [110 mg/kg, NIC] after 15 minutes).

Group III: Diabetic rats + 18 β -glycyrrhetic acid (100 mg/kg/day) [31].

Group IV: Diabetic rats + caffeic acid (40 mg/kg/day) [34].

Group V: Diabetic rats + 18 β -glycyrrhetic acid (100 mg/kg/day) and caffeic acid (40 mg/kg/day).

- 18 β -glycyrrhetic acid and caffeic acid were dissolved in DMSO and orally administered to rats using an intragastric tube daily for a period of 30 days.

Samples collections

After the last treatment, rats were fasted overnight; and all the rats were euthanized by cervical decapitation. Blood samples were collected in tubes containing sodium fluoride for the estimation of plasma glucose. Blood samples were collected in tubes containing ethylene diamine tetraacetic acid. The plasma was obtained after centrifugation at 2000 $\times g$ for 20 minutes and used for various biochemical measurements. Blood samples were collected in serum tubes. The serum was obtained after clotting by centrifugation at 2000 $\times g$ for 20 minutes and used for various biochemical measurements.

The liver was immediately dissected, washed in ice-cold saline to remove the blood. They were then homogenized in Tris-HCl buffer (0.1 mol/L, pH 7.5), centrifuged (3000 $\times g$) for 10 minutes, and the supernatant was collected. Biochemical estimations were carried out in the homogenates.

Biochemical parameters

Determination of glucose

Plasma glucose was determined by using a commercial kit derived from Elitech Clinical Systems, France [35].

Determination of serum insulin

Serum insulin was determined by using a commercial kit derived from SIEMENS Company, USA [36].

Determination of glutathione reductase (GR)

Plasma GR was determined by using a commercial kit derived from Biodiagnostic Company, Egypt [37].

Determination of glutathione peroxidase (GPx)

Tissue GPx was determined by using a commercial kit derived from Biodiagnostic Company, Egypt [38].

Determination of total antioxidant (TAO)

Plasma TAO was determined by using a commercial kit derived from Biodiagnostic Company, Egypt [39].

Determination of lipid peroxidase (malondialdehyde [MDA])

Tissue lipid peroxidase (MDA) was determined by using a commercial kit derived from Biodiagnostic Company, Egypt [40].

Determination of catalase (CAT)

Plasma CAT was determined by using a commercial kit derived from Biodiagnostic Company, Egypt [41].

Determination of superoxide dismutase (SOD)

Tissue SOD was determined by using a commercial kit derived from Biodiagnostic Company, Egypt [42].

Histopathological study

Pancreas specimen was fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. After deparaffinization and dehydration, the paraffin blocks were stained with hematoxylin and eosin for microscopic examination. Light microscopy was used to evaluate the pathological changes in the tissues according to Lillie method [43].

Statistical analysis

All statistical analyses were done by a statistical for social science package "SPSS" 14.0 for Microsoft Windows, SPSS Inc. [44] and considered statistically significant at a two-sided $p < 0.05$. Numerical data were expressed as mean \pm standard deviation. The levels of markers were analyzed by analysis of variance. $p < 0.01$ was considered significant.

RESULTS

Plasma glucose and insulin

Table 1 shows the levels of plasma glucose and insulin of normal and experimental rats. There was a significant ($p < 0.001$) elevation in plasma glucose, while the levels of insulin significantly ($p < 0.001$) decreased in the diabetic control animals as compared with normal group. A significant ($p < 0.001$) decrease in plasma glucose and increase in insulin levels were observed in diabetic rats treated with 18 β -glycyrrhetic acid or caffeic acid. On the other hand, the effect of the combination of 18 β -glycyrrhetic acid and caffeic acid brought plasma glucose and insulin toward near normal values, while normal rats did not exhibit any significant alterations in plasma glucose and insulin levels during the experimental period.

Effect on antioxidants

The antioxidants (GR, GPx, SOD, and CAT) activities and TAO level were found to be lower in diabetic rats compared to that of the control rats. These antioxidant levels in diabetic rats treated with 18 β -glycyrrhetic acid and caffeic acid either used individually or in combination significantly ($p < 0.001$) increased to a level closer to the normal values (Figs. 1-5) and 18 β -glycyrrhetic acid and caffeic acid treatment significantly decreased levels of MDA in diabetic rats ($p < 0.001$) (Fig. 6).

Table 1: Changes in the levels of glucose and insulin in all studied groups

Groups	Glucose (mg/dl)		Insulin (μ U/ml)		p*
	Mean \pm SD	% Change	Mean \pm SD	% Change	
Group I	88.7 \pm 4.11	-	11.512 \pm 0.84	-	-
Group II	303.5 \pm 8.69	243.18	2.932 \pm 0.39	74.53	<0.001
Group III	94.7 \pm 7.54	68.89	8.345 \pm 0.53	184.62	<0.001
Group IV	99.6 \pm 6.06	67.28	8.192 \pm 0.28	179.40	<0.001
Group V	91.3 \pm 5.49	70.01	9.196 \pm 0.23	213.64	<0.001

Values are means \pm SD for 10 rats. * $p < 0.001$ against the control value, SD: Standard deviation

Histopathological study

Group I showed normal pancreas with normal islets of Langerhans with normal morphology of acinar cells (pyramidal in shape, having very small acinar lumen). Group II showed large edematous area with peri-acinar leukocyte cells infiltration. Group III showed cystic dilation of the pancreatic duct. Group IV showed congestion of some peri-acinar capillaries. Group V showed normal pancreatic acini (Fig. 7).

DISCUSSION

In the present study, diabetic rats (Group II) showed increased plasma glucose level and decreased insulin level when compared with the normal rats [45]. In Type 2 diabetic rats treated with 18β-glycyrrhethinic acid

and caffeic acid showed that the combined administration of 18β-glycyrrhethinic acid and caffeic acid to diabetic rats showed the levels of plasma glucose and insulin towards near normally when compared with their individual effects (Table 1). Recent researches have shown that 18β-glycyrrhethinic acid and caffeic acid decreased blood glucose concentration and increased insulin release in diabetic rats. 18β-glycyrrhethinic acid and caffeic acid reduce blood glucose level by virtue of their ability to inhibit glucose-6-phosphatase activity, with the related effects on hepatic glucose production and enhancing the GK activity in the liver [31]. Hepatic GK has a major effect on glucose homeostasis and is a potential target for pharmacological treatment of Type 2 diabetes. The increase of hepatic GK can cause an increased utilization of the blood glucose for energy production or glycogen storage in the liver [19]. Glycyrrhethinic acid enhanced glucose-stimulated insulin secretion and induced mRNA levels of insulin receptor substrate-2, pancreas duodenum homeobox-1 [30].

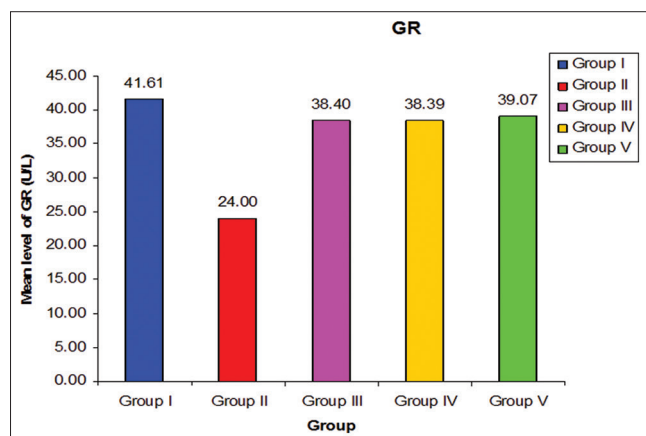


Fig. 1: Effects of 18β-glycyrrhethinic acid and caffeic acid on glutathione reductase level in all studied groups

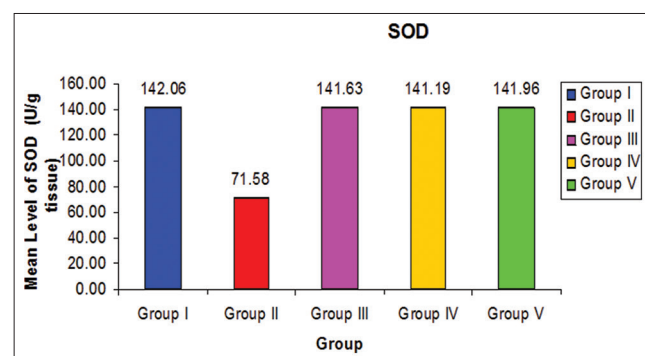


Fig. 4: Effects of 18β-glycyrrhethinic acid and caffeic acid on superoxide dismutase level in all studied groups

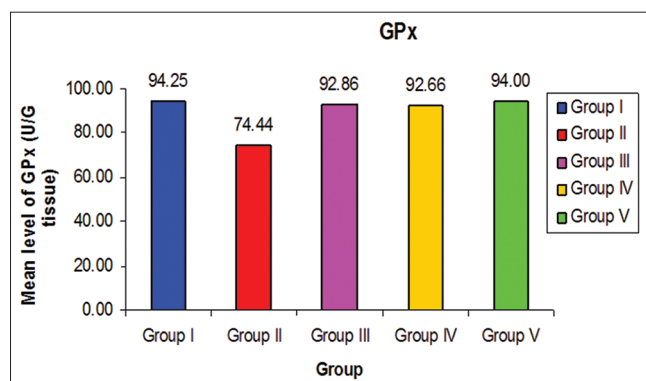


Fig. 2: Effects of 18β-glycyrrhethinic acid and caffeic acid on glutathione peroxidase level in all studied groups

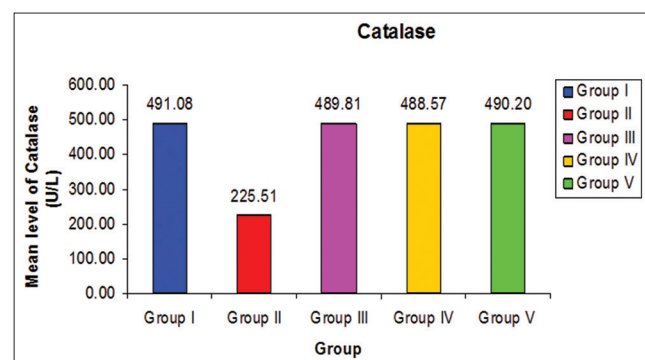


Fig. 5: Effects of 18β-glycyrrhethinic acid and caffeic acid on catalase level in all studied groups

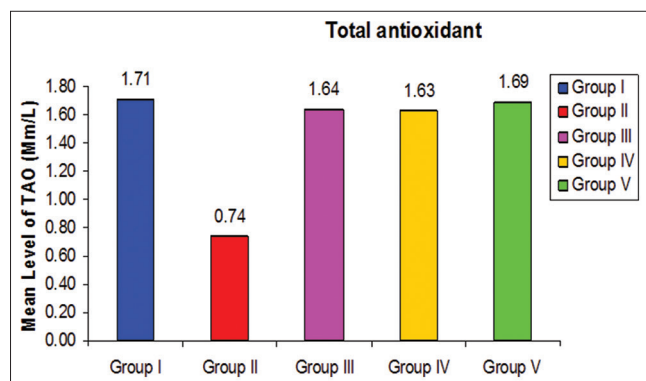


Fig. 3: Effects of 18β-glycyrrhethinic acid and caffeic acid on total antioxidant level in all studied groups

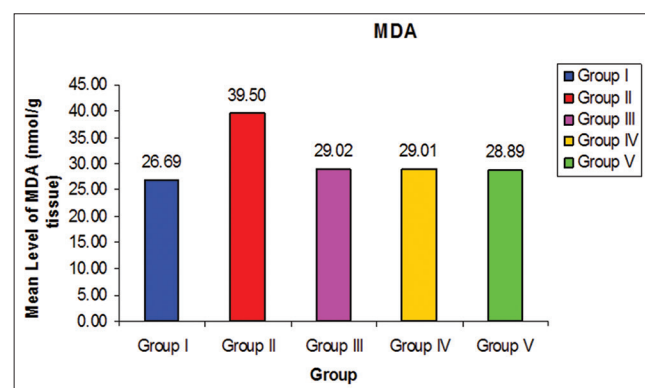


Fig. 6: Effects of 18β-glycyrrhethinic acid and caffeic acid on malondialdehyde level in all studied groups

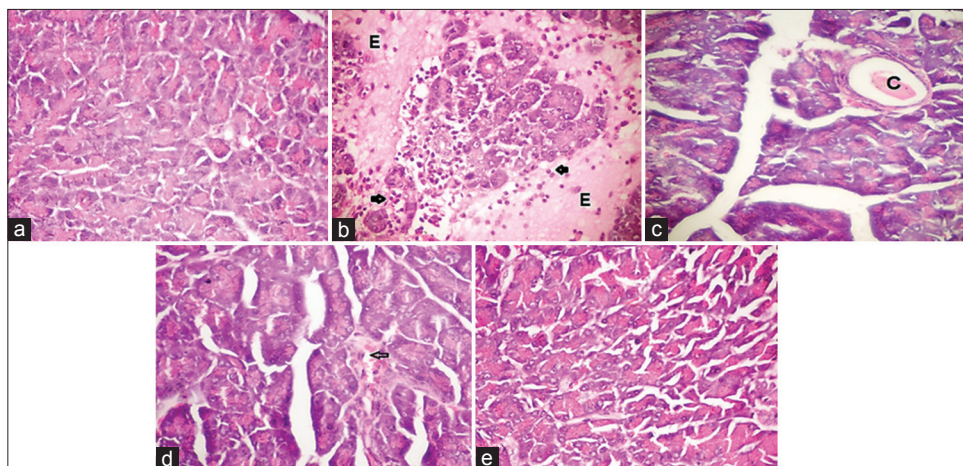


Fig. 7: A photomicrograph of pancreas (H and E, ×400), (a) “negative control Group I” showing normal pancreas with normal islets of Langerhans with normal morphology of acinar cells, (b) “diabetic Group II” showing large edematous area (E) with periacinar leukocyte cells infiltration, (c) “Group III” showing cystic dilation of the pancreatic duct, (d) “Group IV” showing congestion of some periacinar capillaries (arrow), (e) “Group V” showing normal pancreatic acini

However, C-peptide has a longer half-life than insulin and thus may better represent insulin secretion than insulin levels do [46].

Mechanisms involved in the increased oxidative stress in diabetes include different mechanisms result in changing in the activity of antioxidant defense systems [47]. The cytotoxic action of STZ is associated with the generation of reactive oxygen species (ROS) causing oxidative damage that culminates β -cell destruction through the induction of apoptosis and suppression of insulin biosynthesis [45]. β -cells are particularly susceptible to oxidative stress [48]. In Type 2 diabetes, excessive ROS could promote the inhibition of insulin synthesis [49]. It has been confirmed from *in vivo* studies that high glucose concentrations induce mitochondrial ROS, which suppresses the first phase insulin secretion [50].

In the diabetic state, LPO can be induced by protein glycation and glucose auto-oxidation that can further lead to the formation of free radicals. The main free radicals that occur in this disease state are superoxide (O_2^-), hydroxyl (OH) and peroxy (LOO) radicals. The damage that these radicals inflict on cells might be quantitatively determined by measurement of levels of MDA, a product of LPO [51]. Products of lipid oxidation, such as oxidized cholesterol and oxidized unsaturated fatty acyl groups of phospholipids, may affect structure and function of the membrane. In general, the overall effect of LPO is to decrease membrane fluidity, deformability, viscoelasticity and life span of erythrocytes which may cause complications in Type 2 diabetes [52].

A major protective mechanism against oxidative damage is the membrane integrity. Oxidation induces a change in the membrane permeability resulting in hemolysis would relate to the degree of intravascular red blood cell (RBC) destruction. Extravascular mechanisms of RBC destruction may involve changes in cell deformability and antigenicity. LPO causes polymerization of membrane components and decreases cell deformability [53].

Certain enzymes play an important role in the antioxidant defense, to maintain viable reproductive ability; a protective mechanism against oxidative stress is of importance. These enzymes include SOD, GPx, GR and CAT, which convert free radicals or reactive oxygen intermediates to non-radical products. SOD and GPx are major enzymes that scavenge harmful ROS in male reproductive organs [54].

Our present study observed decreasing in antioxidant activities (GR, GPx, SOD, and CAT) and TAO level in diabetic rats (Figs. 1-5) [55-58]. And also, we reported increasing in the MDA level in diabetic rats (Fig. 6) [59]. From our results, the administration of 18 β -glycyrrhetic acid

and caffeic acid to diabetic rats resulted in a significant ($p < 0.001$) increase in the antioxidant enzyme (GR, GPx, SOD and CAT) activities and TAO level either used individually or in combination when compared to diabetic group and inhibit LPO (MDA) when compared to diabetic group. Glycyrrhetic acid has a reduced carboxylic acid group, and some additional functional changes exhibited strong antioxidant activity [60]. The antioxidant activities of caffeic acid are based on carbonyl group separated from the aromatic ring [61]. The antioxidant mechanism of action is assumed to be through its radical-scavenging activity that is linked to their hydrogen- or electron-donating ability and to the stability of the resulting phenoxyl radicals [62]. It has been documented that 18 β -glycyrrhetic acid and caffeic acid possess non-enzymatic antioxidant activity such as scavenging free radicals, and enzymatic antioxidant activity such as increasing protein level of antioxidant enzymes [22,63].

In our study, histopathological examination of pancreas showed that the damage is restored near to normal in the treated groups (Groups III, IV and V) (Fig. 7) as caffeic acid and 18 β -glycyrrhetic acid either used individually or in combination preserved islet and β -cell architecture relatively better compared with the control groups [19,22]. The combined administration was more benefit than the individual administration which supports the biochemical analysis.

CONCLUSION

Our findings demonstrated that 18 β -glycyrrhetic acid and caffeic acid either used individually or in combination to diabetic rats has an antidiabetic effect and a good antioxidant property. From the results, the combined dose of 18 β -glycyrrhetic acid and caffeic acid to diabetic rats showed the promising antidiabetic effect and antioxidant property compared to individual treatments.

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