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PROTECTIVE EFFECT OF CAFFEIC ACID AND 18 B -GLYCYRRHETINIC ACID AGAINST STREPTOZOTOCIN–NICOTINAMIDE INDUCED DIABETES

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ABSTRACT

Diabetes mellitus is associated with the production of reactive oxygen species and consequently oxidative stress. Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. Caffeic acid (40 mg/kg/body weight) and 18β-glycyrrhetinic acid (100mg/kg/body weight) were orally administered to diabetic rats for a period of 30 days. Liver and kidney function tests, total cholesterol, triglyceride, low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), and malondialdehyde (MDA) were significantly increased, whereas high density lipoprotein-cholesterol (HDL-C) and Antioxidants (glutathione reductase (GR), glutathione peroxidase (GPx), total antioxidant (TAO), catalase , and superoxide dismutase (SOD)) were decreased significantly in diabetic rats. Though the diabetic rats treated with caffeic acid and 18β-glycyrrhetinic acid individual exerts beneficial effects in all the biochemical parameters in diabetic rats while the combined treatment with caffeic acid and 18β-glycyrrhetinic acid normalized all the above-mentioned biochemical parameters in STZ-induced diabetic rats.

Key Words: Diabetes, caffeic acid, 18β-glycyrrhetinic acid, Streptozotocin, Antioxidants.

INTRODUCTION

Diabetes is one of the oldest known diseases (Ripoll *et al.*, 2011). Diabetes mellitus is associated with the production of reactive oxygen species (ROS) and consequently oxidative stress, which promotes not only an alteration in the cellular redox state in the presence of chronic hyperglycaemia, but also reduces the ability of tissues to utilize carbohydrates, leading to disturbances in the metabolism of fat and protein. Oxidative stress may occur as a consequence of abnormalities in glucose and lipid metabolism, which favour hyperglycaemia and dyslipidaemia (Almeida *et al.*, 2012). The products of an oxidative stress could play an important role in diabetic complications which involve micro and macroangiopathic processes through lipid peroxidation (low density lipoprotein oxidation) and the production of advanced glycosylation end-products (AGEs), which are responsible for producing fragmentation, cross-linking and damage of basic structures, carbohydrates, lipids, proteins and DNA (Kostic *et al.*, 2008).

In developing countries, herbal medicines are important in the primary health care, while in international traditional trades, herbal medicine products have increased (Jaikang *et al.*, 2011).

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 (Sakakibara, 2003; Jiang *et al.*, 2005). Its derivatives

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including amides, esters, sugar ester and glycosides are widely distributed in honey, propolis, coffee beans and olive (Fiuza *et al.,* 2004). Caffeic acid is commonly produced by extraction from plant sources, such as coffee beans. Chemical or enzymatic hydrolysis of caffeoylquinic acid derivatives is also employed to produce caffeic acid (Wang *et al.,* 2009; Yoshimoto *et al.,* 2005). Caffeic acid has exhibited pharmacological antimutagenic activities (Okutan *et al.,* 2005), antioxidant (Mori *et al.,* 2009; Gulcin, 2006), antivirus (Ikeda *et al.,* 2011), anticancer (Rajendra-Prasad *et al.,* 2011), antiinflammatory properties (Chao *et al.,* 2009) and antidiabetic effect (Hsu *et al.,* 2000; Okutan *et al.,* 2005; Park *et al.,* 2006; Jung *et al.,* 2006).

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 (Jatav *et al.,* 2011). Glycyrrhizin (glycyrrhizic acid; glycyrrhizinate) constitutes 10–25% of licorice root extract and is considered the primary active ingredient. (Kaur *et al.,* 2013). After oral administration or intravenous injection, glycyrrhizin has been shown to be hydrolyzed by the glucuronidase in intestinal bacteria to its active principal aglycone, 18ß-glycyrrhetinic acid, which is then absorbed into the blood (Kalaiarasi *et al.,* 2011). Glycyrrhizin and 18ß-glycyrrhetinic acid have been shown to possess several beneficial pharmacological activities, which include anti-inflammatory (Gao *et al.,* 2011), antiviral activity (Wang *et al.,* 2010; Hardy *et al.,* 2012), anti-tumorigenic, anti-ulcerative, and anti-hepatotoxic activity in vitro and in vivo (Armanini *et al.,* 2002) . Clinical trials clearly show that glycyrrhetinic acid has a good effect on all types of dermatitis (Saeedi *et al.,* 2003), purulent scar disease (Li *et al.,* 2011). Takii *et al.* (2001) reported the antidiabetic effect of glycyrrhizin in genetically diabetic KK-AY mice. Other studies indicate that glycyrrhetinic acid enhanced glucose-stimulated insulin secretion and induced mRNA expression of insulin receptor substrate-2, pancreas duodenum homeobox-1, and glucokinase (Ko *et al.* 2007) and the antidiabetic and hypolipidemic effect of 18ßglycyrrhetinic acid in streptozotocin-diabetic rats (Kalaiarasi and Pugalendi, 2009; Kalaiarasi *et al.,* 2009).

The aim of the present work is to investigate the protective effect of caffeic acid and 18 β -glycyrrhetinic acid against streptozotocin–nicotinamide induced diabetes

MATERIALS AND METHODS

Chemicals

Streptozotocin, nicotinamide, caffeic acid, 18β-glycyrrhetinic acid and other chemicals were purchased from Sigma-Aldrich, Germany.

Animals

Adult male albino rats weighting 70 to 100 g body weight were housed at the experimental animal house of the faculty of Science, Zagazig University. The animals were maintained in 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 Streptozotocin (65 mg/kg, STZ) in overnight fasting rats followed by the i.p administration of Nicotinamide (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. Animals showing fasting blood glucose higher than 280 mg/dl were used for the further study (Kakadiya *et al.,* 2010).

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 ten each.

Group I: Normal control rats; received saline solution.

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

Group III: Diabetic rats + 18β-glycyrrhetinic (100 mg/ kg/day) (Kalaiarasi and Pugalendi, 2009).

Group IV: Diabetic rats + Caffeic acid (40 mg/kg/day) (Jayanthi *et al.,* 2010).

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

- 18β-glycyrrhetinic 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 tetra acetic acid (EDTA). The plasma was obtained after centrifugation at 2000×g for 20 min and used for various biochemical measurements. Blood samples were collected in serum tubes. The serum was obtained after clotting by centrifugation at 2000×g for 20 min and used for various biochemical measurements.

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 ×g) for 10 min, and the supernatant was collected. Biochemical estimations were carried out in the homogenates.

Biochemical Parameters

Plasma Glutathione Reductase (GR) activity was determined according to the method described by Goldberg *et al.,* (1983) using a commercial kit derived from Biodiagnostic Company, Egypt.

Tissue Glutathione Peroxidase (GPx) activity was determined according to the method described by Paglia *et al.,* (1967) using a commercial kit derived from Biodiagnostic Company, Egypt.

Plasma Total Antioxidant (TAO) level was determined according to the method described by Koracevic *et al.,* (2001) using a commercial kit derived from Biodiagnostic Company, Egypt.

Tissue Lipid Peroxidase (Malondialdehyde (MDA)) was determined according to the method described by Ohkawa *et al.,* (1979) using a commercial kit derived from Biodiagnostic Company, Egypt.

Plasma Catalase (CAT) activity was determined according to the method described by Aebi (1984) using a commercial kit derived from Biodiagnostic Company, Egypt.

Tissue Superoxide Dismutase (SOD) activity was determined according to the method described by Nishikimi *et al.,* (1972) using a commercial kit derived from Biodiagnostic Company, Egypt.

Liver Function tests

Serum samples were screened for liver function tests including ALT, AST, total bilirubin, albumin, and total protein according to the methods Zilva *et al.,* (1979), Henry (1974), Young (1990), Doumas *et al.,* (1971), and Grant *et al.,* (1987) respectively.

Kidney function tests

Serum samples were screened for kidney function tests including urea and creatinine according to the methods Kaplan (1984), and Young (1995) respectively.

Lipid Profile

Serum cholesterol, and triglycerides were estimated by the methods of Young (2001), and Stein (1987) respectively. Serum high density lipoprotein (HDL) was estimated by the method of Gotto (1988). Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by Nordestgaard *et al.,* (2009) formula.

Statistical Analysis

All statistical analyses were done by a statistical for social science package "SPSS" 14.0 for Microsoft Windows, SPSS Inc. (Levesque, 2007).

RESULTS

Antioxidants

As shown in Tables 1, 2 The antioxidant 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 18β-glycyrrhetinic acid and caffeic acid either used individually or in combination significantly ($p < 0.001$) increased to a level closer to the normal values Figures (1,2,3,4 and 5) and 18β-glycyrrhetinic acid and caffeic acid treatment significantly decreased levels of MDA in diabetic rats ($p < 0.001$) Figure 6.

Liver function tests

Table 3 indicate significant ($p < 0.001$) difference in the activity of AST, ALT, biluribin, total protein and albumin levels of diabetic rats, respectively, compared to negative control group Figures (7 and 8)**.**

Kidney function tests

Table 4 indicate significant ($p < 0.001$) difference in plasma blood urea nitrogen and creatinine levels of diabetic rats compared to negative control group Figures (9 and 10).

Lipid profile

Table 5 showed the levels of total cholesterol, triglycerides, high density lipoprotein-C (HDL-C), low density lipoprotein-C (LDL-C) and very low density lipoprotein-C (VLDL-C) in the plasma of diabetic rats, respectively. The diabetic rats had significantly ($p < 0.000$) elevated levels of plasma total cholesterol, triglycerides, low densitylipoprotein-C (LDL-C), an d very low density lipoprotein-C (VLDL-C) and significantly ($p < 0.001$) decreased level of high density lipoprotein-C (HDL-C). Treatment with 18β-glycyrrhetinic acid, caffeic acid either used individually or in combination prevented the above changes in diabetic rats and improved towards normal levels (Figure 11).

Table 1. Changes in the levels of GR, GPx, TAO in all studied groups

Groups	GR (U/L)		GPx (U/g tissue)		TAO (Mm/L)		P*
	Mean ± SD.	% Change	Mean ± SD.	% Change	Mean ± SD.	% Change	
Group I	41.61± 2.32	-----	94.25 ± 2.40	-----	1.71 ± 0.12	-----	------
Group II	24.00± 2.15	42.32	74.44± 3.65	21.02	0.74± 0.092	56.56	<0.001
Group III	38.40 ± 3.63	60.02	92.86± 1.08	24.75	1.64± 0.10	120.22	<0.001
Group IV	38.39 ± 2.29	59.95	92.66 ± 0.58	24.48	1.63± 0.083	119.81	<0.001
Group V	39.07± 2.22	62.81	94.00 ± 1.77	26.28	1.69 ± 0.12	127.22	<0.001

Values are means ± S.D. for ten rats; * $p < 0.001$ compared to control value.

Table 2. Changes in the levels of SOD, CAT, MDA in all studied groups

Groups	SOD (U/g tissue)		CAT (U/L)		MDA (nmol/g tissue)		P*
	Mean ± SD.	% Change	Mean ± SD.	% Change	Mean ± SD.	% Change	
Group I	142.06 ± 6.86	-----	491.08 ± 5.22	-----	26.69 ± 0.45	-----	------
Group II	71.58 ± 3.10	49.61	225.513 ± 2.52	54.08	39.50 ± 0.36	47.99	<0.001
Group III	141.63 ± 5.35	97.85	489.81 ± 3.58	117.20	29.02 ± 0.26	26.53	<0.001
Group IV	141.19 ± 6.72	97.23	488.57± 3.24	116.65	29.01± 0.27	26.56	<0.001
Group V	141.96 ± 6.31	98.32	490.19 ± 3.87	117.37	28.89 ± 0.24	26.87	<0.001

Values are means ± S.D. for ten rats.

Table 3. Effects of 18β-glycyrrhetinic acid and caffeic acid on liver function tests in all studied groups

Parameters		Group I	Group II	Group III	Group IV	Group V	P*
AST (U/l)	**Mean ± SD.**	85.79 ± 3.08	162.82 ± 3.52	88.16 ± 3.87	88.99 ± 2.47	86.04 ± 2.68	< 0.001
	% Change	------	89.79	45.85	45.34	47.16	
ALT (U/l)	**Mean ± SD.**	35.07 ± 2.47	61.77 ± 3.88	36.09 ± 2.96	36.59 ± 2.10	35.67 ±2.51	< 0.001
	% Change	------	76.13	41.57	40.76	42.25	
Biluribin (mg/dl)	**Mean ± SD.**	0.59 ± .090	1.573 ± 0.08	0.58 ± .076	0.60 ± 0.082	0.59 ± .082	< 0.001
	% Change	------	166.61	62.87	62.17	62.43	
Total protein (g/dl)	**Mean ± SD.**	7.19 ± 0.37	4.34 ± 0.25	7.16 ± 0.41	7.17 ± 0.47	7.28 ± 0.34	< 0.001
	% Change	------	39.57	64.92	65.01	67.56	
Albumin (g/dl)	**Mean ± SD.**	3.81 ± 0.14	2.34 ± 0.42	3.79 ± 0.13	3.73 ± 0.12	3.83 ± 0.18	< 0.001
	% Change	------	38.52	61.55	59.07	63.41	

Values are means ± S.D. for ten rats; * p < 0.001 compared to control value.

Table 4. Effects of 18β-glycyrrhetinic acid and caffeic acid on kidney function tests in all studied groups.

Group		Urea(mg/dl)	Creatinine (mg/dl)	P*
Group I	**Mean ± SD.**	29.64 ± 0.53	0.76 ± 0.11	< 0.001
	% Change	------	------	
Group II	**Mean ± SD.**	53.12 ± 4.46	1.64 ± 0.12	< 0.001
	% Change	79.23	115.92	
Group III	**Mean ± SD.**	32.62 ± 0.5	0.78 ± 0.092	< 0.001
	% Change	38.58	52.47	
Group IV	**Mean ± SD.**	32.74 ± 0.47	0.77 ± .09	< 0.001
	% Change	38.37	53.08	
Group V	**Mean ± SD.**	31.78 ± 0.66	0.78 ± .09	< 0.001
	% Change	40.17	52.47	

Values are means ± S.D. for ten rats; * p < 0.001 compared to control value

Table 5. Effects of 18β-glycyrrhetinic acid and caffeic acid on lipid profile in all studied groups

Parameters		Group I	Group II	Group III	Group IV	Group V	P
Total cholesterol (mg/dl)	**Mean ± SD.**	89.31 ± 4.11	140.09 ± 2.83	89.82 ± 3.41	90.19 ± 3.97	89.59 ± 3.93	< 0.001
	% Change	------	56.85	35.88	35.62	36.05	
Triglycerides (mg/dl)	**Mean ± SD.**	76.26 ± 2.41	143.21 ± 8.48	80.32 ± 2.32	80.59 ± 2.35	79.89 ± 1.72	< 0.001
	% Change	------	87.80	43.92	43.72	44.21	
HDL-C (mg/dl)	**Mean ± SD.**	49.63 ± 3.17	30.03 ± 2.02	48.84 ± 3.24	48.79 ± 2.41	48.93 ± 3.17	< 0.001
	% Change	------	39.48	62.60	62.47	62.93	
LDL-C (mg/dl)	**Mean ± SD.**	24.44 ± 3.77	81.42 ± 4.35	24.93 ± 4.19	25.27 ± 4.26	24.68 ± 5.34	< 0.001
	% Change	------	233.16	69.38	68.96	69.69	
VLDL-C (mg/dl)	**Mean ± SD.**	15.25 ± 0.48	28.64 ± 1.69	16.06 ± 0.46	16.11 ± 0.47	15.98 ± 0.34	< 0.001
	% Change	------	87.80	43.92	43.72	44.21	

Fig 1. Effects of 18β-glycyrrhetinic acid and caffeic acid on GR activity in all studied groups

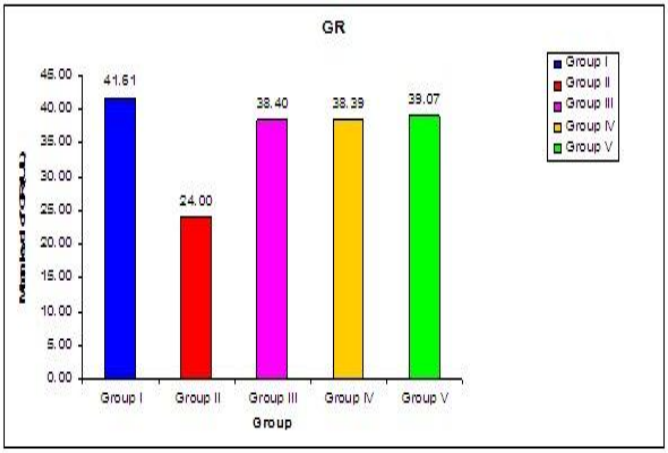
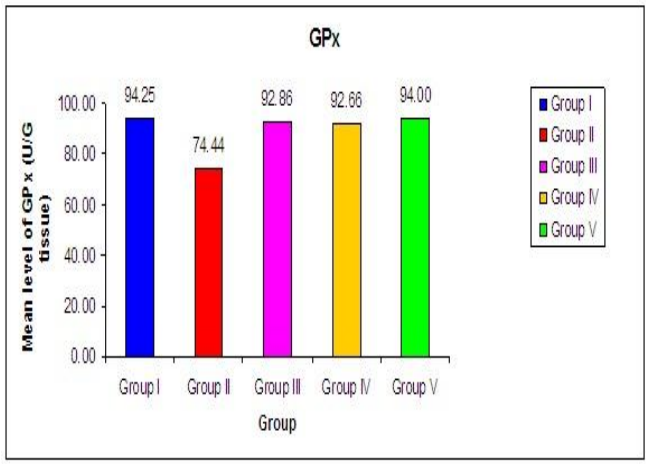


Fig 2. Effects of 18β-glycyrrhetinic acid and caffeic acid on GPx activity in all studied groups



* $p < 0.001$ compared to control value

Fig 3. Effects of 18β-glycyrrhetinic acid and caffeic acid on TAO level in all studied groups

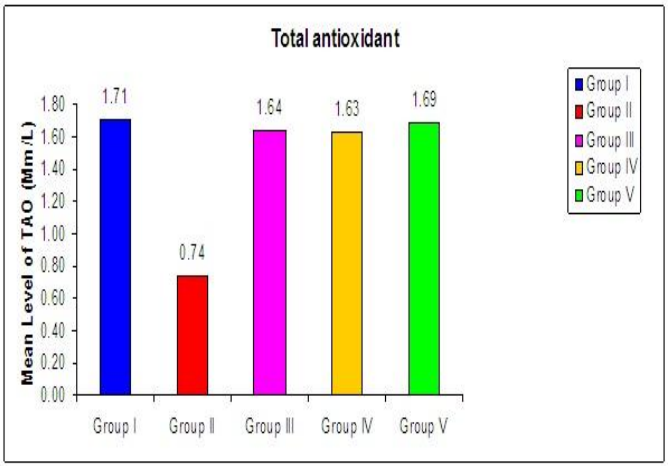


Fig 4. Effects of 18β-glycyrrhetinic acid and caffeic acid on SOD activity in all studied groups.

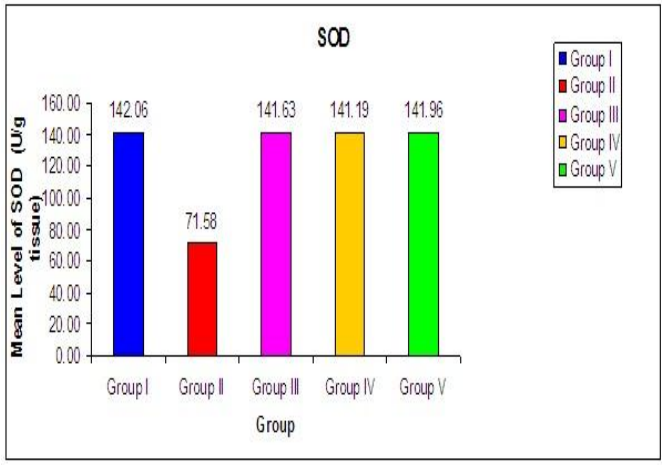


Fig 5. Effects of 18β-glycyrrhetinic acid and caffeic acid on CAT activity in all studied groups

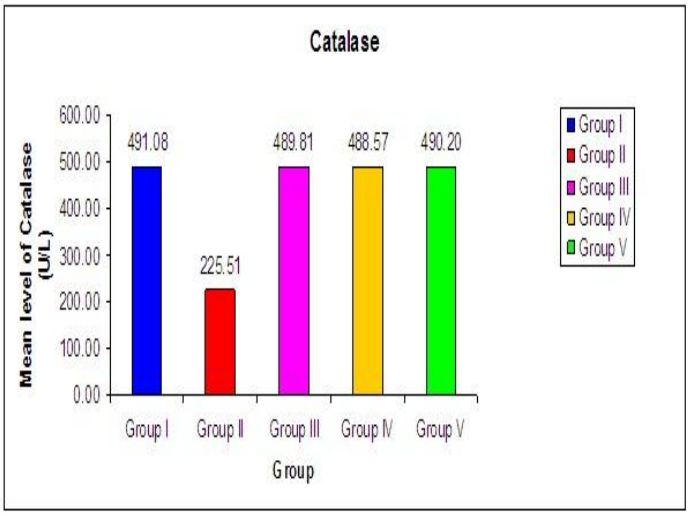


Fig 6. Effects of 18β-glycyrrhetinic acid and caffeic acid on MDA level in all studied groups

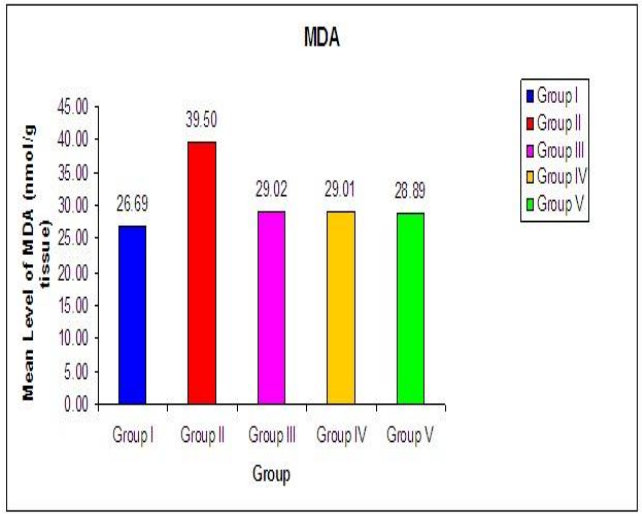


Fig 7. Effects of 18β-glycyrrhetinic acid and caffeic acid on Liver enzymes (AST and ALT) activities in all studied groups

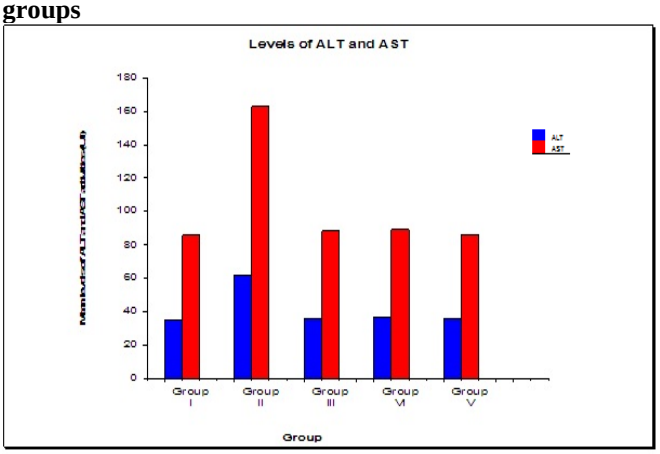


Fig 8. Effects of 18β-glycyrrhetinic acid and caffeic acid on albumin, total protein and biluribin levels in all studied groups

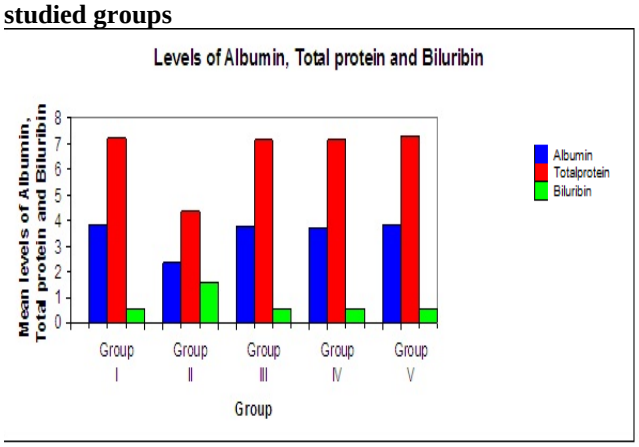


Fig 9. Effects of 18β-glycyrrhetinic acid and caffeic acid on Blood urea nitrogen levels in all studied groups

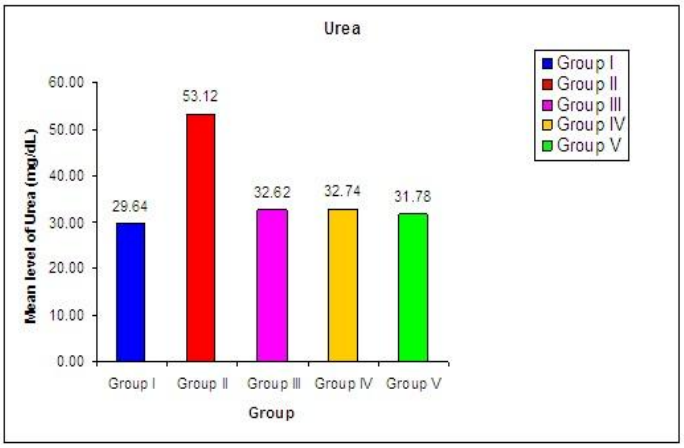


Fig 10. Effects of 18β-glycyrrhetinic acid and caffeic acid on Creatinine levels in all studied groups

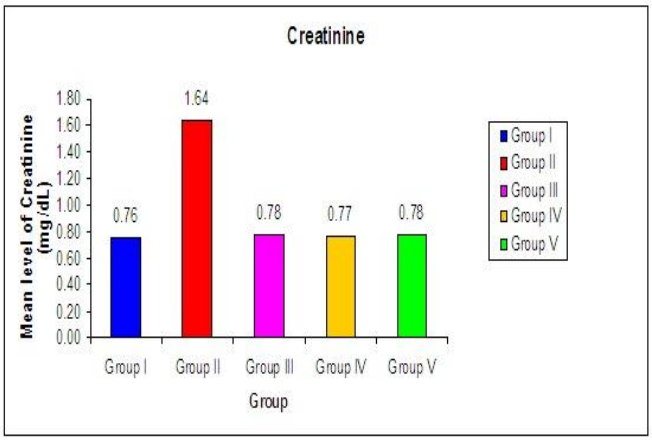
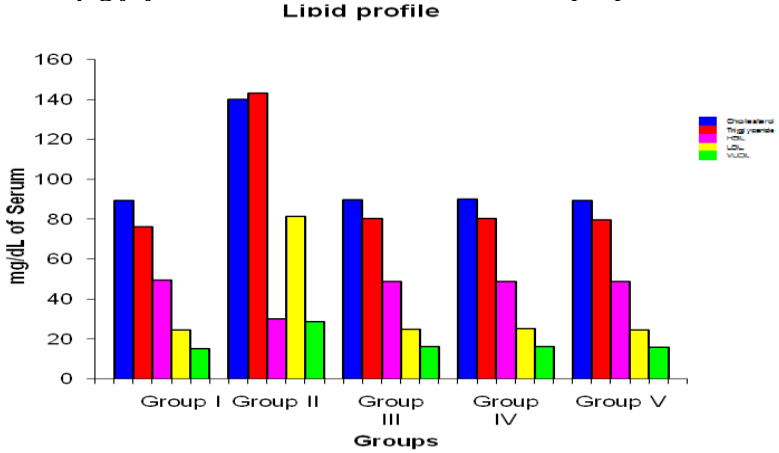


Fig 11. Effects of 18β-glycyrrhetinic acid and caffeic acid on Lipid profile in all studied groups



DISCUSSION

Diabetes mellitus is a metabolic disease characterized by multivarious groups of disorders that disturbs the metabolism of carbohydrates, fat and protein (Rawi *et al.*, 2011).

Oxidative stress induces the production of highly reactive oxygen species that are toxic to the cell, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides (Kalaiarasi and Pugalendi, 2011).

Mechanisms involved in the increased oxidative stress in diabetes include different mechanisms result in changing in the activity of antioxidant defense systems (Madhikarmi *et al.,* 2013). 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 (Karthikesan *et al.,* 2010 b). β-cells are particularly susceptible to oxidative stress (Kataja-Tuomola, 2011). In type 2 diabetes, excessive ROS could promote the inhibition of insulin synthesis (Robertson and Harmon, 2007). It has been confirmed from *in vivo* studies that high glucose concentrations induce mitochondrial ROS, which suppresses the first phase insulin secretion (Sakai *et al.* 2003).

In the diabetic state, lipid peroxidation (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 diseased state are superoxide (O2), hydroxyl (OH) and peroxyl (LOO) radicals. The damage that these radicals inflict on cells might be quantitatively determined by measurement of levels of MDA, a product of LPO (Armagan *et al.,* 2006). 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 lipid peroxidation is to decrease membrane fluidity, deformability, visco elasticity and life span of erythrocytes which may cause complications in type 2 diabetes (Hisalkar *et al.,* 2012).

A major protective mechanism against oxidative damage is the membrane integrity. Oxidation induces 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. Lipid peroxidation causes polymerization of membrane components and decreases cell deformability (Prakash and Sudha, 2012).

Certain enzymes play an important role in antioxidant defense, to maintain viable reproductive ability; a protective mechanism against oxidative stress is of importance. These enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (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 (Roy *et al.,* 2013).

Our present study observed decreasing in the activity of (GR, GPx, SOD and CAT) by 42.32 %, 21.02 %, 49.61 % and 54.08 %, respectively, and TAO level by 56.56 % in diabetic rats when compared with normal values (tables 1, 2) and this is in agreement with Nishanthini and Mohan, (2013) who reported decreasing in GR activity in diabetic rats by 44.06 % when compared with the normal group, Abdullah and Al-Assaf, (2012) who reported decreasing in GPx activity and TAO level in diabetic rats by 19.11 % and 23.84 %, respectively, when compared with the normal group, Shalaby and Kamal, (2014) who reported decreasing in SOD activity in diabetic rats by 51.48 % when compared with the normal group and Ashour *et al*., 2011 who reported decreasing in CAT activity in diabetic rats by 41.89 % when compared with the normal group. And also, we reported increasing in the MDA levels in diabetic rats by 47.99 % when compared with normal value (table 2) and this is in agreement with Kumar *et al*., 2011 who reported increasing in level of MDA in diabetic rats by 81.53 % when compared with the normal group. From our results, the administration of 18β-glycyrrhetinic acid and caffeic acid to diabetic rats resulted in a significant ($p < 0.001$) increase in the antioxidant enzyme activities and TAO level either used individually or in combination, GR by 60.02% in group III, 59.95% in group IV and 62.81in group V, GPx by 24.75% in group III, 24.48% in group IV and 26.28 in group V, SOD by 97.85% in group III, 97.23% in group IV and 98.32% in group V, CAT by 117.20% in group III, 116.65% in group IV and 117.37% in group V and TAO by 120.22% in group III, 119.81% in group IV and 127.22% in group V when compared to diabetic group and inhibit lipid peroxidation (MDA) by 26.53% in group III, 26.56% in group IV and 26.87% in group V when compared to diabetic group. Glycyrrhetinic acid has a reduced carboxylic acid group and some additional functional changes exhibited strong antioxidant activity (Chintharlapalli *et al.,* 2007). The antioxidant activities of caffeic acid are based on carbonyl group separated from the aromatic ring (LeBlanc *et al.,* 2012). It has been documented that 18ß-glycyrrhetinic 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 (Kalaiarasi and Pugalendi, 2011; Makena and Chung, 2007).

In this study, the diabetic rats showed significantly decreased levels of total protein to 4.34 ± 0.25 g/dl when compared with normal group 7.19 ± 0.37 g/dl and albumin to 2.34 ± 0.42 g/dl when compared with normal group 3.81 ± 0.14 g/dl, this is in agreement with Abd El-Ghany *et al*., 2014 who reported that the normal value of total protein was 8.86 ± 0.92 g/dl and the normal value of albumin was 4.04 ± 0.34 g/dl; in diabetic rats, the total protein level was statistically highly significant decreased to 5.28 ± 1.57 g/dl when compared with the normal group and albumin level was a statistically highly significant decreased to 3.01 ± 0.31 g/dl when compared with the normal group. And also, diabetic rats showed increased in total bilirubin to 1.573 ± 0.08 mg/dl by 166.61% when compared to normal group 0.59 ±

0.090mg/dl, this is in agreement with Abd El-Ghany *et al*., 2014 who reported that the normal value of total bilirubin was 0.45 ± 0.04 mg/dl and its value was statistically highly significant increased to 1.51 ± 0.07 mg/dl by 235.5% in diabetic rats when compared with the normal group. The decreased rate of total protein may be due to several reasons like increased rate of amino acids conversion to glucose, decreased amino acids uptake, and increased conversion rate of glycogenic amino acids to CO2 and H2O. Another group of investigators postulated protein decrease to a decrease in the amount and availability of mRNA, a loss of transitional factor, reduction of ribosomal protein synthesis as a result of insulin deficiency and decreased defensive mechanism (Rawi *et al.,* 2011). The administration of 18β-glycyrrhetinic acid and caffeic acid either used individually or in combination to diabetic rats caused a significant increase ($p < 0.001$) in total protein by 64.92 % in group III, 65.01 % in group IV and 67.56 % in group V, albumin by 61.55% in group III, 59.07 % in group IV and 63.41 % in group V, and decrease in total bilirubin by 62.87 % in group III, 62.17 % in group IV and 62.43 % in group V when compared with diabetic group (table 3) which indicates stabilization of plasma membrane and protection of liver cell membrane (Luke *et al.,* 2013). This improvement could be attributed to increased protein synthesis, increasing incorporation of certain amino acids as a result of increasing insulin secretion, increase of hepatic uptake of glycogenic amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzing amino acids transamination. Also, good correlation between protein synthesis and insulin level has been recorded by (El- Shenawy and Abdel- Nabi, 2006).

The present data indicates significant increase in the activity of AST 162.82 ± 3.52 U/l and ALT 61.77 ± 3.88 U/l of diabetic rats when compared with normal rats 85.79 ± 3.08 U/l and 35.07 ± 2.47 U/l, respectively, which are in agreement with Luke *et al*., 2013 who reported that the normal values of AST 75.07 ± 6.75 U/l and ALT 26.13 ± 1.71 U/l and these values were statistically highly significant increased to 121.84 ± 6.92 U/l and 61.21 ± 7.47 U/l, respectively, in diabetic rats when compared with the normal group. These enzymes are usually elevated in acute hepatotoxicity or mild hepato-cellular injury, but tend to decrease with prolonged intoxication due to liver damage. AST and ALT were used as markers to assess the extent of liver damage in Streptozotocin induced diabetic rats (Daisy and Jeeva kani, 2013). The elevation of AST and ALT levels may be due to the destructive changes in the hepatic cells as a result of toxemia (Kim *et al*., 2006). The present study revealed that treatment of diabetic rats with 18β-glycyrrhetinic acid and caffeic acid either used individually or in combination caused a significant decrease ($p < 0.001$) of the AST activity by 45.85 % in group III, 45.34 % in group IV and 47.16 % in group V and ALT activity by 41.57 % in group III, 40.76 % in group IV and 42.25 % in group V when compared to diabetic group (table 3). This decrease is attributed to the good hepatoprotective and antioxidant activity of 18β-glycyrrhetinic acid (Chen *et al*., 2013) and caffeic acid (Jayanthi *et al*., 2010). Since antioxidants are known to reduce the development of chemically induced liver damage (Hui-Yin Chen and Gow-Chin Yen, 2007).

Our study indicates significant increases in blood urea nitrogen level 53.12 ± 4.46 mg/dl and creatinine level 1.64 ± 0.12 mg/dl of diabetic rats compared to normal group 29.64 ± 0.53 mg/dl 0.76 ± 0.11 mg/dl respectively, this is in agreement with Al-Blooni, 2014 who reported that the normal value of blood urea nitrogen level was 36.5 ± 0.5 mg/dl and normal value of creatinine was 0.63 ± 0.62 mg/dl and in diabetic rats, blood urea nitrogen level was a statistically highly significant increased to 51 ± 1.73 mg/dl when compared with the normal group and creatinine level was statistically highly significant increased to 1.59 ± 0.01 mg/dl when compared with the normal group. An increase in serum urea and creatinine levels in diabetic rats may indicate diminished ability of the kidneys to filter these waste products from the blood and excrete them in the urine (Ugwu *et al.,* 2013). The treatment with 18β-glycyrrhetinic acid and caffeic acid either used individually or in combination caused a significant ($p < 0.001$) decrease blood urea nitrogen level by 38.58 % in group III, 38.37 % in group IV and 40.17 % in group V and creatinine level by 52.47 % in group III, 53.08 % in group IV and 52.47 % in group V when compared to diabetic group (table 4). These results may indicate that 18β-glycyrrhetinic acid and caffeic acid can enhance the ability of the kidneys to remove these waste products from the blood and thus, confer a protective effect on the kidney of diabetic rats (Ugwu *et al.,* 2013). Our results are in agreement with Yamabe *et al.,* (2006) who reported that antioxidants and good control of diabetes led to improved renal functions and urinary albumin excretion.

Type 2 diabetes mellitus is frequently associated with dyslipidemia, which is a significant risk factor for the development of cardiovascular disease. Elevated serum or tissue lipids and lipoproteins are characteristics of uncontrolled diabetes (Rao *et al*., 2011). A deficiency of insulin is associated with increase in cholesterol levels due to the enhanced mobilization of lipids from the adipose tissue to the plasma (Sharmila Banu *et al.,* 2009). In the present study, we have observed higher levels of cholesterol 140.09 ± 2.83 mg/dl, triglycerides 143.21 ± 8.48 mg/dl, LDL-C 81.42 ± 4.35 mg/dl, VLDL-C 28.64 ± 1.69 mg/dl and decreased in the level of HDL-C 30.03 ± 2.02 mg/dl in the diabetic rats when compared with normal rats 89.31 ± 4.11 mg/dl, 76.26 ± 2.41 mg/dl, 24.44 ± 3.77 mg/dl, 15.25 ± 0.48 mg/dl and 49.63 ± 3.17 mg/dl, respectively, this is in agreement with Kumar *et al*., 2012 who reported that the normal value of cholesterol 71.58 ± 6.95 mg/dl, triglycerides 63.14 ± 3.47 mg/dl, LDL-C 18.90 ± 5.86 mg/dl, and HDL-C 40.05 ± 1.78 mg/dl; in diabetic

rats, cholesterol, triglycerides and LDL-C levels were statistically highly significant increased to 183.77 ± 25.84 mg/dl, 155.91 ± 9.37 mg/dl and 129.22 ± 4.93 mg/dl, respectively, when compared with the normal group and HDL-C level was statistically highly significant decreased to 23.36 ± 1.77 mg/dl when compared with the normal group. Normally circulating low density lipoprotein- C undergoes reuptake in the liver via specific receptors and gets cleared from the circulation (Moussa *et al.,* 2009). This increased low density lipoprotein concentration in the plasma of diabetic rats might be due to the defect in low density lipoprotein-C receptor either through failure in its production (or) function. High density lipoprotein-C is protective by reversing cholesterol transport, inhibiting the oxidation of low density lipoprotein-C and by neutralizing the atherogenic effects of oxidized low density lipoprotein-C. A greater increase of low density lipoprotein-C and very low density lipoprotein-C may also cause a greater decrease of high density lipoprotein-C as there is a reciprocal relationship between the concentration of very low density lipoprotein-C and high density lipoprotein-C. Decreased high density lipoprotein-C may also be due to diminished lecithin cholesterol acyl transferase activity (Al-Numair, 2010). In our study, the diabetic rats treated with 18β- glycyrrhetinic acid and caffeic acid either used individually or in combination showed a significant ($p < 0.001$) elevation in high density lipoprotein-C by 62.60% in group III, 62.47% in group IV and 62.93% in group V and a significant ($p < 0.001$) decrease in cholesterol by 35.88% in group III, 35.62% in group IV and 36.05% in group V, low density lipoprotein-C by 69.38% in group III, 68.96% in group IV and 69.69% in group V and very low density lipoprotein-C by 43.92% in group III, 43.72% in group IV and 44.21% in group V when compared with diabetic group (table 5). Moreover, hypertriglyceridemia is a common finding in patients with diabetes mellitus associated with vascular complications (Karthikesan *et al.,* 2010 a). Kalaiarasi *et al.,* 2009 reported that deficiency of lipoprotein lipase (LPL) activity may contribute significantly to the elevation of TGs in diabetes and the treatment of diabetes with insulin served to lower plasma triglyceride levels by returning LPL activity to normal. Thus a significant ($p < 0.001$) decrease in triglyceride level following 18β- glycyrrhetinic acid, caffeic acid treatment to diabetic rats either used individually by 43.92% in group III and 43.72% in group IV or in combination by 44.21% in group V when compared to diabetic group (table 5) might be due to the increased insulin secretion, which in turn increase lipoprotein lipase activity. In addition, enhancement of insulin secretion also inhibits hormone sensitive lipase and increases the utilization of glucose and thereby decreasing the mobilization of free fatty acids (FFAs) from the fat depots (Kalaiarasi *et al.,* 2009).

In conclusion, our findings demonstrated that 18β-glycyrrhetinic acid and caffeic acid either used individually or in combination to diabetic rats have hypolipidemic effect, as evidenced by decreasing the levels of cholesterol, triglycerides, LDL-c, and VLDL-c, and increasing the level of HDL-c. And also, have a good antioxidant property, as evidenced by increased antioxidants status and decreased lipid peroxidation, which may protect from the risk of diabetic complications.

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