Biochemical alterations of inflammatory markers in experimentally induced diabetes in rats

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A B S T R A C T

This study was evaluated the protecting effect of rutin on the glycemic condition, lipid profile, in streptozotocin (STZ)-induced experimental diabetic in rats. With respect to an orally administrated rutin on serum glucose, the result is showing a significant decrease in glucose concentration when compared to diabetic group, While, a significant increase in insulin concentration when compared to diabetic. Moreover, the results revealed that, an administration of streptozotocin (STZ) showing significant increase in triacylglycerol, total cholesterol, LDL-c and VLDL-C concentration when compared with control group, while, showing significant decrease in HDL-C concentration and serum AST and ALT activity when compared to the control group. The data revealed also significant increase in serum L-MDA concentration. While, significant decrease in serum calcium concentration when compared to the control group.

Keywords: Streptozotocin, Rutin, Glucose, Insulin, L-MDA, Calcium

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1. INTRODUCTION

Diabetes is a serious chronic disease worldwide and is caused by defects in insulin production, insulin secretion, and insulin signaling (Skyler, 2007). There are two types of diabetes: type 1 diabetes (T1D) is due to self-destruction of the insulin producing beta cells in the pancreas, and type 2 diabetes (T2D) is caused by defects in insulin action and insulin production (Cohen and Horton, 2007). The number of patients suffering from diabetes (DM) worldwide has increased rapidly over the past few years and for example it increased from 171 million people suffering from diabetes in 2000, to 382 million in 2013 and may reach to 592 million people by 2035 (IDF, 2013). Hyperglycemia is involved in the pathogenesis of diabetic neuropathy, retinopathy, nephropathy, and macro vascular disease via multiple mechanisms, of which increased aldose reductase activity (Kato et al., 2003), nonenzymatic glycation and glycooxidation (Vlassara and Palace, 2003), activation of protein kinase C (PKC) (Way et al., 2001), and oxidative-nitrosative stress (Pennathur and Heinecke, 2004). Also, diabetic patients with poor glycemic control are particularly at risk for developing associated pathologies described in humans and in animal models, such as cataracts, retinopathy, nephropathy, neuropathy, micro- and macro-vascular diseases, cardiomyopathy, and impaired tissue healing (Stirban et al., 2006). Oxidative stress resulting from enhanced free radical formation and/or a defect in antioxidant defenses has been implicated in the pathogenesis of experimental diabetic neuropathy. Reactive oxygen species (superoxide radical, hydrogen peroxide and hydroxyl radical) and reactive nitrogen species (peroxynitrite) contribute to pathophysiological changes in diabetic

neuropathy (Vincent et al., 2004). Antioxidant enzyme defense system (superoxide dismutase, catalase and glutathione peroxidase) is also attenuated in peripheral nerves of diabetic animals indicating the vital role of oxidative stress in diabetic neuropathy (Low et al., 1997). Furthermore, diabetes mellitus is associated with an increased production of reactive oxygen species and a reduction in antioxidant defenses, which is partly responsible for diabetic complications, although some minerals and vitamins or cofactors, such as lipoic acid, are also able to exert antioxidant activity. The beneficial effects of α-lipoic acid, both in the prevention and treatment of diabetes, have been suggested by different investigators (Haak et al., 2000) and at least one study indicates that α-lipoic acid has beneficial effects on diabetic neuropathy partly due to its actions as an antioxidant and also by improving the circulation in the small blood vessels that supply nerve tissue (Packer et al., 2001). Protective effects of exogenously administered antioxidants have been extensively studied in animal models within recent years, thus providing some insight into the relationship between free radicals, diabetes, and its complications Maritim et al. (2003). Rutin is a common dietary flavonoid that possess a wide spectrum of biochemical and pharmacological actions attributed, at least partially, to their antioxidative and free-radical scavenging properties (Kampkotter et al., 2007). It is assumed to exert beneficial effects in various diseases including cancer, cardiovascular and neurodegenerative disorders (Williams et al., 2004). Rutin has potent antioxidant activity and is clinically used to treat diabetic neuropathy. Moreover, it enhances insulin-stimulated glucose disposal; to improve peripheral microcirculation and reduce neuropathic symptoms, possibly through attenuated oxidative stress (Vessal et al., 2003). Ozansoy and Akin (2004) recorded that, blood glucose, plasma triglyceride, cholesterol, low-density lipoprotein (LDL) cholesterol and thiobarbituric acid reactive substances (TEARS) levels were markedly increased in diabetic rats. Sri Balasubashini et al. (2003) recorded that, the levels of blood glucose and plasma triglycerides (TG), cholesterol and phospholipids were elevated during diabetes in rats induced with streptozotocin. Joice et al. (2008) observed that, significant increase in MDA levels and decrease in SOD activities were recorded in diabetic rats. The aim of this study was to evaluate the protecting effect of Rutin on the glycemic condition, lipid profile, in streptozotocin-induced diabetic in rats. Moreover, determination of its antioxidant effects on inflammatory markers in diabetes disease in rats.

2. MATERIALS AND METHODS

2.1. Animals

The study was carried out on 120 Male albino rats, aging (6-8 weeks) and of approximate weights ranging from (150-180 gm). The animals were purchased from "The Laboratory Animals Research Center", Faculty of Veterinary Medicine, Benha University. Rats were housed in separate wire mesh cages, exposed to good ventilation, humidity and to a 12-hr light/dark cycle. Provided with a constant supply of standard pellet diet (its composition is explained in table the below) and plenty of fresh, clean drinking water ad-libitum. Rats were left for 15 days' adaptation period prior to the inception of experiment. And kept at constant environmental and nutritional conditions throughout the period of the experiment

2.2. Chemicals and dosage:

Physical properties: Rutin called also rutoside, quercetin-3-O-rutinoside and sophorin, is a citrus flavonoid glycoside between the flavonol quercetin and the disaccharide rutinose, with the molecular formula $C_{27}H_{30}O_{16}$, molecular weight 450.27 , melting point 242ºC , yellowish powder and soluble in propylene glycol. Rutin (purity ~99%) was manufactured by

EIPICO (Egyptian International Pharmaceutical Industries Company), 10th of Ramadan City, Egypt. Preparation and dose of Rutin: Rutin was dissolved with in propylene glycol solution, (Tongjaroenbuangam et al., 2011), and administered to rats at a dose of (200 mg/kg body weight) through oral intubation between 7 and 8 a.m. once a day for 45 days (Abdel-Raheem, 2010).

2.3. Induction of diabetes:

Rats were fasted for 18 hours and allowed free access of water. The experimental induction of diabetes in male rats was induced by a single intraperitoneal (i.p) injected dose of 50 mg/kg bw of Streptozotocin (STZ) (sigma Chemical Co. P.O. Box. 14508, St. Low is, U.S.A.) freshly dissolved in citrate buffer, pH 4.5. Control rats were received an equivalent amount of buffer alone. A week later, STZ-treated rats were fasted for 12 hours, and blood samples were collected from the orbital venous sinus for glucose determination. Only those rats in diabetic group (group II) with blood glucose levels higher than 250 mg/dl were considered diabetic Baynes and Thorpe (1999). Streptozotocin (STZ) is widely used in experimental animal models to induce diabetes. Its cellular action includes irreversible changes in genetic material causing lethal alterations in the metabolism of cells Delfino et al. (2002).

2.4. Animal grouping:

Group I: Control group Included 20 of normal male rats. Group II: Diabetic rats "STZ group" A total number of 40 male diabetic rats kept in a separate metal. Diabetic rats were administered with propylene glycol only (diabetic control group). Group III: Rutin treated group: Diabetic rats were received Rutin was dissolved in propylene glycol and given alone at a dose of (200 mg/kg) body weight, orally through intra-gastric intubation, daily for 45 days.

2.5. Sample Collection:

At the end of the 15th, 30th and 45th days of experimental period rats were fasted overnight, then, blood samples were collected from the retro-orbital venous plexus by heparinized capillary tubes. Blood samples were divided into two portions; the first portion was collected on EDTA anticoagulant. The rest of blood samples were collected in dry, clean test tubes and incubated for 1/2 hr at room temperature to allow clotting for serum separation. Clear sera were separated by centrifugation at 3500 r.p.m. for 15 minutes and then collected in Eppendorf's tubes using automatic micropipettes. Part of serum samples were used immediately for measuring the activity of the following biochemical parameters: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Glucose, and Insulin. The rest amount of the serum were kept in deep freezer at (-20ºC) for analysis of the remaining biochemical parameters.
Determination of serum glucose (Trinder, 1969); insulin (Finlay and Dillard, 2007); total cholesterol (Allain et al., 1974); triacylglycerols (Fossati and Prencipe, 1982); HDL-C and LDL-C (Friedewald et al., 1972); VLDL-C (Bauer, 1982); Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) (Reitman and Frankel, 1957); L-Malondialdehyde L-MDA (Ohkawa et al., 1979) and calcium (McLean and Hastings, 1935)

2.6. Statistical analysis:

The Statistical analysis was carried out using ANOVA (Steel et al. 1997).

3. RESULTS

Serum glucose concentration (mg/dl):

The data presented in Table (1) revealed that, an administration of Streptozotocin (STZ) showing significant increase in glucose concentration when compared.

With respect to an orally administrated rutin on serum glucose, the result is showing a significant decrease in glucose concentration when compared to Diabetic group. Also, with respect to an orally administrated rutin on serum glucose, the result showing a significant increase in insulin concentration when compared to Diabetic. The data presented in Table (2) revealed that, an administration of Streptozotocin (STZ) showing significant increase in triacylglycerol, total cholesterol, LDL-c and VLDL-C concentration when compared with control group, while, showing significant decrease in HDL-C concentration when compared to the control group. Data in Table (2) showing a significant decrease in triacylglycerol, total cholesterol, LDL-C, concentration when compared to diabetic group. With respect to an orally administrated of rutin on HDL-C concentration. The result is showing a significant increase in HDL-C concentration when compared to Diabetic group. With respect to an orally administrated rutin on serum LDL-C concentration, the result showing a significant decrease in LDL-C concentration in when compared to diabetic group. With respect to an orally administrated a 0.2 ml from the prepared solution at a dose 200 mg/kg b.w. of rutin on some plasma of Albino rats showing a significant difference decrease in VLDL-C concentration when compared to rutin treated diabetic rats with Diabetic group during all the period of the experiment.

The data presented in Table (3) revealed that, an administration of Streptozotocin (STZ) showing significant increase in serum AST and ALT activity when compared to the control group. With respect to an orally administrated rutin showing a significant decrease in serum AST and ALT activities when compared to diabetic group**.**

The data presented in Table (3) revealed that, an administration at of Streptozotocin (STZ) showing significant increase in serum L-MDA concentration when compared to the control group. While, showing significant decrease in serum calcium concentration when compared to the control group.

Table (1): The effect of STZ and or rutin on serum glucose and insulin (mean ± S.E.)

Parameter	Duration (day)	Group: Control (G1)	Experimental Diabetes (G2)	Rutin treated diabetic rats (G3)
Glucose (mg/dl)	0	85.13±1.93^{C}	202.3±6.17^{A}	140.13±3.27^{B}
	15	89.37±2.78^{g}	170.88±1.15^{c}	155.04±2.06^{d}
	30	81.16±1.46^{g}	189.12±1.66^{b}	146.91±1.42^{e}
	45	84.86±0.95^{g}	246.9±1.39^{a}	118.45±0.92^{f}
Insulin (mg/dl)	0	17.01±0.4^{A}	7.89±0.17^{C}	9.89±0.28^{B}
	15	15.21±0.48^{b}	8.37±0.19^{de}	9.75±0.29^{cd}
	30	18.33±0.16^{a}	7.61±0.15^{e}	10.25±0.27^{c}
	45	17.5±0.27^{a}	7.68±0.16^{e}	9.69±0.3^{cd}

Table (2): The effect of STZ and or rutin on serum lipid profile (mean ± S.E.)

Parameter	Duration (day)	Group Control (G1)	Experimental Diabetes (G2)	Rutin treated diabetic rats (G3)
Triacylglycerol (TG) (mg/dl)	0	107.10±1.47^{B}	226.98±6.44^{A}	105.83±2.37^{B}
	15	103.40±1.04^{f}	192.65±1.55^{c}	117.70±1.45^{d}
	30	105.26±1.43^{ef}	215.8±2.15b	108.49±1.33^{ef}
	45	112.63±1.45^{de}	272.48±1.28^{a}	91.30±0.99^{g}
Total cholesterol (TC) (mg/dl)	0	96.98±1.40^{C}	242.35±7.63^{A}	157.22±3.32^{B}
	15	93.64±0.87^{h}	197.48±1.07^{c}	174.09±1.02^{d}
	30	95.34±1.20^{gh}	234.26±2.00^{b}	162.14±1.73^{e}
	45	101.96±1.64^{g}	295.31±1.35^{a}	135.43±1.56^{f}
HDL-C (mg/dl)	0	39.85±1.38^{B}	24.17±0.75^{C}	45.54±1.05^{A}
	15	37.88±1.83^{b}	23.76±0.89^{c}	48.31±1.09^{a}
	30	40.76±1.14^{b}	24.51±0.73^{c}	47.41±0.71^{a}
	45	40.93±1.14^{b}	24.24±0.71^{c}	40.90±0.83^{b}
LDL-C (mg/dl)	0	32.48±1.16^{C}	97.85±3.22^{A}	55.91±1.73^{B}
	15	33.39±1.07^{f}	82.72±0.76^{c}	49.86±1.27^{e}
	30	31.92±1.35^{f}	92.54±2.69^{b}	52.31±1.18^{e}
	45	32.14±1.14^{f}	118.28±0.77^{a}	65.55±1.15^{d}
VLDL-C (mg/dl)	0	21.33±0.66^{B}	45.02±1.54^{A}	20.90±1.01^{B}
	15	22.09±0.94^{de}	37.66±1.02^{c}	23.43±1.09^{d}
	30	21.22±0.61^{de}	44.06±0.77^{b}	20.88±1.07^{de}
	45	20.69±0.35^{de}	53.34±1.18^{a}	18.40±0.69^{e}

Table (3): The effect of STZ and or rutin on serum AST, ALT, L-MDA and calcium activities (mean ± SE).

Parameter	Duration (day)	Group Control (G1)	Experimental Diabetes (G2)	Rutin treated diabetic rats (G3)
AST activity (U/l)	0	80.75±1.11^{C}	123.22±1.99^{A}	90.38±1.74^{B}
	15	78.11±1.11^{d}	118.84±1.93^{b}	94.13±0.86^{c}
	30	81.24±0.96^{d}	124±2.04^{ab}	95.73±1.17^{c}
	45	82.9±1.18^{d}	126.81±1.93^{a}	81.3±1.75^{d}
ALT activity (U/l)	0	100.29±1.9^{C}	153.82±5.15^{A}	119.27±2.07^{B}
	15	96.79±1.12^{e}	130.35±2.47^{c}	118.85±1.93^{d}
	30	101.74±2.43^{e}	143.71±2.7^{b}	115.46±1.74^{d}
	45	102.34±1.95^{e}	187.41±2.5^{a}	123.5±2.43^{cd}
L-MDA (mg/dl)	0	5.02±0.15^{B}	8.28±0.23^{A}	4.72±0.19^{B}
	15	4.92±0.16^{d}	6.98±0.11^{c}	5.2±0.13^{d}
	30	5.01±0.18^{d}	8.47±0.16^{b}	5.24±0.12^{d}
	45	5.14±0.13^{d}	9.39±0.15^{a}	3.71±0.15^{e}
Calcium (mg/dl)	0	8.29±0.11^{B}	6.03±0.22^{C}	9±0.18^{A}
	15	8.27±0.12^{cd}	5.2±0.11^{e}	8.56±0.17^{bc}
	30	8.27±0.12^{cd}	7.59±0.09^{d}	9.29±0.18^{a}
	45	8.32±0.11^{c}	5.3±0.08^{e}	9.15±0.19^{ab}

4. DISCUSSON

The results revealed that, I.P. injection of STZ to male albino rats induced pathophysiological symptoms as occur in experimental diabetic rats. Myers et al. (1990) who reported that, serum glucose levels was elevated three-fold in the diabetic animals group compared to normal. In contrary, (Ogihara et al., 1984) reported that, in streptozotocin (STZ)-diabetic rats, the plasma glucose concentration decreased gradually during prolonged starvation, while it did not change in normal rats. Our results showed a significant decrease in plasma insulin concentration was observed in streptozotocin-induced diabetic rats allover the periods of the experiments. These results are nearly similar to those reported by Vinik and Vinik (2003) who reported that, high levels of circulating glucose and FFA found in type II diabetic patients are toxic to β-cells. Prolonged hyperglycemia in individuals with diabetes causes not only β-cell dysfunction but also decreased β-cell mass due to induction of apoptosis. Our results showed a significant increase in plasma glucose concentration was observed in streptozotocin-induced diabetic rats allover the periods of the experiments. These results are nearly similar to those reported by Yimaz et al. (2004) reported that glucose concentration in the blood plasma of streptozotocin-treated rats was significantly higher than in the normal control group. Our results showed a high significant increase in group two diabetic rats at 15, 30 and 45 days and significant increase in group 3 Rutin treated diabetic rats at 15 and 30 days. Our results showed a high significant decrease in plasma insulin concentration was observed in group two diabetic rats at 15, 30 and 45 days. Furthermore, the increased presence of circulating fatty acids in combination with hyperglycemia has also been implicated in the induction of β-cell apoptosis, resulting in decreased β-cell mass and function. Moreover, (Robertson, 2004) reported that, diabetes mellitus comprises a group of chronic diseases characterized by hyperglycaemia or diminished insulin secretion or both and profound effects on lipid metabolism. The obtained data revealed that, a significant increase in plasma triacylglycerols concentration was observed in streptozotocin induced diabetic rats in group two diabetic rats after 15 days, and a high significant increase after 30 and 45 days. The pronounced increase in Plasma cholesterol levels in diabetic rats is in agreement with results reported previously by Black et al. (1993) observed that, glucose, triglyceride, and cholesterol concentrations were significantly elevated in streptozocin (STZ)-induced (55 mg/kg intravenously [IV]) diabetic male Wistar rats.

In contrary, (Nishida et al., 2002) observed that, the untreated diabetic rats had the increased plasma levels of triglycerides, cholesterol, insulin and leptin at 35 wk, as compared with the healthy control rat. Our results revealed that, a significant increase in plasma total cholesterol concentration was observed in streptozotocin- induced diabetic rats after 15 days and a high significant increase in plasma total cholesterol concentration was observed after 30 and 45 days of the experiments in group two diabetic rats and a significant increase in group three after 15 days. Hypercholesterolemia is common in diabetes, contributing to the high prevalence of coronary heart disease (Ali et al., 2004). Also, (Nishida et al., 2002) observed that, the untreated diabetic rats had the increased plasma levels of triglycerides, cholesterol, insulin and leptin at 35 wk, as compared with the healthy control rat. Moreover, (Thirunavukkarasu et al., 2004) reported that, streptozotocin-induced diabetes increases TC, LDL-C, and triglycerides as well as decreases HDL-C concentrations. The mechanism is possibly due to reduction in lipoprotein lipase activity secondary to reduced plasma insulin levels. Cholesteryl ester transfer protein, which is important in regulating

lipoprotein lipid composition was increased in DM condition and may also contributed to the dyslipidemia Wasan et al. (1998). Hypertriglyceridemia is a common metabolic disorder associated with STZ-induced diabetes (Nielsen et al., 2002). The pronounced increase in plasma triglycerides levels in diabetic rats is in agreement with results reported previously by Ahmed et al. (2001) showed that, there was a significant increase in plasma non-esterified cholesterol, triglycerides and phospholipids in STZ-induced diabetic rats, accompanied by a decrease in high density lipoprotein (HDL)-cholesterol. Furthermore, (Anandh Babu et al., 2006) reported that, there was an increase in the serum and cardiac triglyceride levels in diabetic rats. The reported changes in TG could be related to the mild but significant insulin deficiency resulted in mild hypertriglyceridemia, linked to impaired triglyceride removal rather than to an overproduction of VLDL-triglyceride, despite elevated levels of plasma free fatty acids. Also it could be attributed to the disturbed tissue lipases system which regulated by insulin were suppressed by STZ increasing TG (Gorska et al., 1990). This suggestion was confirmed by Yoshino et al. (1992) who suggested that, the removal of triglyceride from the circulation, as well as its entry into the circulation, was impaired in mildly insulin-deficient rats. Our results revealed that serum HDL-concentration decreased significantly after 30 and 45 days. Our results revealed that, a high significant increase in plasma LDL- concentration was observed in streptozotocin induced diabetic rats in group two diabetic rats after 30 and 45 days, and a significant increase after 15 days. A significant increase in Serum transaminases enzymes (AST and ALT) activities was observed in streptozotocin-induced diabetic rats allover the periods of the experiments. These results are nearly similar to those reported by Mohammad et al. (2006) who showed that, the aminotransferases (AST and ALT) levels were significantly increased in the liver of STZ-treated animals. The increase in aminotransferases levels may be due to the cellular damage in the liver caused by STZ-induced diabetes. Moreover, (Voss et al., 1988) proposed that STZ in hyperglycemic animals caused a time dependent rise in AST, ALT, and ALP levels. In contrary, (Okada et al., 1997) reported that, AST activity was lower than the amount of enzyme in diabetic rat tissues. It is suggested that this may be due to the inactivation of cytosolic AST in the diabetic rat tissues by a glycation reaction, accompanied by impairment in glucose utilization in STZ induced diabetes.

Many workers explained the observed reduction of serum Ca^{+2} level in diabetics to be due to reduction in duodenal Ca^{+2} transport (Schneider and Schedl, 1972) or impaired renal reabsorption of Ca^{+2} Cheug (1980). Our results showed a significant increase in serum L-malondialdehyde concentration was observed in streptozotocin-induced diabetic rats allover the periods of the experiments. These results are nearly similar to those reported by Sundaram et al. (1996) who demonstrated that, plasma MDA showed 80 % increase in the early stages of diabetes, and more progressive increase later which explained as the factors favouring the formation of reactive oxygen species may catalyze lipid peroxidation in the plasma and other tissues and in poorly controlled diabetic, glucose oxidation through the pentose phosphate pathway initiates excessive formation of NADPH, this in turn can promote lipid peroxidation in the presence of cytochrome P- 450 system. The recorded results could be related to the inactivation or inhibition of antioxidant enzymes through glycation, in poorly controlled diabetes mellitus, may give rise high lipid peroxidation rate, evidence of lipid peroxidation had been observed in many diabetic complications increased level of plasma lipid peroxidation products, measured a thiobarbituric acid reactive substance, have been found to be higher in diabetes and the most harmful free

radical are unpaired oxygen (stable molecules contain paired electrons), hydroperoxides and superoxide anions (Diplock, 1994). Malondialdehyde (MDA) formed by the breakdown of oxidized polyunsaurated fatty acids, has been considered as a marker of the free oxygen radical formation in red blood cells (RBCs) of diabetic patient and alloxan diabetic rats and the hyperglycemia can accelerate lipid peroxidation of human (RBCs) Awaji et al. (1995).

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