Research Article

Antioxidant properties of swertiamarin, from *Enicostemma littorale* blume. leaves studied in high fat diet fed and low dose streptozotocin induced diabetic rats

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is an endocrine disorder characterized by insulin resistance, β cell dysfunction, visceral obesity, inflammation and chronic oxidative stress. However, insulin resistance is predominant in type 2 DM which results in persistent hyperglycemia. Chronic hyperglycemia promotes auto-oxidation of glucose to form free radicals. The generation of free radicals beyond the scavenging abilities of endogenous antioxidant defenses results in the development of both macro- and microvascular complications. **Objective:** In present study, we have evaluated the antioxidant property of swertiamarin, a secoiridoid glycoside present in the *Enicostemma littorale* blume leaves in High fat fed and -Low dose streptozotocin induced type 2 diabetic rats. **Materials and Methods:** High-fat diet-fed and low-dose STZ was used to induce experimental type 2 diabetes in rats. Diabetic rats were orally treated with swertiamarin (50 mg/kg b.w./rat/day) for 30 days. The levels of oxidative stress markers such as lipid peroxides, hydroperoxides and protein carbonyls were determined. The levels of both enzymatic as well as non enzymatic antioxidants were evaluated. **Results and conclusion:** Diabetic rats showed significantly increased levels of oxidative stress markers and declined levels of both enzymatic and non enzymatic antioxidants. Oral treatment of swertiamarin at a concentration of 50mg/kg bw/rat/day for 30 days decreased the levels of oxidative stress markers and improved the status of antioxidants in diabetic rats indicating the antioxidant properties of swertiamarin in addition to its antidiabetic properties.

Keywords: Swertiamarin, high fat, oxidative stress, antioxidant

Introduction

Type 2 diabetes mellitus (T2DM) is an endocrine disorder characterized by insulin resistance, β cell dysfunction, visceral obesity, inflammation and oxidative stress. Insulin resistance coupled with defect in insulin secretion by the pancreatic β cells paves the way for the progression of hyperglycemia. Chronic hyperglycemia is suggested to be a major cause of diabetic complications, which not only deteriorate the quality of life, but also pose a financial burden on health care systems. In order to overcome the primary and secondary complications of diabetes, understanding its pathogenesis and molecular mechanisms may create a foundation for novel treatment strategies. Oxidative stress play a pivoted role in hyperglycemia-induced tissue injury and other related events of T2DM (Nowotny et al., 2015).

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Abnormal regulation of the metabolic pathways leads to a wide spectrum of diseases in human. Thus, identification of molecules as drugs for the treatment of multi-genic diseases such as cancer, diabetes and inflammatory disorders is more challenging (Sangeetha et al., 2017).

Although the primary cause of type 2 diabetes is unknown, it is clear that insulin resistance plays an early role in its pathogenesis which occurs via multiple mechanisms. Among them, elevated free fatty acid (FFA) levels are the prime cause of insulin resistance along with an altered glucose output and uptake (Rajala et al., 2003). Increased levels of plasma free fatty acids promote insulin insensitivity by impairing the anti-oxidant system by the detonated production of reactive oxygen species, and increased expression of inflammatory markers (Wellen et al., 2003). Inflammation and oxidative stress are the prime factors that implicate adipogenesis and insulin resistance (Evans et al., 2003). Antioxidants such as N-acetylcysteine, vitamin C and α-lipoic acid are effective in ameliorating the diabetic complications, indicating that it may be beneficial either by

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ingestion of natural antioxidants or through dietary supplementation. Increased incidence of obesity is also a major contributing factor in the escalating rates of type 2 diabetes. Drug discovery efforts are therefore crucially dependent on recognizing the individual molecular targets and validating their significance to human ailments.

Plant derived phytochemicals serves as therapeutic agents owing to their structural diversity, biochemical specificity and maximum therapeutic efficacy with minimal side effects for the treatment of non communicable diseases (Cragg and Newman, 2013). Most of these bioactive principles originate from edible plants; possess antidiabetic as well as antioxidant potential. Phytochemicals such as flavonoids, alkaloids, glycosides, saponins, dietary fibres, steroids, xanthone, coumarins, iridoids, alkyl disulphides, and guanidines of plant origin exerts anti-diabetic as well as antioxidant activity (Patel et al., 2012).

Enicostemma littorale Blume (*E. littorale*), a medicinal plant which belongs to the family of Gentianaceae*,* possess significant antidiabetic properties. Swertiamarin, a secoiridoid glycoside, originally isolated from the leaves of *E. littorale* was reported to be responsible for most of the medicinal properties of *E. littorale* such as anti-atherosclerotic and anti-inflammatory properties (Leong et al., 2016). Recently, we have reported the antidiabetic as well antidyslipidemic properties of swertiamarin (Selvam et al., 2018a &b). In the present study, the antioxidant efficacy of swertiamarin was studied in high fat diet (HFD) fed-low dose STZ induced type 2 diabetes in rats.

Materials and methods

Experimental animals

Male Wistar rats weighing about 160-180 g, procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, India, were housed in clean, sterile, polypropylene cages under standard vivarium conditions (12 h light/dark cycle) and temperature (25 ± 2°C) with ad libitum access to water and standard rat chow (Hindustan Lever Ltd., Bangalore, India) with a composition of 5% fat, 21% protein, 55% nitrogen-free extract and 4% fiber (w/w) with adequate minerals and vitamins for the animals. The animals were acclimatized to the laboratory conditions for 2 week prior to the inception of experiments. Animal experimentations were premeditated and executed in accordance with the ethical norms approval by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines

High Fat Diet fed-low dose streptozotocin induced diabetes

The rats were divided into two dietary regimens by feeding either normal or high fat diet (HFD) for the initial period of two weeks (Srinivasan et al., 2005). The ingredients and chemical composition of the HFD was followed as before reported. After two weeks of dietary manipulation, the groups of rats fed with HFD were intraperitoneally injected with freshly prepared low dose of streptozotocin (35 mg/kg b.w) dissolved in 0.1M ice cold citrate buffer, ph 4.5. One week after the administration of with STZ, the experimental rats were screened for fasting blood glucose level. Rats having fasting blood glucose (FBG) >250mg/dl that exhibited random hyperglycemia and glycosuria were selected for the experiment. The rats were allowed to continue to feed on the respective diets until the end of the experiments.

Experimental protocol

The rats were divided into four groups each group comprising six rats.

Group 1: Control rats.

Group 2: Diabetic rats.

Group 3: Diabetic rats treated with swertiamarin (50 mg/kg b.w/rat/dayfor 30 days.).

Group4: Diabetic rats treated with metformin (50 mg/kg b.w/ rat/dayfor 30 days.)

At the end of 30 days of experimental period, the rats were fasted overnight, anaesthetized (ketamine, 80 mg/kg b.w. I.p.) And sacrificed by cervical decapitation. The blood was collected with or without anticoagulants for plasma or serum separation, respectively. The liver, kidney and pancreatic tissues were dissected out, washed in ice-cold saline and used for further experimental studies.

Estimation of oxidative stress markers

The levels of total lipid peroxides (Ohkawa et al., 1979), hydroperoxides (Jiang et al., 1992) and protein carbonyls (Uchida and Stadtman, 1993) were determined in plasma and tissue homogenates.

Determination of antioxidant status

The activities of enzymatic antioxidants such as SOD (Misra and Fridrovich, 1972), catalase (Takahara, et al., 1960), gpx (Rotruck et al., 1973), GST (Habig et al., 1974) were assayed in the tissue homogenate of control and experimental groups of rats. The levels of non-enzymatic antioxidants, vitamin C (Omaye et al., 1979), vitamin E (Desai, 1984), ceruloplasmin (Ravin, 1961) and GSH (Sedlak and Lindsay, 1968) were determined.

Results

Tables 1, 2, 3 and 4 exemplify the levels of lipid peroxides, hydroperoxides and protein carbonyls in pancreatic, hepatic and kidney tissues of control and experimental groups of rats. The significant ($p<0.05$) increased observed on the levels of lipid peroxides, hydroperoxides and protein carbonyls in plasma, pancreatic, hepatic and kidney tissues of HFD-STZ diabetic rats were declined ($p<0.05$)

Table 1. The levels of lipid peroxides, hydroperoxides and protein carbonyls in plasma of control and experimental groups of rats after 30 days of experimental period

Groups	Lipid peroxides	Hydroperoxides	Protein carbonyls
Control	4.01 ± 0.23	10.10 ± 0.50	7.20 ± 0.22
Diabetic control	9.00 ± 0.34 a*	25.00 ± 1.62 a*	25.70 ± 1.80 a*
Diabetic + Swertiamarin	4.92 ± 0.10b*	18.00 ± 1.00 b*	14.22 ± 0.63b*
Diabetic + Metformin	4.11 ± 0.26b*	17.56 ± 1.38b*	13.10 ± 0.70b*

Units are expressed as: nm/ml for lipid peroxides; 10^{-5} mm/dl for hydroperoxides; nm/mg of protein for protein carbonyls. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats; [c]diabetic rats treated with metformin. Values are statistically significant at *P<0.05

Table 2. Effects of swertiamarin on the levels of lipid peroxides, hydroperoxides, and protein carbonyls in pancreatic tissues of experimental groups of rats

Groups	Lipid peroxides	Hydroperoxides	Protein carbonyls
Control	34.00 ± 2.50	14.44 ± 0.81	5.45 ± 0.20
Diabetic control	72.00 ± 3.81 a*	31.50 ± 1.66 a*	22.73 ± 0.78a*
Diabetic + Swertiamarin	48.00 ± 2.45b*	19.40 ± 1.20b*	14.50 ± 0.33b*
Diabetic + Metformin	43.44 ± 2.66b*	18.51 ± 0.81b*	12.09 ± 0.55 b*

Units are expressed as: mm/ 100 g of wet tissue for lipid peroxides and hydroperoxides; nm/mg of protein for protein carbonyls. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats. Values are statistically significant at *P<0.05.

Table 3. Effects of swertiamarin on the levels of lipid peroxides, hydroperoxides, and protein carbonyls in hepatic tissues of control and experimental groups of rats

Groups	Lipid peroxides	Hydroperoxides	Protein carbonyls
Control	2.01 ± 0.13	45.30 ± 3.21	5.00 ± 0.24
Diabetic control	4.10 ± 0.24 a*	96.40 ± 5.20 a*	15.50 ± 0.51 a*
Diabetic + Swertiamarin	2.40 ± 0.13b*	78.40 ± 3.10b*	8.10 ± 0.13b*
Diabetic + Metformin	2.21 ± 0.23b*	73.88 ± 2.45b*	7.42 ± 0.22b*

Units are expressed as: mm/ 100 g of wet tissue for lipid peroxides and hydroperoxides; nm/mg of protein for protein carbonyls. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats; Values are statistically significant at *P<0.05.

significantly to near normalcy by the treatment of swertiamarin as well as metformin to diabetic groups of rats.

Table 5-7 depicts the activities of enzymatic antioxidants such as SOD, catalase, gpx and GST in pancreatic, hepatic and renal tissues of control and experimental groups of rats. The activities were significantly ($p<0.05$) diminished in the all the tissues of diabetic group of rats. Oral treatment of swertiamarin significantly ($p<0.05$) attenuated the altered activities of these enzymic antioxidants to near normalcy in the tissues of diabetic rats.

The levels of plasma non-enzymatic antioxidants such as vitamin C, vitamin E and ceruloplasmin (Table 8), and pancreatic, hepatic and renal GSH (Table 9) content are represented. Similarly, Table 9 shows the level of GSH in pancreas, liver and kidney tissues of control and experimental groups of rats. HFD-STZ diabetic rats showed a significant ($p<0.05$) decrease in these levels when compared with control group of rats. Conversely, administration of swertiamarin as well as metformin to HFD-STZ diabetic rats significantly ($p<0.05$) increased the levels to near control values.

Discussion

Chronically elevated plasma levels of glucose and fatty acids contribute to relentless β-cell dysfunction by endorsing processes known as as glucolipotoxicity. Mechanisms related to glucolipotoxicity include endoplasmic reticulum (ER) stress, oxidative stress,

Table 4. Effects of swertiamarin on the levels of lipid peroxides, hydroperoxides, and protein carbonyls in renal tissues of control and experimental groups of rats

Groups	Lipid peroxides	Hydroperoxides	Protein carbonyls
Control	1.13 ± 0.06	54.30 ± 2.10	3.55 ± 0.20
Diabetic control	4.10 ± 0.14 ^{a*}	83 .50 ± 3.00 ^{a*}	17.32 ± 0.81 ^{a*}
Diabetic + Swertiamarin	2.20 ± 0.14 ^{b*}	60.00 ± 3.20 ^{b*}	9.00 ± 0.58 ^{b*}
Diabetic + Metformin	2.00 ± 0.13^{b*}	55.40 ± 3.51^{b*}	7.52 ± 0.69^{b*}

Units are expressed as: mm/ 100 g of wet tissue for lipid peroxides and hydroperoxides; nm/mg of protein for protein carbonyls. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats; Values are statistically significant at *P<0.05

Table 5. Activities of Superoxide dismutase (SOD), Catalase, Glutathione peroxidase (gpx) and Glutathione-S-transferase (GST) in pancreatic tissues of control and experimental groups of rats

Groups	SOD	Catalase	Gpx	GST
Control	10.10 ± 0.20	23.30 ± 1.50	7.50 ± 0.21	6.00 ± 0.20
Diabetic	2.30 ± 0.10 ^{a*}	6.88 ± 0.43^{a*}	4.24 ± 0.23 ^{a*}	1.70 ± 0.15 ^{a*}
Diabetic + Swertiamarin	6.15 ± 0.20^{b*}	14.00 ± 0.33^{b*}	6.70 ± 0.28^{b*}	4.33 ± 0.21^{b*}
Diabetic + metformin	7.00 ± 0.12^{b*}	15.80 ± 0.58^{b*}	7.10 ± 0.18^{b*}	5.00 ± 0.24^{b*}

Activities of enzymes are expressed as: 50% of inhibition of epinephrine autoxidation/min for SOD; mm of hydrogen peroxide decomposed/min/mg of protein for catalase; mm of glutathione oxidized/min/mg of protein for gpx; U/min/mg of protein for GST. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats; Values are statistically significant at *P<0.05

Table 6. Activities of Superoxide dismutase (SOD), Catalase, Glutathione peroxidase (gpx), Glutathione-S-transferase (GST) and Glutathione reductase (GR) in hepatic tissues of control and experimental groups of rats

Groups	SOD	Catalase	Gpx	GST	GR
Control	12.00±0.48	81.30 ± 2.32	11.50±0.38	9.11 ± 0.24	26.50±2.00
Diabetic	4.50±0.19^{a*}	38.40±2.08^{a*}	3.80±0.16^{a*}	3.51± 0.18*	12.63 ± 0.61^{a*}
Diabetic+Swertiamarin	8.40±0.20^{b*}	63.11± .42^{b*}	7.22±0.12^{b*}	6.71±0.21^{b*}	20.60 ± 1.63^{b*}
Diabetic + metformin	7.31±0.22^{b*}	60.20±3.10^{b*}	7.00 ± .10^{b*}	6.00±0.24^{b*}	24.29 ± 1.44^{b*}

Activities of enzymes are expressed as: 50% of inhibition of epinephrine autoxidation/min for SOD; mm of hydrogen peroxide decomposed/min/mg of protein for catalase; mm of glutathione oxidized/min/mg of protein for gpx; U/min/mg of protein for GST; µm of DTNB-GSH conjugate formed/min/mg of protein for GR. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats; Values are statistically significant at *P<0.05.

mitochondrial dysfunction and islet inflammation. Oral antidiabetic drugs such as metformin and sulfonylurea, improve glycaemic control to certain extent. However, these drugs do not alter the continuous decline in β-cell function in diabetic individuals (van Raalte and Diamant, 2011). Hence search for an alternative therapy for diabetes continues from natural sources.

Chronic oxidative stress mediated by glucolipotoxicity has been implicated to play a chief role in the onset and progression of diabetes and its associated complications. Hyperglycemia along with increased lipid content elicits excessive reactive oxygen species (ROS) generation by several well established mechanisms (Fiorentino et al., 2013). Type 2 diabetes mellitus is primarily associated with insulin resistance. As a result, there is an increased formation of advanced glycation end products (ages) and lipid peroxidation products that intensify intracellular oxidative stress, resulting in the loss of molecular integrity, disruption in cellular signaling and homeostasis, followed by inflammation and tissue injury.

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. Oxidative stress occurs due to an imbalance between ROS generation and the availability of

Table 7. Activities of Superoxide dismutase (SOD), Catalase, Glutathione peroxidase (gpx), Glutathione-S-transferase (GST) and Glutathione reductase (GR) in renal tissues of control and experimental groups of rats

Groups	SOD	Catalase	Gpx	GST	GR
Control	14.20 ± 0.76	43.70 ± 3.10	7.70 ± 0.21	6.40 ± 0.12	37.10 ± 1.72
Diabetic	9.50± 0.32[a*]	18.30±0.81[a*]	3.51±0.17[a*]	2.31 ± 0.13 [a*]	12.00±0.60[a*]
Diabetic+Swertiamarin	13.58±0.33[b*]	37.00±1.42[b*]	7.00±0.20[b*]	4.42±0.21[a*b*c]	27.00±1.10[b*]
Diabetic + metformin	14.29 ± 0.50[b*]	34.36 ± 2.15 [b*]	6.15 ± 0.10 [b*]	5.01 ± 0.24[b*]	25.45 ± 1.21 [b*]

Activities of enzymes are expressed as: 50% of inhibition of epinephrine autoxidation/min for SOD; mm of hydrogen peroxide decomposed/min/mg of protein for catalase; mm of glutathione oxidized/min/mg of protein for gpx; U/min/mg of protein for GST; µm of DTNB-GSH conjugate formed/min/mg of protein for GR. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats. Values are statistically significant at [*]P<0.05.

Table 8. Effect of Swertiamarin on the levels of Vitamin E, Vitamin C and Ceruloplasmin in plasma of control and experimental groups of rats

Groups	Vitamin E	Vitamin C	Ceruloplasmin
Control	0.92 ± 0.055	1.80 ± 0.060	14.06 ± 0.46
Diabetic	0.45 ± 0.024 [a*]	0.49 ± 0.045 [a*]	5.40 ± 0.30[a*]
Diabetic + Swertiamarin	0.73 ± 0.029 [b*]	0.86 ± 0.024 [a*b*c]	9.13 ± 0.40[b*]
Diabetic + metformin	0.78 ± 0.022[b*]	0.80 ± 0.033[b*]	9.49 ± 0.38[b*]

Units: mg/dl. Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats; Values are statistically significant at [*]P<0.05.

Table 9. Effect of Swertiamarin on the level of reduced glutathione in pancreas, liver and kidney tissues of control and experimental groups of rats

Groups	Reduced glutathione		
	Pancreas	Liver	Kidney
Control	24.00 ± 0.70	49.20 ± 1.80	35.12 ± 1.61
Diabetic	8.20 ± 0.38 [a*]	23.81 ± 0.94 [a*]	21.14 ± 0.66 [a*]
Diabetic + Swertiamarin	16.04 ± 0.24[b*]	32.50 ± 2.10 [b*]	29.60 ± 1.40 [b*]
Diabetic + metformin	17.00 ± 0.52 [b*]	38.40 ± 1.83 [b*]	32.18 ± 0.78[b*]

Units: mg/100 g of wet tissue.

Values are given as mean ± S.E.M for groups of six rats in each. One-way ANOVA followed by post hoc test LSD. Statistical significance was compared within the groups as follows: [a]control rats; [b]diabetic control rats. Values are statistically significant at [*]P<0.05.

antioxidants (Betteridge, 2000). A certain amount of oxidative stress/ ROS is necessary for the normal metabolic processes since ROS play various regulatory roles in cells (Gomes et al., 2012). ROS are produced by neutrophils and macrophages during the process of respiratory burst in order to eliminate antigens (Freitas et al., 2010). Due to oxidative stress the metabolic abnormalities of diabetes cause mitochondrial superoxide overproduction in endothelial cells of both large and small vessels, as well as in the myocardium (Giacco and Brownlee, 2010). Oxidative stress acts as a mediator of insulin resistance and its progression to glucose intolerance and installation of diabetes mellitus, subsequently favoring the appearance of atherosclerotic complications and contributes to rise in many micro- and macrovascular complications (Negre-Salvayre et al., 2009).

Lipid peroxidation plays a major role in the development of cardiovascular disease in diabetes (Januszewski et al., 2003). Lipid peroxidation is the formation of lipid peroxides via enzymatic and/or non-enzymatic mechanisms. ROS generation due to chronic hyperglycaemia is the primary cause for the initiation of lipid peroxidation (Cosentino et al., 1997; Evans et al., 2002). Lipids are reported as one of the primary targets of ROS. Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic

hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes, such as malondialdehyde (MDA), that damage cell membranes (Lobo et al., 2010). Peroxidation of lipids produces highly reactive aldehydes, including MDA, acrolein, 4-hydroxynonenal (HNE), 4- oxononenal (ONE), and isolevuglandins (isolgs) (Guo et al., 2012). The peroxyl radicals remove hydrogen from lipids resulting in the production of hydroperoxides resulting in the free-radical mediated pathway (Lobo et al., 2010). MDA is a primary biomarker of free radical mediated lipid damage and oxidative stress (Shodehinde and Oboh, 2013). Increased lipid peroxidation is associated with the high glycemic levels and oxidative stress in diabetes mellitus (Bandeira et al., 2012; Salgueiro et al., 2013). In the present study elevated levels of oxidative markers were observed. Upon treatment with swertiamarin, the levels of oxidative stress markers were decreased which indicate the oxyradical-scavenging effect of swertiamarin.

The benefit of antioxidants is not only attributed to their radical scavenging but to their ability to interact with many basic cellular activities (Liu et al., 2005). Antioxidant-rich foods derived primarily from plants containing a large number of health promoting components make them a positive addition to the human diet (Wolfe et al., 2007). Antioxidant enzymes such as SOD, CAT, gpx and GST, form the first line of defense against ROS in the organism, play an important role in scavenging the toxic intermediate of incomplete oxidation.

During oxidative stress, endogenous mechanisms, enzymes and antioxidant molecules are deployed to destroy the excessive generation of reactive oxygen species and reduce the harmful effects of oxidants. In normal conditions, these mechanisms are sufficient to counteract free radical production, but in diabetes, they are overwhelmed because of an increased oxidative stress. The vulnerability of each tissue to oxidative stress varies depending upon their expressed antioxidant enzymes. In addition to decreased levels of antioxidants in the pancreatic β cells, the supraphysiological glucose concentration is notorious to provoke oxidative stress in hepatocytes, which can cause hepatic tissue damages (Ling et al., 2003).

Liver is the chief organ of oxidative and detoxifying processes as well as free radical reactions and the biomarkers of oxidative stress are elevated in the liver at an early stage in many diseases, including diabetes mellitus (Stadler et al., 2003). In experimental diabetes, streptozotocin exerts its toxic effects on liver and other organs in addition to pancreatic β cells. The insulin insufficiency and hyperglycemia that result from β cell necrosis further augment liver damage through reactive free radicals mediated lipid peroxidation of hepatocellular membrane (Kume et al., 2004).

Superoxide dismutase (SOD) is the antioxidant enzyme that catalyses the dismutation of superoxide anion ($O2^{-}$) into hydrogen peroxide and molecular oxygen (Faraci and Didion, 2004; Wang et al., 2012). SOD protects cellular and histological damages that occur due to oxidative stress. It facilitates the conversion of superoxide radicals into hydrogen peroxide and in the presence of other enzymes it converted into oxygen and water (Davari et al., 2013). Increased expression of SOD or the supplements of antioxidants including SOD mimetics, t overcomes oxidative stress, reduces ROS generation, and increases enzymatic antioxidant thereby ameliorating prevent diabetes mellitus (Wang et al., 2011).

Catalase is an antioxidative enzyme present in all living organisms. It acts against oxidative stress-generated complications that occurs in cancer, diabetes and cardiovascular diseases (Chelikani et al., 2004). Hydrogen peroxide is a highly reactive molecule formed as a natural by-product of energy metabolism. High levels of hydrogen peroxide cause significant damages to proteins, DNA, RNA and lipids (Takemoto et al., 2009). Catalase enzymatically processes hydrogen peroxide into oxygen and water and thus neutralizes it. Catalase deficiency results in oxidative stress leading to β-cell dysfunction. β-cells are rich in mitochondria and thus this organelle might be a source of ROS (Goth and Eaton, 2000).

Gpx, a selenium-containing peroxidase is concerned in the detoxification of hydrogen peroxide and lipid peroxide by using GSH as a hydrogen donor and acts as a peroxynitrite reductase. Persistent hyperglycemia increase oxidative stress through diverse mechanisms; the defective antioxidant function of gpx is a hall mark in the diabetic state. The low activity of gpx could be directly explained by the low content of GSH found in patients with type 2 diabetes, since GSH is a substrate and cofactor of gpx. Enzyme inactivation could also contribute to low gpx activity. Gpx is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress. Inactivation of this enzyme may occur through glycation governed by prevailing glucose concentration (Rahbani-Nobar et al., 1999). Increased activity of GR may be a compensatory response to oxidative stress. Changes in glutathione peroxidase and glutathione reductase activity can be considered an adaptation of antioxidant defense against ROS. However, the altered levels of enzymatic antioxidants were improved upon swertiamarin treatment indicating the effective antioxidant as well as tissue protective nature of swertiamarin.

Vitamin C, a hydrophilic antioxidant sequesters the singlet oxygen radical, stabilize the hydroxyl radical and regenerate reduced vitamin E back to the active state. Vitamin E, a lipophilic antioxidant, transfers its phenolic

hydrogen to a peroxyl free radical of peroxidized polyunsaturated fatty acids, thereby breaking the radical chain reaction and averting the peroxidation of membrane lipids (Opara, 2002). Ceruloplasmin is a a powerful non-enzymatic antioxidant that inhibits lipid peroxidation by binding with copper. The observed decline in plasma ceruloplasmin in diabetic rats may be due to elevated lipid peroxidation which was normalized upon treatment with swertiamarin.

Glutathione (GSH), a tripeptide, γ-Lglutamyl-L-cysteinylglycine, is present in all mammalian tissues at 1–10 mm concentrations (highest concentration in liver) as the most abundant nonprotein thiol that defends against oxidative stress (Lu, 2013). GSH can maintain SH groups of proteins in a reduced state, participate in amino acid transport, detoxify foreign radicals, act as coenzyme in several enzymatic reactions, and also prevent tissue damage (Tsai et al., 2012). It is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level (Rizvi and Chakravarty, 2011). Decreased GSH level is a factor in the oxidative DNA damage in type 2 diabetes mellitus (Dincer et al., 2002). Hyperlipidemia, inflammation, and altered antioxidant status are the common factors in diabetes mellitus accompanied with a decreased GSH/GSSG ratio (Das et al., 2012). Altered GSH status is involved in β-cell dysfunction and in the pathogenesis of long-term complications of diabetes. Similarly the lowered GSH levels observed in diabetic rats were increased upon oral administration of swertiamarin further strengthening the antioxidant potential of swertiamarin.

Conclusion

In the present study, the elevated levels of lipid peroxides and hydroperoxides in plasma, pancreatic, hepatic and renal tissues of diabetic rats were significantly altered upon oral administration of swertiamarin which demonstrates the anti-lipidperoxidative property of swertiamarin under oxidative stress environment. Also, swertiamarin treated improved the antioxidant status in pancreatic, hepatic and renal tissues of diabetic rats. Thus, it can be concluded that swertiamarin recuperates antioxidant status and protects the pancreatic, hepatic and renal tissues from hyperglycemia mediated oxidative stress indicating its antioxidant potential as well as tissue protective nature.

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Authors' contributions

All the three authors have equal contribution in the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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