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Valproic acid displays anti-diabetic and pro-antioxidant effects in high-fat diet and streptozotocin-induced type 2 diabetic rats

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ABSTRACT

Introduction: Valproic acid (VPA) has been demonstrated to attenuate inflammatory responses which are associated with types 1 and 2 diabetes mellitus. The hypoglycemic, antilipidemic and antioxidant effects of VPA has been reported in animal model of type 1 diabetes mellitus.

Objective: This study investigated the anti-diabetic and antioxidant effects of VPA in type 2 diabetic rats.

Materials and methods: Type 2 diabetes was induced in rats with high-fat diet and 35 mg/kg body weight streptozotocin. Non-diabetic female Wistar rats were treated with water (control) while the diabetic female Wistar rats were treated with water, varying doses of VPA (100, 300 and 600 mg/kg body weight) and metformin (100 mg/kg body weight) for two weeks.

Results: VPA or metformin normalised the elevated fasting blood glucose level and percentage glycosylated haemoglobin, without affecting plasma insulin of the diabetic rats. Treatment with VPA or metformin normalised the reduced hepatic glycogen content and hexokinase activity of the diabetic rats. VPA or metformin increased or normalised the reduced activities of catalase and glutathione peroxidase, and glutathione concentration in both liver and serum but normalised the elevated level of malondialdehyde in the serum of the diabetic rats.

Conclusion: These results suggest that VPA displays anti-diabetic and pro-antioxidant effects in type 2 diabetic rats.

Keywords: Valproic acid, anti-diabetic, antioxidant, type 2 diabetes

Introduction

Diabetes mellitus is a diverse group of metabolic disorders that is often associated with a high disease burden in both developed and developing countries (Ogbera and Ekpebeh, 2014). Globally, an estimated 422 million adults are living with diabetes (WHO, 2016).

In 2013, it was estimated that about 4 million people are diabetic in Nigeria which represent a fifth of all diabetes cases in Sub-Saharan Africa (Atlas, 2015). There are two main types of diabetes mellitus which are type 1 diabetes (insulin dependent diabetes mellitus) and type 2 diabetes (non-insulin dependent diabetes mellitus). Type 2 diabetes accounts for 90-95% of diabetes cases (Gonazalez *et al*., 2009) and mortality in diabetic patients (Alberti *et al*., 2004). Type 2 diabetes mellitus involves at least two primary pathogenic mechanisms: a progressive decline in pancreatic islet cell function resulting in reduced insulin secretion and inadequate suppression of glucagon secretion (Cernea and Dobreanu, 2013)Type 2 diabetes mellitus involves at least two primary

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pathogenic mechanisms: a progressive decline in pancreatic islet cell function resulting in reduced insulin secretion and inadequate suppression of glucagon secretion (Cernea and Dobreanu, 2013), and peripheral insulin resistance resulting in a decrease in the metabolic responses to insulin (Diabetes, 2006). The basic effect of insulin lack or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain (Guyton and Hall, 2006). As a result of this, blood glucose concentration increases (hyperglycemia), cell utilization of glucose falls increasingly lower, and utilization of fats and proteins increases.

Hyperglycemia as one of the features that predominates type 2 diabetes results in the generation of oxidative stress by inhibiting glyderaldehyde-3-phosphate dehydrogenase (Brownlee, 2001). It is well known that increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Ceriello, 2000; Walton, 2017). Mechanisms by which increased oxidative stress is induced in the diabetic complications are activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C (Ullah *et al.*, 2016). Despite the advances in the development of pharmacological therapies for the management of diabetes, it still remains a global health issue due to high rate of morbidity and mortality. This therefore necessitates a further search for more potent and less toxic drug candidates for diabetes. Histone deacetylases (HDACs) have been reported to be potential target for the treatment of diabetes because of their role in the regulation of glucose metabolism, insulin resistance, and insulin expression and secretion (Christensen *et al*., 2011). Valproic acid (VPA), a histone deacetylase inhibitor, has been demonstrated to attenuate inflammatory responses (Cardinale et al., 2010) which are associated with types 1 and 2 diabetes (Zhang *et al*., 2008; Pollack *et al.*, 2016). It has also been reported to promote insulin expression in β-cells (Lenoir *et al*., 2011). Although the hypoglycemic, antilipidemic and antioxidant effects of VPA has been reported in animal model of type 1 diabetes (Akindele *et al*., 2015), some anti-diabetic indices such as percentage glycosylated haemoglobin, hepatic glycogen content and activities of glucose metabolising enzymes were not considered. In addition, the anti-diabetic and antioxidant effects of VPA in type 2 diabetes has not been reported to the best of our knowledge. This study was therefore aimed at investigating the anti-diabetic and antioxidant effects of VPA in type 2 diabetic rat.

Materials and methods

Chemicals and reagents

Valproic acid sodium salt and Streptozotocin used for this study are products of Sigma Aldrich, United Kingdom. Metformin is a product of Wells Biosciences Pvt. Ltd. Manimajra, Chandigarh, India. All other reagents used for this study were of analytical grade and were prepared using distilled water.

Feed formulation

The normal diet and high-fat diet were formulated according to the modification of the method described by Nishina *et al*. (1990). The compositions of both high-fat diet and normal diet are contained in Table 1.

Table 1: Feed Composition

Ingredients	Normal Diet (g/kg)	High-Fat Diet (g/kg)
Corn Starch	506	276
Cellulose (Rice husk)	40	40
Soy beans	280	180
Sucrose	80	80
Soy oil	50	50
Vitamin/Mineral premix	40	40
D-Methionine	4	4
Fat (Lard)	-	330
Total	1000g	1000g

Experimental animals and induction of type 2 diabetes mellitus using the high-fat diet and streptozotocin model

Adult female Wistar rats weighing 150-210 g were used for this study. The rats were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, and were handled in accordance with the Guide for the Care and Use of laboratory animals, as approved by the Animal Ethics Committee of the institution. The rats were housed in experimental cages under a 12 hrs light-dark cycle at standard condition of temperature, and were allowed to acclimatize for 7 days, during which they were fed normal diet and given water *ad libitum*. To induce type 2 diabetes, rats (excluding the normal control group), were fed high-fat diet (HFD; 38 % fat, 18.4 % protein, 39.6 % carbohydrate and 4% vitamin/mineral, as a percentage of total kcal) for 2 weeks, and then injected with a single dose of Streptozotocin (STZ) (35 mg/ kg body weight, intraperitoneally, in citrate buffer; pH 4.5) (Abeeleh et al., 2009). The development of hyperglycemia in rats was confirmed by testing fasting blood glucose (FBG) 48h post-STZ injection. Rats that maintained FBG higher than 200 mg/dL were considered diabetic, and selected for the study.

Animal grouping and drug administration

Group I (normal control) comprised of 5 rats which were fed normal diet throughout the experimental period. 25 high-fat diet and STZ-induced diabetic rats were divided into 5 groups of 5 animals each as follows: group II (diabetic control) rats were fed HFD; group III (diabetic + 100mg/kg bw VPA) rats were fed HFD and treated with 100mg/kg body weight VPA; group IV (diabetic + 300mg/kg bw VPA) rats were fed with HFD and treated with 300mg/kg body weight VPA; group V (diabetic + 600mg/kg bw VPA) rats were fed with HFD and treated with 600mg/kg body weight VPA and group VI (diabetic + 100mg/kg bw metformin) rats were fed with HFD and treated with 100 mg/kg bw metformin. The treatment lasted for 14 days, during which FBG was measured at 0, 7th, and 14th day of the study using blood from rats' tail vein. At the end of the treatment, the rats were fasted overnight, and sacrificed. Then their blood and liver samples were collected for biochemical assays.

Sample preparation

The venous blood was collected from the experimental animals as earlier described (Narayanan *et al*., 1984). The serum and plasma were prepared by centrifuging the blood samples at 3000 rpm for 5 minutes and collected by pipetting. The animals were thereafter quickly dissected and the liver removed. The liver was suspended in ice-cold 0.25 M sucrose solution (1:5 w/v) and homogenized. The homogenates were kept frozen overnight to ensure maximum release of the enzymes (Ngaha *et al*., 1989).

Biochemical assays

Fasting blood glucose (FBS) was determined by using glucose meter. Glycosylated haemoglobin (HbA1c) in the blood was estimated by the method based on the phenol sulphuric acid reaction of carbohydrates (Nayak and Pattabiraman, 1981). The plasma insulin concentration was determined based on the interaction of antibody and insulin (Gerbitz, 1980). A colorimetric micro-method that is based on a colour reaction which occurs when a dilute solution of glycogen is heated with concentrated sulphuric acid was adopted for the determination of liver glycogen content (Kemp and Van Heijningen, 1954). Liver hexokinase activity was determined by measuring the rate of disappearance of glucose (Brandstrup *et al*., 1957). The protein concentration of the liver homogenates was determined using the Biuret method (Gornall *et al*., 1949). A simple colorimetric assay using $K_2Cr_2O_7$/acetic acid

reagent was used to determine catalase activity (Sinha, 1972). The activity of glutathione peroxidase (GPx) in tissues was determined by measuring the rate of disappearance of glutathione (Rotruck *et al*., 1973). The levels of reduced glutathione (GSH) in the tissues were estimated by the method of Beutler *et al*. (1963). The rate of lipid peroxidation in tissues was assayed by quantifying malondialdehyde (MDA) levels (Varshney and Kale, 1990).

Statistical Analysis

All data are presented as mean of five replicates ± standard error of mean (S.E.M.). Statistical analysis was done using one-way analysis of variance and Duncan Multiple Range test (SPSS 16.0 version statistical package program, SPSS Inc., Chicago IL). Differences in mean were considered statistically significant at $p<0.05$.

Results

Anti-diabetic effect of valproic acid in high-fat diet and streptozotocin-induced diabetic rats.

High-fat diet and streptozotocin-induced diabetes significantly increased ($p<0.05$) fasting blood glucose level in rats (Figure 1). Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw; the standard anti-diabetic drug used in this study) significantly reduced ($p<0.05$) and normalised the elevated fasting blood glucose level in the diabetic rats after 7 and 14 days (Figure 1).

High-fat diet and streptozotocin-induced diabetes significantly increased percentage glycosylated haemoglobin (Figure 2). Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly reduced ($p<0.05$) and normalised the elevated percentage glycosylated haemoglobin in the diabetic rats after 14 days (Figure 2).

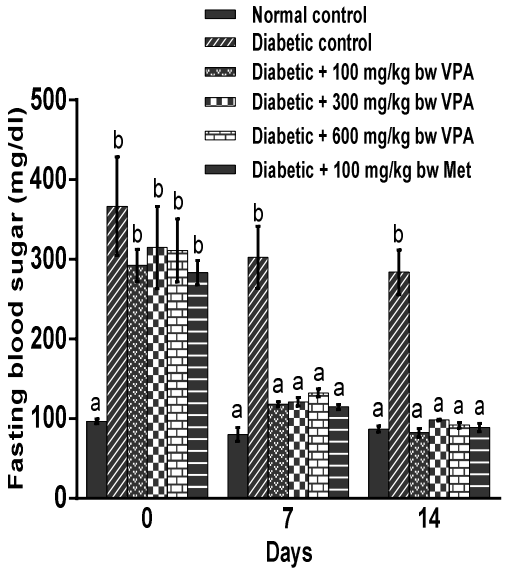

Figure 1: Effect of valproic acid on fasting blood sugar in high-fat diet and streptozotocin-induced diabetic rats. Bars with different superscripts are significantly different ($p<0.05$).

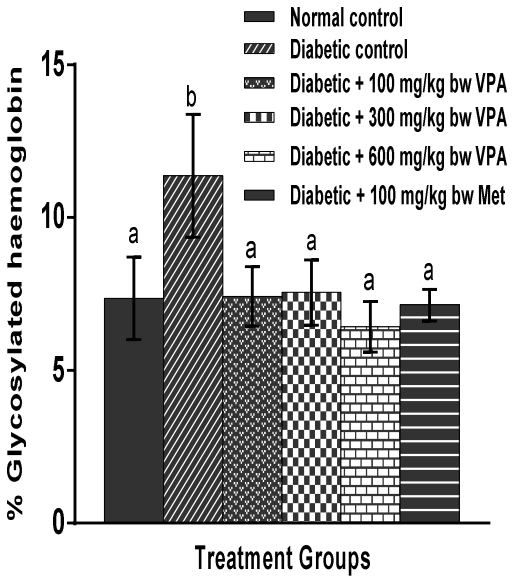

Figure 2: Effect of valproic acid on percentage glycosylated haemoglobin in high-fat diet and streptozotocin-induced diabetic rats. Bars with different superscripts are significantly different ($p<0.05$).

The plasma insulin concentration was not significantly affected ($p>0.05$) by diabetic induction caused by high-fat diet and streptozotocin (Figure 3). Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) did not affect plasma insulin concentration of the diabetic rats after 14 days (Figure 3).

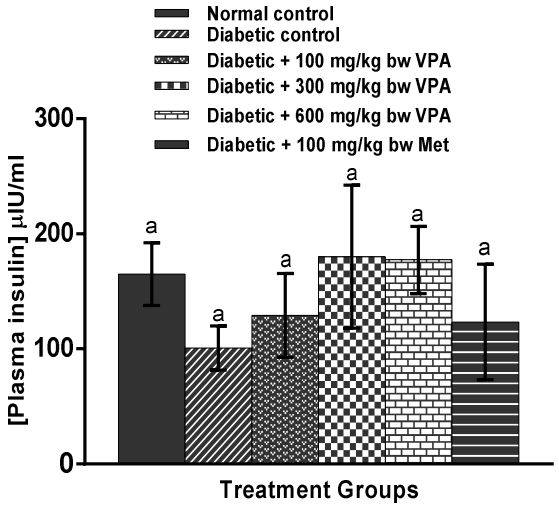

Figure 3: Effect of valproic acid on plasma insulin concentration in high-fat diet and streptozotocin-induced diabetic rats. Bars with different superscripts are significantly different ($p<0.05$).

Hepatic glycogen content and hexokinase activity were significantly reduced following high-fat diet and streptozotocin-induced diabetes (Figures 4 & 5). VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly increased ($p<0.05$) the reduced hepatic glycogen content in the diabetic rats (Figure 4). 100 mg/kg bw VPA did not significantly affect ($p>0.05$) the hepatic hexokinase activity of the diabetic rats. However, treatment with 300 and 600 mg/kg bw VPA or 100 mg/kg bw metformin significantly increased ($p<0.05$) and normalised the reduced hexokinase activity of the diabetic rats (Figure 5).

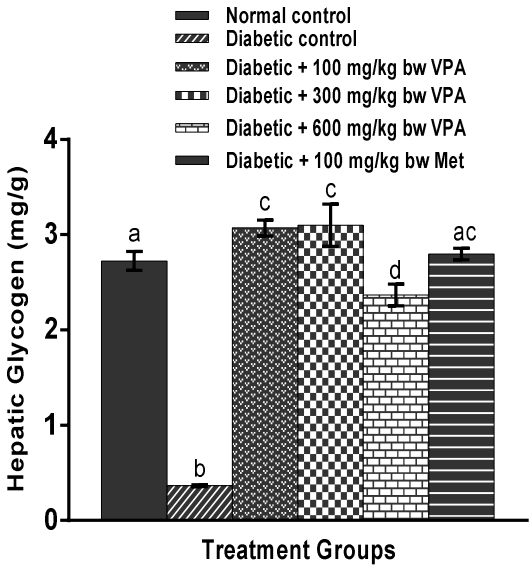

Figure 4: Effect of valproic acid on hepatic glycogen content in high-fat diet and streptozotocin-induced diabetic rats. Bars with different superscripts are significantly different ($p<0.05$).

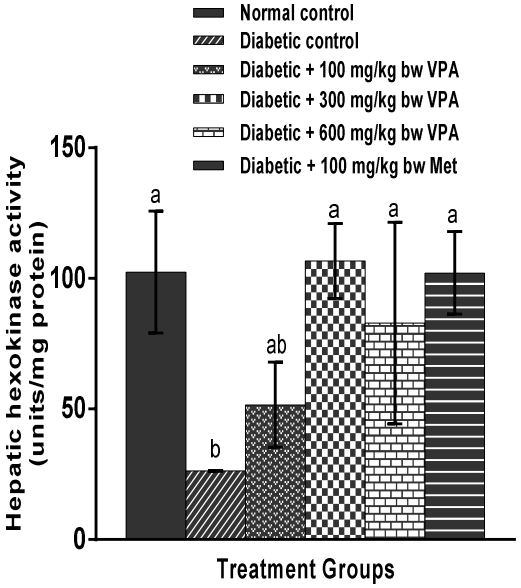

Figure 5: Effect of valproic acid on hepatic hexokinase activity in high-fat diet and streptozotocin-induced diabetic rats. Bars with different superscripts are significantly different ($p<0.05$).

Antioxidant effect of valproic acid in high-fat diet and streptozotocin-induced diabetic rats

High-fat diet and streptozotocin-induced diabetes significantly reduced ($p<0.05$) the activities of hepatic catalase and glutathione peroxidase in rats. Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly increased ($p<0.05$) and normalised the reduced activities of hepatic catalase and glutathione peroxidase in the diabetic rats (Table 2).

Table 2: Effect of valproic acid on hepatic antioxidants indices of high-fat diet and streptozotocin-induced diabetic rats.

Treatment Groups	Hepatic antioxidant indices		
	Catalase activity (units/mg protein) x 10^{-2}	Glutathione peroxidase activity (units/mg protein) x 10^{-2}	Reduced glutathione concentration (µmol/l) x 10^{-2}
Normal control	15.40± 01.487[a]	66.01± 7.257[a]	4.06 ± 0.092[a]
Diabetic control	2.62± 0.427[b]	23.89± 0.705[b]	3.02 ± 0.021[c]
Diabetic + 100mg/kg bw VPA	12.84± 1.392[a]	64.74± 4.481[a]	4.01 ± 0.106[a]
Diabetic + 300mg/kg bw VPA	15.07± 1.955[a]	58.41± 3.247[a]	3.62 ± 0.078[b]
Diabetic + 600mg/kg bw VPA	14.15± 2.114[a]	57.27± 5.086[a]	3.73 ± 0.128[ab]
Diabetic + 100mg/kg bw Met	10.75± 0.427[a]	62.44± 3.594[a]	3.87 ± 0.168[ab]

Values along the same column with different superscripts are significantly different ($p<0.05$).

The reduced glutathione concentration in the liver was significantly reduced ($p<0.05$) following high-fat diet and streptozotocin-induced diabetes. Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly increased ($p<0.05$) the concentration of reduced glutathione in the liver of the diabetic rats (Table 2).

The activities of catalase and glutathione peroxidase in the liver of high-fat diet and streptozotocin-induced diabetic rats was significantly reduced ($p<0.05$) when compared to normal control (Table 3).

Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly increased ($p<0.05$) the serum catalase activity of the diabetic rats. VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly increased ($p<0.05$) and normalized the serum glutathione peroxidase activity of the diabetic rats (Table 3). The reduced glutathione concentration in the serum was significantly reduced ($p<0.05$) following high-fat diet and streptozotocin-induced diabetes. Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly increased ($p<0.05$) and normalised the concentration of reduced glutathione in the serum (Table 3).

Serum malondialdehyde level was significantly increased ($p<0.05$) following high-fat diet and streptozotocin-induced diabetes (Table 4). Treatment with VPA (100, 300 and 600 mg/kg bw) or metformin (100 mg/kg bw) significantly reduced ($p<0.05$) and normalised serum malondialdehyde level of the diabetic rats (Table 4).

Table 3: Effect of valproic acid on serum antioxidants indices of high-fat diet and streptozotocin-induced diabetic rats.

Treatment Groups	Serum antioxidant indices		
	Catalase activity (units/mg protein) x 10^{-2}	**Glutathione peroxidase activity (units/mg protein) x 10^{-2}**	**Reduced glutathione concentration (µmol/l) x 10^{-2}**
Normal control	8.19 ± 0.782^{a}	15.97 ± 3.352^{a}	2.85 ± 0.0046^{ab}
Diabetic control	1.90 ± 0.266^{d}	2.3 ± 0.000^{b}	2.78 ± 0.0002^{c}
Diabetic + 100mg/kg bw VPA	5.44 ± 0.470^{c}	14.07 ± 3.694^{a}	2.85 ± 0.0020^{ab}
Diabetic + 300mg/kg bw VPA	7.01 ± 0.254^{ab}	14.49 ± 3.714^{a}	2.86 ± 0.0080^{ab}
Diabetic + 600mg/kg bw VPA	5.90 ± 0.490^{bc}	13.28 ± 3.572^{a}	2.85 ± 0.0089^{b}
Diabetic + 100mg/kg bw Met	7.14 ± 0.552^{ab}	14.92 ± 4.208^{a}	2.84 ± 0.0025^{a}

Values along the same column with different superscripts are significantly different ($p<0.05$).

Table 4: Effect of valproic acid on malondialdehyde concentration of serum lipid profile of high-fat diet and streptozotocin-induced diabetic rats.

Treatment Groups	**Serum Malondiadehyde level (unit/mg protein)**
Normal control	0.96 ± 0.169^{a}
Diabetic control	4.80 ± 0.194^{b}
Diabetic + 100mg/kg bw VPA	1.18 ± 0.115^{a}
Diabetic + 300mg/kg bw VPA	1.16 ± 0.118^{a}
Diabetic + 600mg/kg bw VPA	1.77 ± 0.740^{a}
Diabetic + 100mg/kg bw Met	1.07 ± 0.724^{a}

Values along the same column with different superscripts are significantly different ($p < 0.05$).

Discussion

This study demonstrates that valproic acid (VPA) displays anti-diabetic and pro-antioxidant effects in type 2 diabetic (T2D) rats in the same manner as metformin (the standard anti-diabetic drug used in this study). VPA normalised the elevated fasting blood glucose level and percentage glycosylated haemoglobin, without affecting plasma insulin of the diabetic rats. Treatment with VPA normalised the reduced hepatic glycogen content and hexokinase activity of the diabetic rats. VPA normalised the reduced activities of catalase and glutathione peroxidase, and glutathione concentration in both liver and serum but decreased or normalised the elevated level of malondialdehyde in the serum of the diabetic rats.

Hyperglycemia (high glucose level in the blood) is a hallmark of diabetes. The usage of high-fat diet and low-dose streptozotocin in inducing T2D in animal model had been previously reported (Reed *et al*., 2000). High-fat diet induces insulin resistance (Storlien *et al*., 1993), while streptozotocin (STZ) is a known diabetogen (Muranyi *et al*., 2006), being able to cause the destruction of β-cells of the islets of Langerhans, thereby leading to reduction in the mass of β cells of the pancreas and thus resulting to a massive reduction in insulin release. Deficiency of insulin consequently leads to high glucose level in the blood (hyperglycemia). Insulin deficiency is usually accompanied with decreased utilization of glucose by the tissues, and excessive hepatic glycogenolysis and gluconeogenesis, which constitute the fundamental mechanism underlying hyperglycemia in diabetes mellitus (Jayasri *et al*., 2008). In this study, treatment with VPA normalised the elevated fasting blood glucose level in high-fat diet and STZ-induced diabetic rats when compared with normal control (Figure 1). The normoglycemic effect of VPA may be due to any or combination of the following: reduction of intestinal absorption of dietary carbohydrate (Warter *et al*., 1984), inhibition of glucose-metabolizing enzymes (Johannessen, 2000), improvement of pancreas β-cell function (Kim *et al*., 2005), and stimulation of insulin secretion and action (Luef *et al*., 2003). The finding in this study corroborates some previous studies that show that VPA possesses antihyperglycemic effects in treated patients (Ebbesen *et al*., 2000), reduced fasting blood sugar of STZ-diabetic rats (Akindele *et al*., 2015), and modulates glucose metabolism in patients with epilepsy after first exposure (Rakitin *et al*., 2015).

Glycosylation of proteins is another critical feature of diabetic conditions (Selvin *et al*., 2004). Under diabetic conditions, an elevated level of glycosylated haemoglobin (HbA1c) has been reported. Measurement of percentage glycosylated haemoglobin was the first clinically used method to assess the levels of hyperglycaemia in T2D patients (Koenig *et al*., 1976). Elevated glycosylated haemoglobin is strongly associated with long-term microvascular complications (Stratton *et al*., 2000), and assessment of glycosylated haemoglobin is used for monitoring effective glycaemic levels (Saudek *et al*., 2006). In this study, VPA normalised the elevated percentage glycosylated haemoglobin in high-fat diet and STZ-induced diabetic rats when compared with normal control (Figure 2). This might be due to the normoglycemic effect of VPA as observed in this study.

Insulin, a hormone synthesized and produced by β-cells in the pancreas, plays a key role in the regulation of blood glucose levels (Wang *et al*., 2006). A lack of insulin or insulin resistance can lead to the development of symptoms of diabetes (Sonksen and Sonksen, 2000). Insulin disturbance is pathologic in type 1 diabetes, type 2 diabetes and other metabolic conditions (Koeslag *et al*., 2003). In this study, high-fat diet and STZ produced hyperglycemia (Figure 1) without significantly affecting ($p<0.05$) plasma insulin concentration (Figure 3), suggesting insulin resistance which is a characteristic feature of type

2 diabetes. This observation correlates with earlier induction of type 2 diabetes mellitus using high fat-diet and low-dose streptozotocin model (Ren *et al*., 2000; Srinivasan *et al*., 2005). Treatment with VPA did not affect plasma insulin concentration (Figure 3) though it normalised the elevated fasting blood glucose level in the diabetic rats (Figure 1). These results suggest that VPA might be exerting its anti-diabetic effect by increasing insulin sensitivity.

Glycogen level is a good index for assessing anti-hyperglycemic activity of therapeutic agents (Grover *et al*., 2000). Glycogen metabolism is regulated through reciprocal modulation of glycogen phosphorylase and glycogen synthase, such that activation of glycogen phosphorylase is tightly linked to inhibition of glycogen synthase, and vice versa (Cline *et al*., 1999). Insulin enhances intracellular glycogen deposition by stimulating activities of glycogen synthase and inhibiting glycogen phosphorylase (Shivanna *et al*., 2013). In this study, VPA normalised the reduced hepatic glycogen content produced by high-fat and STZ (Figure 4). The reduction of hepatic glycogen content by high-fat diet and STZ as observed in this study is in agreement with Ahmed and colleagues who reported that STZ-induced diabetes reduced hepatic glycogen content in diabetic rats (Ahmed *et al*., 2010). The increase in hepatic glycogen content following administration of VPA may be due to an enhanced insulin sensitivity causing activation of glycogen synthase and inhibition of glycogen phosphorylase leading to increased glycogen deposition in the liver.

Hexokinase, one of the key enzymes in the catabolism of glucose which catalyses the phosphorylation of glucose to glucose-6-phosphate, plays a major role in the control of blood glucose homeostasis in the liver, and has a very high control on hepatic glucose disposal (Pari and Murugan, 2005; Agius, 2008). Hexokinase is both insulin dependent and insulin-sensitive enzyme and is almost completely inhibited or inactivated in the diabetic rat liver in the absence of insulin (Tan *et al*., 2005). Hexokinase insufficiency in diabetic rats can cause decreased utilization of glucose for energy production. In this study, VPA normalised the reduced liver hexokinase activity in high-fat diet and STZ-induced diabetic rats when compared with normal control (Figure 5). The normalisation of hexokinase activity following treatment with VPA as observed in this study might be due to increase in insulin sensitivity since high-fat diet and STZ-induced diabetic rats produced insulin resistance. Thus, increased insulin sensitivity which promote glucose uptake into the cells may account for the decrease in fasting blood glucose level of the diabetic rats following treatment with VPA. Consequently, increase in hepatic hexokinase activity may increase glucose utilization for energy production.

Chronic hyperglycemia in diabetes is usually accompanied by increased production of reactive oxygen species (ROS) and impaired antioxidant defense system, which precipitate oxidative stress. Increased production of ROS promotes the depletion of non-enzymatic antioxidants (including reduced glutathione), and reduces the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) (Zhang *et al*., 2010). SOD, CAT and GPx constitute the front line of the body's endogenous antioxidant enzyme defense system, for scavenging free radicals (Ji, 1999). In this study, the attenuated activities of CAT and GPx and depleted GSH level (a non-enzymatic antioxidant) was observed in the liver and serum of diabetic control rats when compared with the control group (Table 2), indicating impaired antioxidant status arising from exacerbated ROS formation. However, VPA normalised the reduced GSH concentration and activities of CAT and GPx in both liver (Table 2) and serum (Table

3). This result suggests that VPA has the potential to ameliorate oxidative stress and its associated damages that characterize T2D.

Diabetic monocytes are induced by oxidative stress to generate large amount of superoxide anion (O^{2-}) which in turn leads to peroxidation of plasma and tissue lipids to generate peroxidation products. Studies have shown that malondialdehyde (MDA) concentration, a product of lipid peroxidation, is elevated in diabetic conditions (Altomare *et al*., 1992). MDA can impair membrane function, inactivate membrane bound receptors and enzymes, and increase tissue permeability (Abdel-Rahman et al., 2003). In this study, the elevated level of malondialdehyde in the serum of the diabetic rats was normalised in the VPA-treated groups, as well as in those treated with metformin. This further suggests that VPA has the potential to prevent oxidative damage in diabetic condition.

In conclusion, the findings from this study suggest that valproic acid displays anti-diabetic and pro-antioxidant effects in high-fat diet and streptozotocin-induced type 2 diabetic rats.

Disclosure statement

There is no conflict of interest reported by the authors

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