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COAST
JOURNAL OF THE SCHOOL OF SCIENCE
Volume 5, Issue 2. December, 2023 ISSN: 2714-3716
Coast, J. Sch. Sci. 5 (2): 875- 892

HEPATO-PROTECTIVE ROLE OF DIPHENYL DISELENIDE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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https://doi.org/10.61281/coastjss.v5i2.1

Abstract
Diabetic hepatopathy prevalence as a result of prolonged hyperglycemia is increasing with the expanding size of the diabetes population around the world. In this present study, the hepato-protective activity of diphenyl diselenide (DPDSe) in a persistent diabetic state was studied. Diabetes mellitus were induced with streptozotocin (STZ) (60 mg/kg) intraperitoneally and after 60 days without treatment, the rats were administered diphenyl dislenide (DPDSe) (10 mg/kg) orally for 35 days. Animals were euthanized liver and serum were used for experimental determinations such as glycemic level, non enzymic and enzymic redox statuses, hepatic biomarkers, hematological and lipid profile. The results showed that the fasting glucose levels and thiobarbituric reactive substances were reduced at a significant level of $p < 0.05$. Furthermore, a concomitant rise in the percentage of DPPH free radicals scavenged, ferric reducing antioxidants property, protein and non-protein thiols were observed in hepatic tissue. The activities of delta–aminolevulinate dehydratase and antioxidant enzymes were improved at a significant level of $p < 0.05$. Additionally, there was significant reduction ($p < 0.05$) in the activity of the purinergic enzymes, hepatic biomarkers while there was an increase in the albumin level. Furthermore, the hematological and lipid profile of were refurbished. This study showed that DPDSe was able to reverse the biochemical alterations related with hepatic tissue in STZ-induced diabetic rats.
Keywords: Diphenyl diselenide, streptozotocin, purinergic enzymes, hepatic biomarkers, hematological profile

Introduction

Diabetes and other associated diseases are major health problems in modern society. Diabetes is no longer a disease of primarily rich nations and its commonness is steadily on the increase across the globe (WHO, 2016); gender, socioeconomic status and ethnicity of the individual nonetheless (Okoduwa *et al.*, 2015). According to the latest International Diabetes Federation (IDF), the global prevalence of T2DM in adults was 536.6 million people (10.5%) in 2021, and that there would be 783.2 million people (12.2%) living with diabetes worldwide by 2045 (Sun *et al.*, 2022). Diabetes mellitus (DM) can be defined as a group of hormonal and metabolic disorders with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion or insulin action (Mahmoud *et al.*, 2015). Etiologically diabetes can be categorized into Type 1 and Type 2; generally, type 2 diabetes

(T2DM) is found in 95% of population, and 5% have type 1 diabetes (T1DM) (American Diabetes Association, 2018). It has been reported that chronic hyperglycemia, a clinical trademark to diabetes causes severe metabolic instability and pathological alteration in liver (Akpan *et al.*, 2013) and may therefore increase the menace of both chronic liver diseases and hepatocellular carcinoma (Byrne, 2012). Furthermore, liver enzymes reveal hepatocyte integrity or cholestasis, the later increases alkaline phosphatase level (ALP) (Giannini *et al.*, 2005), while alanine transaminase (ALT) is prominent whenever there is hepatocyte injury (Newsome *et al.*, 2018). Understanding the pathogenesis of DM is a possible lead to developing novel anti-diabetic agents with less side-effects, this has stimulated countless research on synthetic drugs. Most therapeutics drugs used for the management of diabetes are oral hypoglycemic agents and insulin injection with various limitations. Therefore, there is need to discover antioxidant drug not only for the management of diabetes but that will also reduce its associated complications in liver.

Selenium, an essential element with physiological antioxidant properties occurs in the body as selenocysteine, a structural component of several enzymes involved in peroxide decomposition, including glutathione peroxidase (Nogueira *et al.*, 2004). Additionally, the organic forms of selenium have been suggested as possible antioxidant agents because they exhibit glutathione peroxidase-like activity (Mugesh *et al.*, 2001a; Nogueira *et al.*, 2004). Interestingly, selenium-containing compounds such as diphenyl diselenide has been reported to have both antioxidant and insulin-mimetic properties *in vivo* (Kade *et al.*, 2009 a, b). Furthermore, Barbosa et al. (2006) reported that DPDSe and ebselen possessed anti-hyperglycemic and antioxidants effects *via* subcutaneous administration to streptozotocin-induced diabetic rats.

Although, studies have reported the STZ-induced diabetes mellitus and its association with specific diabetic complications and disturbances in various organs, such as liver and kidney. However, only limited information is available on the hepato-protective activity of diphenyl diselenide in a prolonged and untreated diabetes state. Therefore, the aim of this present study was to investigate the influence of DPDSe on hepatic tissue of STZ-induced diabetic animals with emphasis on non-enzyme, enzyme redox dependent homeostasis and some other biochemical functional indices of liver.

2.0 Materials and Methods

2.1. Chemicals

Diphenyl diselenide, streptozotocin (STZ), 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), sodium azide, calcium chloride, magnesium chloride, adenosine triphosphate (ATP) and δ-aminolevulinic acid (δ-ALA) were obtained from Sigma (St. Louis, MO). All other chemicals were of analytical grade obtained from standard commercial suppliers

2.2. Animals

Adult wistar rats (200 –250 g) were used in the experiment. Animals were kept in separate animal cages, with free access to food and water. The animals were used according to standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

2.3 Diabetes induction

Diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg) freshly prepared in 0.1 M citrate-buffer (pH 4.5). Control rats received an equivalent amount of buffer. Diabetic state was checked 72 h after induction with STZ. The animal's glucose levels were analyzed using

glucometer (FINTEST GLUCOMETER). Animals were considered diabetic when blood glucose level exceeded 250 mg/dL.

2.4 Experimental Design

After the successful induction of diabetes, the diabetic and control animals were left for 60 days to develop hepatic complications associated with a prolonged and persistent diabetic state before treatment.

The animals were divided randomly into the following groups:

- Control: Animals in this group were given only food and water.
- DPDSe: This group contains normal animals which were administered diphenyl diselenide (10mg/kg) only orally for 35 days.
- STZ: This is the diabetic group which were injected with streptozotocin (60mg/kg) intraperitoneally and left untreated.
- STZ+ DPDSe: Groups 4 was administered DPDSe by gavage at a dose of 10 mg/kg (once/day) for 35 days. At the end of the experimental period, corresponding control, diabetic and treated animals were anesthetized with ether. Rats were fasted 12 h prior to euthanasia and euthanized by decapitation.

2.5 Hepatic Functions and Lipid profile parameters

Whole blood was collected by cardiac puncture into plain tubes and centrifuge for 10min at 4000rpm for measurement of biochemical parameters. Hepatic function parameters alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of Reitman and Frankel (1957); alanine phosphatase (ALP) was assayed according to the method of Englehart (1970), total bilirubin was evaluated by the method of Jendrassik and Grof (1938) and albumin was estimated by the method of Wolf (1972) Lipid profile test (total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very-low density lipoprotein) were analyzed according to the method of Allain *et al.*, (1974). All assays were analyzed using Randox laboratories limited kits, United Kingdom.

2.6 Hematological Parameters

Heamatological parameters were analyzed using heamatology analyzer (Model MINDRAY BC, China).

2.7. Preparation of Tissue Homogenate

The liver was rapidly removed, placed on ice and weighed. The liver was immediately homogenized in cold 50 mM Tris–HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged for 10 min at 4000 g to yield a pellet that was discarded and a low-speed supernatant (S1). This was used for all assays. The amount of protein in S1 was carried out by the method of Lowry *et al.,* (1951).

2.8 Lipid Peroxidation Assay

An aliquot of 100 µl of Supernatant (S1) was used, follow by the addition of 200 µl 8.1% SDS to S1, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% of thiobarbituric acid (TBA). This mixture was incubated at 100^0C for 30 minutes Productions of TBARS was determined as described by method of Ohkawa *et al.,* (1979). TBARS produced were measured at 532nm and the absorbance was compared to that of a standard curve obtained using malondialdehyde (MDA).

2.9 1, 1-Diphenyl-2 picrylhydrazyl DPPH Free Radical Scavenging Ability

The free radical scavenging abilities of the liver tissue homogenate against DPPH (2, 2-diphenyl-1-picrylhydrazyl) was determined according to the method of Brand-Williams *et al.* (1995) with minor modifications. Briefly, 100µl of the protein-free tissue homogenates was mixed with 600µl, 0.3mM methanolic solution containing DPPH radicals. The mixture was left in the dark for

30 minutes, and absorbance values were measured at 517 nm.

2.10 Estimation of Protein-bound and non-protein Thiols

Aliquots (100 – 200 µl) of tissue homogenate, 0.1 M phosphate buffer and 0.1 mM DTNB were used to determine the level of protein thiols while tissue homogenate treated with 4% trichloroacetic acid was used for non-protein thiols estimation. The level of protein and non-protein thiols were estimated according to the method of Ellman (1959).

2.11 Ferric Reducing Antioxidant Properties

Ferric reducing antioxidant property of tissue homogenate against TPTZ (2, 4, 6-Tri(2-pyridyl)-s-triazine was assessed according to the method of Benzie and Strain, (1996). Briefly, 300µl of the protein free tissue homogenates was mixed with 900 µl of TPTZ solution. The mixture was left in the dark for 10 minutes, and absorbance values were measured at 543 nm.

2.12 δ-ALA-D Activity

The activity of hepatic δ-ALA-D was assayed according to the method of Sassa (1982) by measuring the rate of product (porphobilinogen) formation except that 1M potassium phosphate buffer, pH 6.8 and 2.4 mM ð-ALA were used. The reaction was started 10 min after the addition of enzyme by adding the substrate ð-ALA. Incubations were carried out for 1 h at 37 °C. The reaction product was determined using modified Ehrlich's reagent at 555 nm with a molar absorption coefficient of 6.1×104 M−1 for the Ehrlich-PBG salt.

2.13 Assay of Purinergic Enzymes Activity

The activities of Nucleoside triphosphate diphosphohydrolase activity and 5'-nucleotidase in the liver tissue were evaluated. The NTPDase enzymatic assay was carried out as described by Battastini *et al.,* (1991). Briefly, the reaction medium contained 0.1M Tris-HCl buffer, pH 7.4, 30mM calcium chloride ($CaCl_2$), 20mM sodium azide (NaN_3), tissue homogenate in a final volume of 500µl. The reaction was initiated by the addition of ATP to a final concentration of 3.0mM. After 30 minutes of incubation, the reaction was stopped by adding 250µl of 5% trichloroacetic acid (TCA) containing 10mM mercury (II) chloride ($HgCl_2$). Released inorganic phosphate (Pi) was measured by the method of Fiske and Subbarrow (1925). The 5'-Nucleotidase activity was determined essentially by the method of Heymann *et al* (1984). The reaction medium contained 30mM magnesium sulphate ($MgSO_4$), 0.1M Tris-HCl buffer, pH 7.4, 200mM sodium azide (NaN_3), tissue homogenate. The reaction mixture was initiated by the addition of AMP to a final concentration of 3.0mM. After 30 minutes of incubation the reaction was stopped by adding 250µl of 5% trichloroacetic acid (TCA) containing 10mM mercury (II) chloride ($HgCl_2$). The released inorganic phosphate (Pi) was then measured by the method of Fiske and Subbarow (1925).

2.14 Superoxide Dismutase Enzyme Assay

Antioxidant enzymes [Superoxide dismutase was analyzed according to the method of Misra and Fridovich (1972).

2.15 Glutathione Peroxidase Enzyme Assay

Glutathione peroxidase was determined according to the method of Tappel (1978).

2.16 Glutathione Reductase Enzyme Assay

Glutathione reductase was analyzed according to the method of Goldberg and Spooner (1983)] were analyzed using Randox laboratory assay kits from Randox laboratories limited, United Kingdom.

2.17 Catalase Enzyme Assay

Catalase was determined according to the method of Aebi (1974). Sample (70µl) was

mixed with 920µl 0.1M phosphate buffer pH 7.0 containing 0.1mM EDTA (ethylenediaminetetraacetic acid). The reaction was started by adding 10 µl of H_2O_2. The decrease in H_2O_2 concentration was taken by reading the absorbance at 240nm.

2.18 Glutathione Transferase Enzyme Assay

Glutathione transferase was determined according to the method of Habig *et al* (1974) with slight modifications. The reaction was measured by observing the conjugation of 1-chloro, 2, 4-dinitrobenzene (CDNB) with reduced glutathione (GSH). One unit of enzyme will conjugate 10.0 nmol of CDNB with reduced glutathione per minute at 25°C. 100 mM CDNB dissolved in ethanol, 100 mM reduced glutathione assay buffer and 0.1M phosphate buffer adjusted to pH 6.5. For each assay, 1 ml of assay cocktail was prepared, these include 980 µl of phosphate buffer of pH 6.5,10 µl of 100 mM CDNB and 10 µl of 100 mM reduced glutathione. For each sample and a blank, 900 µl of enzyme cocktail was placed into 1.5 ml plastic cuvetes. After, which it was incubated at 30°C in spectrophotometer for 5minutes. To the blank cuvet, 100 µl PBS and 100 µl of sample were added and mix. Absorbance readings were taken at 340 nm for five minutes. One unit of GSTs activity is defined as the amount of enzyme producing 1mol of thioether per min.

2.19 Statistical Analysis

All values obtained were expressed as mean ± SEM. The data were analyzed by appropriate ANOVA followed by Duncan's multiple range tests where appropriate and this is indicated in the text of results. The differences were considered significant when $p < 0.05$.

3.0 Results

Figure 1 revealed that there was a significant reduction in the glucose level of the treated groups (STZ+DPDSe), while there was an elevated level of glucose in the diabetic group compared to the control. The level of thiobarbituric acid reactive substances (TBARS) in liver was markedly increased in diabetic group when compared with the control group as presented in figure 2. Conversely, when diabetic animals were administered with DPDSe there was a marked reduction in the level of TBARS produced. In addition, percentage of DPPH free radicals scavenged and ferric reducing antioxidants property were augmented in the treated animals as presented in Figure 2 compared to the diabetic animals. Figure 3 revealed that there was an increased in the level of protein and non-protein thiols when diabetic animals were treated with DPDSe compared to the diabetic and the control. The result obtained in Figure 4 revealed that DPDSe apparently increase activity of the enzyme in the liver of the treated group, compared to untreated diabetic group where there was a decrease in enzyme activity compared to the control. The results in Figure 5 showed there was a significant decrease in the activity of the purinergic enzymes (Nucleoside Triphosphate Diphosphohydrolase and 5^1 nucleotidase) in the treated, whereas there was an increase in the enzyme activity in untreated diabetic group as compared with the control in the liver.

Furthermore, Figure 6 revealed an elevated level in the superoxide dismutase, glutathione peroxidase, glutathione reductase activities in liver of the treated groups (STZ+ DPDSe), whereas, a decreased in the enzyme activities in the diabetic group was seen compared to the control. The results in Figure 7 showed that there was an increment in the glutathione transferase and catalase activities in liver after DPDSe treatment, whereas there was a reduction in the enzyme activities in the diabetic group compared to the control. Figure 8 revealed that there was a substantial increase in the plasma level of

ALT, ALP, total bilirubin and AST level in the diabetic groups. While there was a reduction in the plasma total bilirubin, ALT, ALP and alkaline phosphatase after DPDSe was administered to diabetic animals compared to the control. The panels in Figure 8 revealed that there was a significant ($p < 0.05$) decrease in the plasma albumin compared to the control, whereas there was a marked rise in albumin level after DPDSe administration compared to the diabetic group. Table 1.0 showed the hematological parameters of both the diabetic and treated groups. However, DPDSe modified the irregularities in the hematological parameters of diabetic animals. Figure 11 revealed that there was an elevated level of high-density lipoprotein, whereas there was a low level of low-density lipoprotein, total cholesterol, triglycerides in the treated diabetic animals compared to the STZ group. Figure 12 revealed that there was a meaningful reduction in the level of very low-density lipoprotein in the treated diabetic animals compared to STZ group and control.

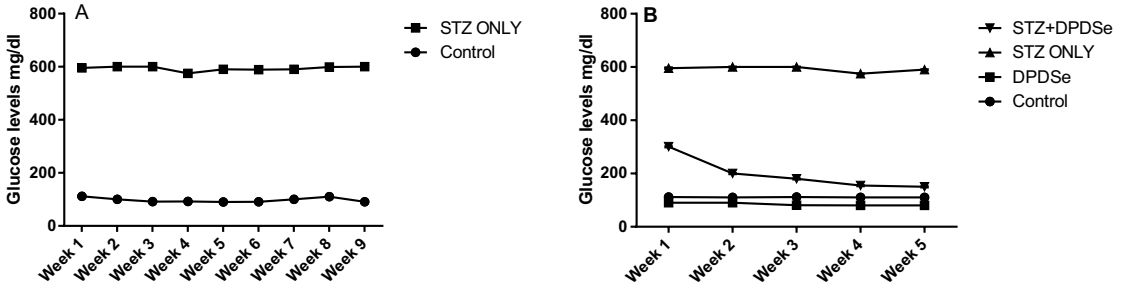

Figure 1: Effect of STZ on glucose level of diabetic animals maintained for 60 days (A) and the effect of DPDSe on glucose level of 60 days persistent diabetic animals (B). Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days.

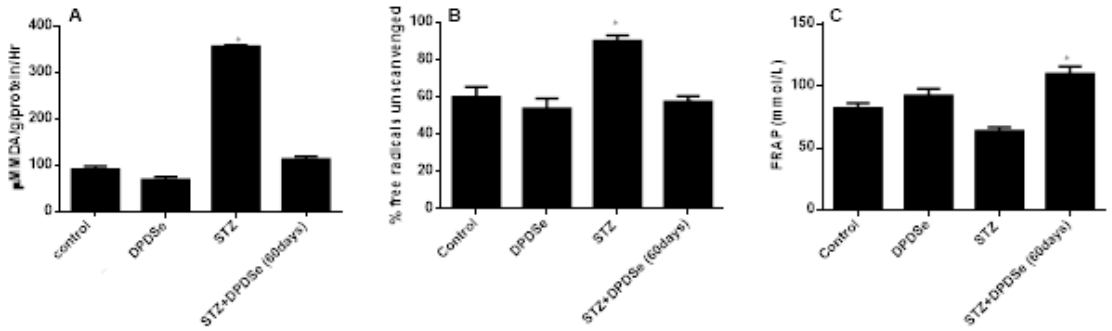

Figure 2: Effect of DPDSe on lipid peroxidation (A), DPPH (B) and FRAP (C) in liver of diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. *Represent significantly higher from control, while # represent significantly lower than control at $p < 0.05$.

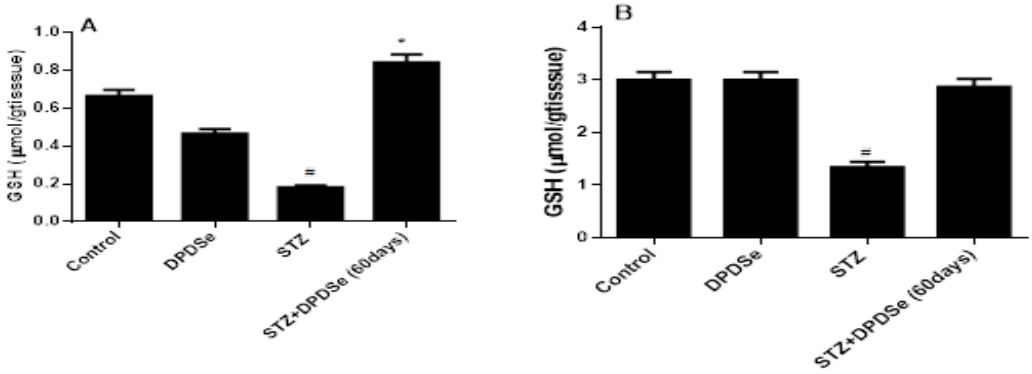

Figure 3: Effect of DPDSe on protein (A) and non- protein thiols (B) in liver of diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. * represent significantly higher from control, while # represent significantly lower than control at $p < 0.05$.

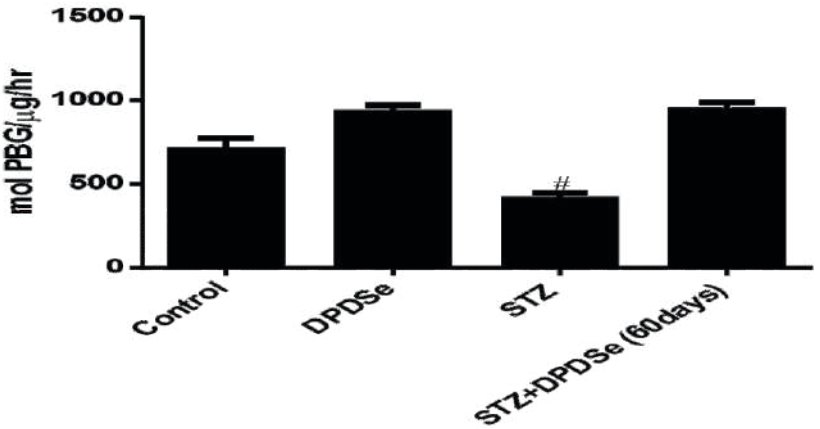

Figure 4: Effects of DPDSe on aminolevulinic acid dehydratase (δ -ALAD) activity in liver of diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. *represent significantly higher from control, while # represent significantly lower than control at $p < 0.05$.

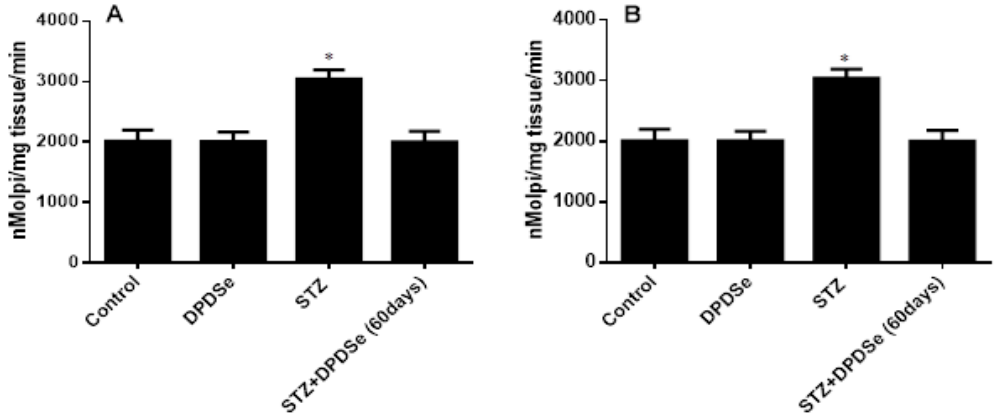

Figure 5: Effect of DPDSe on Nucleoside Triphosphate Diphosphohydrolase (A) and 5^1 nucleotidase (B) in liver of diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. * represent significantly higher than the control at $p < 0.05$.

Antioxidants Enzymes

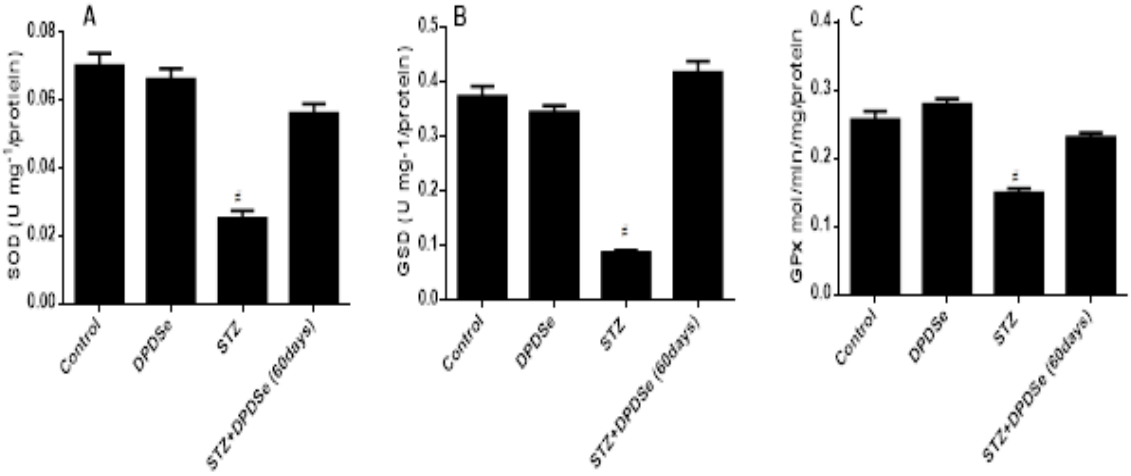

Figure 6: Effects of DPDSe on superoxide dismutase (A), glutathione reductase (B) and glutathione peroxidase (C) activities in liver of the diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. # represent significantly lower than the control at $p < 0.05$.

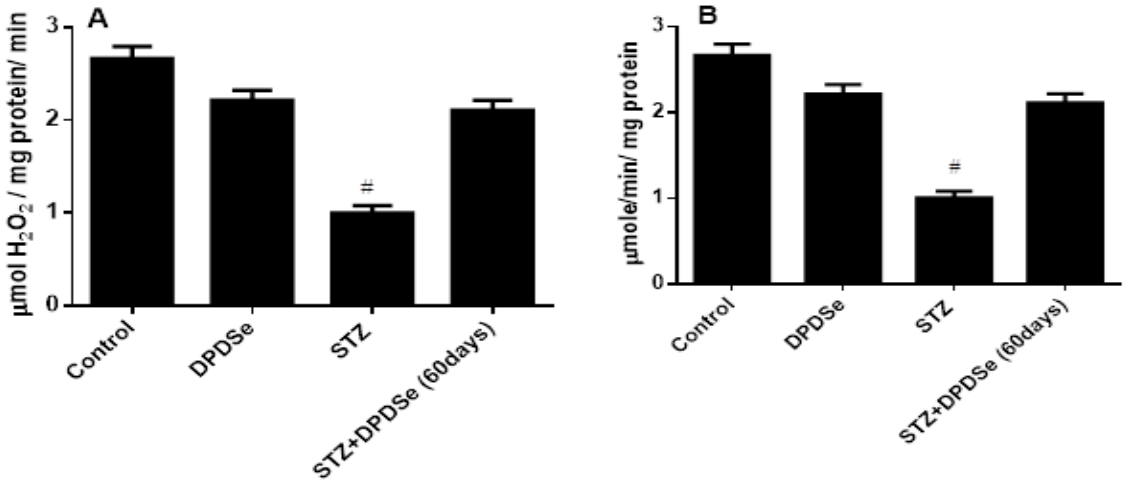

Figure 7: Effects of DPDSe on glutathione transferase (A) and catalase (B) activities in liver of the diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. # represent significantly lower than the control at $p < 0.05$

Hepatic Functions Parameters

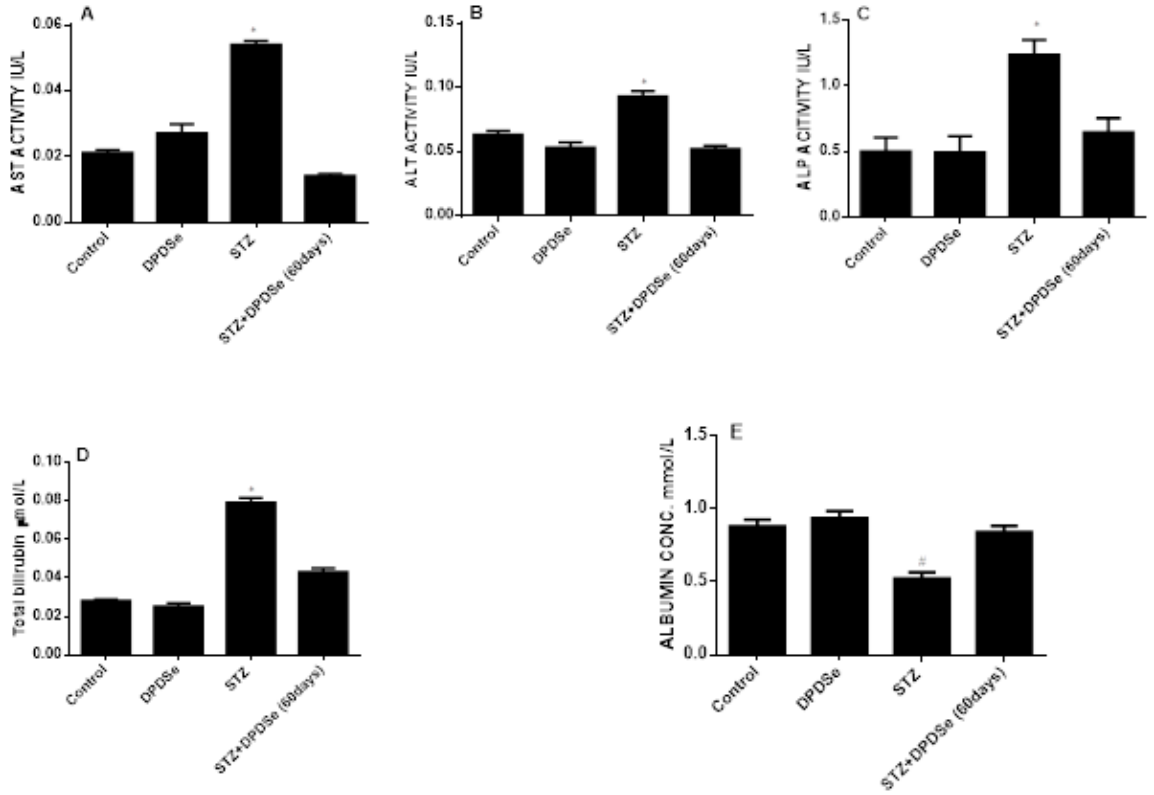

Figure 8: Effects of DPDSe on Aspartate aminotransferase (AST) (A), Plasma alanine aminotransferase (ALT) (B), alkaline phosphatase (C), total bilirubin (panel D) and albumin (panel E) of the diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. * represent significantly higher than the control, while # represent significantly lower than the control at $p < 0.05$.

Table 1.0: Heamatological Parameters

	Control	**DPDSe**	**STZ**	**STZ+DPDSe**
WBC	6.90±0.50	8.70±0.70	15.90±0.90	7±0.60 [a]
MCHC	242.00±5.00	258±5.90	214±5.20 [b]	242±5.80 [a]
Lymph%	32.00±2.00	41.00±2.20	46±2.30	40±2.20 [b]
Eosino%	5.00±0.60	6.00±0.50	4±0.50	4.50±.0.50 [b]
Gran%	63.00±3.20	56.00±3.00	50±2.00 [b]	56.50±2.10 [b]
RBC	8.12±0.50	8.13±0.50	2.97±0.70 [b]	7.79±0.50 [a]
MCH	16.60±0.60	16.20±0.50	15.8±0.50	15.0±0.50 [b]
PLT	759.00±4.00	874.00±4.70 [b]	700±5.00 [b]	738±4.00 [a]
HCT	55.70±1.50	51.20±1.00	50.70±2.00	48.20±1.00 [b]

STZ=streptozotocin, DPDSe=diphenyl diselenide, WBC=white blood cell, MCHC= mean corpuscular hemoglobin concentration, lymph%=lymphocytes percentage, eosino%=eosinophils percentage and gran%=granulocytes percentage, RBC=red blood cell count, MCH=mean corpuscular hemoglobiin, PLT=platelet count and HCT=hematocrit number of animals=8. [b] $p < 0.05$ when experimental groups were compared with the control, while [a] $p < 0.05$ when experimental groups were compared with the diabetic group.

Lipid Profile

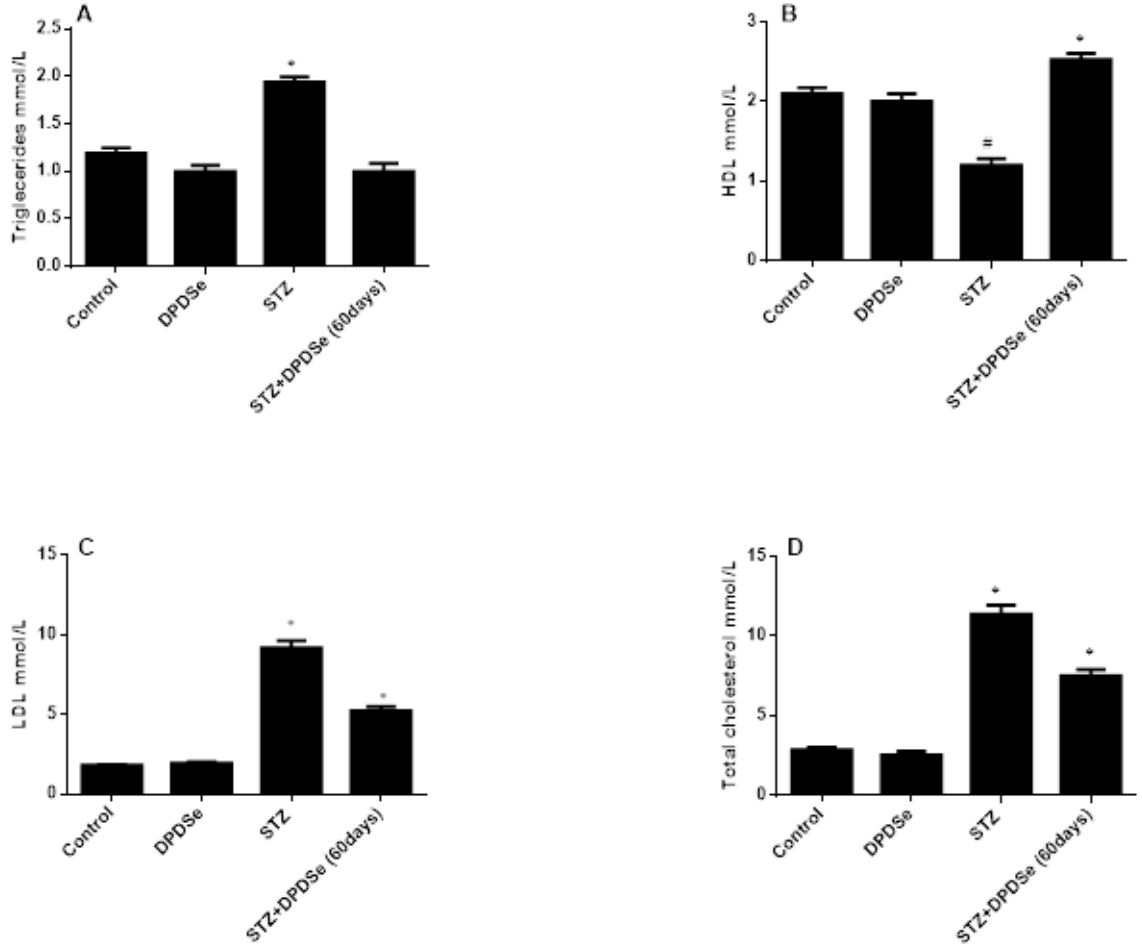

Figure 9: Effects of DPDSe on triglycerides (panel A), Plasma high density lipoprotein (panel B), low density lipoprotein (panel C) and total cholesterol (panel D) of the diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. * represent significantly higher than the control, while # represent significantly lower than the control at $p < 0.05$.

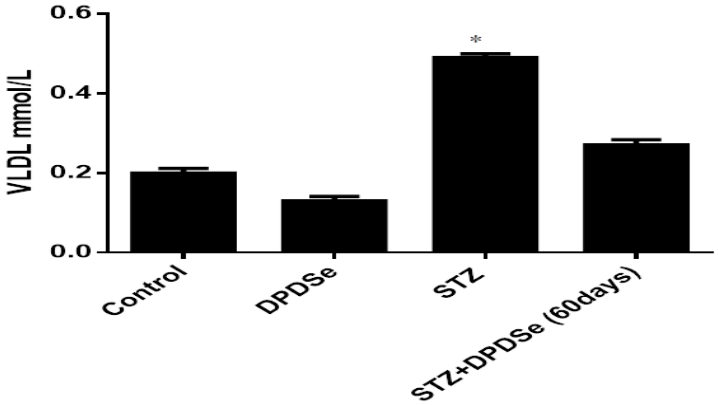

Figure 10: Effects of DPDSe on Plasma very low density lipoprotein of the diabetic rats. Data are presented as mean ± SEM for independent experiments done in duplicate carried out in different days. * Represent significantly higher than the control at $p < 0.05$

4.0 Discussion

Diabetes mellitus, a metabolic syndrome, is primarily characterized by hyperglycemia which is caused by the abnormal insulin secretion, insulin action or both (Aggarwal *et al.*, 2014). Diabetes mellitus is related with higher incidence of liver damage due to generation of free radicals through oxidation of glucose, non-enzymatic protein glycation, deterioration in antioxidant defense system, amplified activity of polyol pathway, stimulation of protein kinase C and cytokine production leads to oxidative stress which plays a crucial role in etiology of diabetes and its various complications (Giacco and Brownlee, 2010). In addition, untreated diabetes can initiate numerous alterations in cell membrane properties for instance enhanced rigidity, permeability for cations, and transmembrane potential in its absolute magnitude (Jaiswal *et al.*, 2013). The most significant diabetes mellitus complication is the extreme elevation of blood glucose level which is caused by insulin hormone synthesis and secretion impairment (Mohammed *et al.*, 2007). Notably, a previous study has showed that chronic administration of DPDSe, at low doses, reduces temporarily the elevated level of blood glucose in streptozotocin-treated rats (Kade *et al.,* 2009). The result presented in Figure 1, revealed that there was an extremely high glucose level in the STZ-diabetic rats, this result is in agreement with the findings of several authors using STZ as diabetogenic agent (Dotzert *et al.*, 2017; Cam *et al.*, 2019; Karigidi *et al.*, 2020). However, there was a decreased in the glucose level when the diabetic animals was administered 10mg/kg of diphenyl diselenide after 60 days without treatment compared to the diabetic group and control, which implies that DPDSe was able to lower the elevated blood glucose in the diabetic animals.

Hyperglycemia is linked with the generation of reactive oxygen species (ROS) and lessening of antioxidant capability, which play a crucial role in the oxidative damage mainly in the liver, kidneys, eyes, nerves, pancreas and blood vessels (Arya *et al.*, 2014). Oxidative stress plays an important role in the pathogenesis, progression of DM and in the diabetic complications. The implication of oxidative stress in insulin resistance and in the pathogenesis of DM is explained not only through the generation of reactive oxygen and nitrogen species (RONS) but also through the alteration of endogenous antioxidant enzymes. Hyperglycemia generates RONS by increasing mitochondrial production of the superoxide anion radicals (Yang *et al.*, 2011). In this study, the level of lipid peroxidation was evaluated in liver of rats exposed to streptozotocin induction and it was observed that lipid peroxidation in the diabetic rats was much higher than the control. However, after diphenyl diselenide administration, the level of TBARS was significantly ($p < 0.05$) decreased rats as presented in Figure 2.

DPPH is a stable and commercially available organic nitrogen free radical that accepts an electron or hydrogen from an antioxidant to become a stable diamagnetic molecule (Omololu *et al.*, 2011). The purple DPPH radical upon reaction with donors of hydrogen changes to yellow color (Kedare and Singh, 2011). The results (Figure 2) from this present study showed that there was a marked increase in the percentage of DPPH free radicals scavenged by diphenyl diselenide in liver. This suggests the potent ability of DPDSe to mop up free radicals in biological systems is related to its DPPH scavenging ability. However, another antioxidant capacity of diphenyl diselenide was evaluated using the ferric reducing antioxidant property assay which depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex

to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant at low pH (Benzie and Strain, 1996). The result obtained in this research are in agreement with Cakatay and Kayali (2006) as they have showed diabetes can cause a reduction in FRAP levels. The result obtained in Figure 2 revealed that the ferric reducing antioxidants properties was increased in liver when diabetic animals were treated with DPDSe.

Glutathione (GSH) has a complex role in antioxidant defense both as a direct scavenger of free radicals and as a co-substrate for peroxide detoxification by glutathione peroxidases (Ewis and Abdel-Rahman, 1995); therefore, a decrease in GSH concentration may be harmful to the cell. STZ administration has been related with a significant decrease in hepatic GSH concentrations (Karigidi and Olaiya, 2020) which may be due to decreased synthesis or increased degradation by oxidative stress (Anusooriya *et al.*, 2014). In this study, the protein and non-protein thiols in the liver were evaluated. Figure 3 revealed that there was decreased in the level of protein and non- protein thiols of the diabetic group. However, after DPDSe treatment there was an increment in the level of protein and non-protein thiols of the treated group in the liver, which implies that the DPDSe did not oxidized protein and non- protein thiols *in vivo*.

Delta–aminolevulinate dehydratase (ð-ALA-D) is a sulfhydryl-containing enzyme that is extremely sensitive to oxidizing agents and plays a fundamental role in most living aerobic organisms by participating in heme biosynthesis (Farina et al., 2003). It has been reported that the decrease in the enzyme activity can be related to pro-oxidant-mediated oxidation of its critical sulfhydryl and –SH groups which play a pivotal role in its for catalytic activities (Schmatz et al.,2012). The results obtained are in accordance with data found in human and experimental diabetes, where a significant inhibition of δ-ALA-D is described and has been related mainly to high glucose levels and overproduction of ROS (Kade *et al.*, 2009b). Moreover, a decrease in the activity d-ALA-D has been observed in the liver diabetic rats (Stefanello *et al.*, 2015). Consequently, it can be imply that the inhibition of δ-ALA-D activity in liver of diabetic rats may be as a result of either the glycation in the lysine residue from the active site of δ-ALA-D, which is involved in the formation of the Schiff basis with the first molecule of ALA (Caballero *et al.*, 1998) or by oxidation of essential reduce cysteinyl residues of the enzyme by ROS (Brito *et al.*, 2007). In this present study, it was observed that STZ caused a significant inhibition in the activity of ð-ALA-D in the liver, and that DPDSe was able to significantly relieve this inhibition as presented in Figure 4. In addition to oxidative stress, the *in vivo* effects of DPDSe on the activity of the purine-dependent signaling enzymes in diabetic rats was also evaluated. It has been reported in the research that signaling molecules, enzymes, and receptors of purinergic signaling plays an important role in diabetes (Fotino, *et al.*, 2018; Stefanello *et al.*, 2016). Furthermore, signaling events induced by extracellular adenine nucleotides, are coordinated by the action of ectonucleotidases, including nucleoside triphosphate diphosphohydrolase (NTPDase and ecto-5'nucleotidase) (Yegutkin, 2008). These enzymes constitute an organized enzymatic cascade for the regulation of nucleotide mediated signalling. Results presented in Figure 5 revealed there was significant increase in the activity of these enzymes (NTPDase and 5[1] - nucleotidase) in the untreated diabetic group. However, after DPDSe treatment, there was a dimunition in the enzyme activities.

Increased oxidative stress is one of the major causes of diabetes in experimental animals

(Liguori *et al.*, 2018) and it is linked to auto-oxidation of glucose, protein glycation, lipid peroxidation, dyslipidemia and lowered activities of enzymic and non-enzymic antioxidants (Lapena *et al.*, 2018). The present results of this research is coherent with these findings as it showed that the liver of diabetic rats had increased level of lipid peroxidation, decreased GSH concentration, altered lipid profile and reduction in the activities of antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), glutathione transferase (GST), catalase and superoxide dismutase (SOD). The activities of these key endogenous antioxidant enzymes are inhibited during the oxidative stress induced by STZ (Kumawat *et al.*, 2013). This suggests that antioxidants may play a vital role in the management of diabetes and its complications. Kaleem *et al.* (2006) suggested that decrease in activities of SOD and CAT in both liver in a diabetic state is probably due to over-production of reactive oxygen species in diabetic animals. The STZ-induced diabetes model exhibits high oxidative stress due to persistent and chronic hyperglycemia, decreasing the activity of the antioxidative defense system and thus promoting generation of free radicals (Eleazu *et al.*, 2013). The results presented in Figures (6 and 7) respectively revealed that there was a decreased in the activities of glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase and glutathione transferase in liver of diabetic groups compared to the control. The activities of SOD, catalase, GST, GR and GPx were lowered in diabetic rats, probably due to glycation of the enzyme due to hyperglycemia. However, DPDSe was able to cause a marked elevation in the levels of these enzyme activities compared to the diabetic groups.

Liver is the vital organ involving in maintaining the optimum the level of blood glucose within narrow limits. Hyperglycemia induced free radical toxicity, thereby causing severe liver damage (Hickman and Macdonald, 2007). ALT a cytoplasmic enzyme found in high amounts in the liver and an increase in ALT in the blood indicates liver damage, while AST is less specific than ALT as an indicator of liver damage (Parmar *et al.*, 2016). Increased activity of liver enzymes such as AST, ALP and ALT in diabetic animals have been reported. The increased transamines level in absence of insulin because of increased amino acid activity in hyperglycemic condition leads to ketogenesis and gluconeogenesis respectively (Atta et al., 2017). Moreover, apart from these enzymes biomarkers for hepatic injury, bilirubin and albumin are also good biomarker for hepatic damage. Bilirubin which is the end product of heme catabolism in the systemic circulation. It is formed by the action of heme oxygenase (HMOX), an enzyme that splits cyclic tetrapyrrole heme into biliverdin, carbon monoxide, and ferrous iron. In this study, the effect of diphenyl diselenide in diabetic rats on plasma [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)] activities and total bilirubin in the experimental animals were presented in Figure 8, which showed that DPDSe was able to modify the plasma level of ALT, AST, ALP and total bilirubin in diabetic animals, which can be suggested that DPDSe is an hepato-protective compound.

Albumin is mainly synthesized in the liver, representing about 25% of total hepatic protein synthesis, and thus, serum albumin is the most abundant circulating protein in the plasma accounting for approximately 60% of total plasma protein, has several important physiological and pharmacological

functions. It is involve in the transportation of metals, fatty acids, cholesterol, bile pigments and drugs. Generally, albumin is also a major antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress (Roche *et al.*, 2008). Furthermore, previous studies have shown that more than 70% of the free radical-trapping activity of serum was majorly due to the presence of serum albumin (Bourdon and Blache, 2001). The implication of this function is that hypoalbuminemic patients have reduced potential for scavenging of oxygen radical (Nicholson *et al.*, 2000). The diabetic animals showed a reduction in the serum level of albumin, which are considered as significant markers of hepatic dysfunction. However, the panel in Figure 8 revealed there was significant increase in the plasma albumin after DPDSe treatment.

Aside the previous biochemical parameters been analyzed in this *in vivo* study, hematological parameters and lipid profile were also evaluated. The link between chronic diseases and anemia is well characterized (Weiss and Goodnough, 2005) and the occurrence of anemia in diabetes mellitus has been well reported due to rise in non-enzymatic glycosylation of red blood cell membrane proteins, which shows a relationship with hyperglycemia (Oyedemi *et al.*, 2011). In this present study, the hematological parameters as presented in Table 1.0, revealed that DPDSe was capable in restoring the irregularities to normal in the hematological indices of the diabetic animals. Diabetes mellitus is associated with a large number of lipid abnormalities. Lipid profile which is altered in diabetic state is one of the significant factors in development of cardiovascular disease (Einarson *et al.*, 2018). The higher lipid [triglycerides (TG), total cholesterol (TC), low density lipoprotein (LDL), and very low density lipoprotein (VLDL)] levels seen in plasma of diabetic rats (Figures 11 and 12) in this present study is in agreement with previous research (Ganjali *et al.*,2017). The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase (Tomkin and Owens, 2017). Significant abnormalities in lipid metabolism and lipoproteins in diabetes are evident which in turn depend on the extent of insulin deficiency, insulin resistance, obesity, diet and the presence of concomitant primary and other secondary causes of hyperlipidemia. Control of hyperlipidemia is a prerequisite for the prevention of diabetic microangiopathy (retinopathy, nephropathy and neuropathy) and macroangiopathy (ischemic heart disease), cerebral vascular disease (CVD) and arteriosclerosis obliterans in diabetes (Arora *et al.*, 2002). In this present study, Figures 11 and 12 revealed that there was a significant decreased in the level of triglycerides, low density lipoprotein, very low density lipoprotein and total cholesterol in the treated diabetic animals compared to the STZ groups when DPDSe was administered to the diabetic animals. However, concomitant increased in the level of high density lipoprotein in the treated diabetic animals was detected, compared to the diabetic and control.

Conclusion

In conclusion, this present study indicates that the administration of 10mg/kg of DPDSe has hypoglycemic, hypo-lipidemic and antioxidant properties. The antioxidant and hepato-protective properties of DPDSe may be due to its selenium moiety intermediates which may be responsible for the reversal of the effect of diabetes on some of the biochemical indices in albino wistar rats. In addition, this *in vivo* study revealed that

DPDSe can be able use as a potential agent in the management of a prolonged and untreated diabetic state.

References

A. Atta, G. Mustafa, M.A. Sheikh, M. Shahid, H. Xiao . (2017). The biochemical significances of the proximate, mineral and phytochemical composition of selected vegetables from Pakistan Mat. *Sci. Pharma,* 1 (1), pp. 06-09

Aebi, H. (1984). Catalase *in vitro*. *Methods in Enzymology*; 105:121–126.

Aggarwal A, Singla SK, Tandon C. (2014). Urolithiasis: phytotherapy as an adjunct therapy. *Indian J Exp Biol*. 52:103–111.

Aguiree, F., Brown, A., Cho, N. and Dahlquist, G. (2013). IDF diabetes atlas. *International Diabetes Federation.*

Akpan, O. U, Ewa, I. D, Etim, B. E. (2013). Ocimumgratissimum alleviates derangements in serum and biliary bilirubin, cholesterol and electrolytes in streptozotocin-induced diabetic rats. *Int J Biochem Res Rev* 3: 171-895.

Allain GC, Poon LS, Chan CS, Richmond W. (1974). Quantitative determination of cholesterol using enzymatic colorimetric method. *Clin Chem*. 20:470e475.

American Diabetes Association, (2018).Classification and diagnosis of diabetes: standards of medical care in diabetes. *Diabetes Care* 41(1): S13–S27.

Anusooriya, P., Malarvizhi, D., Gopalakrishnan, V. K. and K. Devaki, (2014) "Antioxidant and Antidiabetic Effect of Aqueous Fruit Extract of *Passiflora ligularis* Juss. on Streptozotocin Induced Diabetic Rats," *International Scholarly Research Notices*, vol. 2014, Article ID 130342, 10 pages.

Arkkila, P.E., Koskinen, P.J., Kantola, I. M., Ronnemaa, T., Seppanen, E. and Viikari, J.S. (2001). Diabetic complications are associated with liver enzyme activities in people with type-1 diabetes. *Diabetes Res. Clin. Pract.,* 52(2), 113-118.

Arora, P., Jain, B., Kohli, G. K., Sehgal, M. and Goyal, R. K. (2002). Dyslipidemia in type 2 diabetes mellitus. *Indian Medical Gazette*. pp. 258–260.

Arya A, Jamil Al-Obaidi MM, Shahid N, Bin Noordin MI, Looi CY, Wongd WF, Khaing SL and Mustafa MR: (2014). Synergistic effect of quercetin and quinic acid by alleviating structural degeneration in the liver, kidney and pancreas tissues of STZ-induced diabetic rats: A mechanistic study. *Food Chem. Toxicol*. 71, 183–196.

Barbosa NBV, Rocha JBT, Wondracek DC, Perottoni J, Zeni G, Nogueira CW (2006). Diphenyl diselenide reduces temporarily hyperglycemia: Possible relationship with oxidative stress. *Chemico Biological Interactions*;163: 230–238.

Benzie, I.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of Antioxidant power: the FRAP assay. *Analytical Biochemistry*; 239: 70–76.

Bourdon, E. and Blache, D. (2001). The importance of proteins in defense against oxidation. *Antioxidants and Redox Signaling*; 3: 293–311.

Brand-Williams, W., Cuvelier, M.C. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*; 28(1):25–30.

Brito, .B., Folmer, V., Soares, J.C., Silveira, I.D., Rocha, J.B., (2007). Long-term

sucrose andglucose consumption decreases the delta-aminolevulinate dehydrataseactivity in mice, *Nutrition* 23: 818-826.

Byrne CD (2012) Non-alcoholic fatty liver disease, insulin resistance and ectopic fat: a new problem in diabetes management. *Diabetes Med*; 29: 1098-1107.

Caballero, F.A. , Gerez, E.N., Polo, C.F. , Vazquez, E.S., Batlle, A.M.C. (1998). Reducing sugars triggered aminolevulinate dehydratase inactivation: evidence of in vitroaspirin prevention, *Gen. Pharmacol*. 31:441-445.

Cakatay U, Kayali R. (2006). The evaluation of altered redox status in plasma and mitochondria of acute and chronic diabetic rats. *Clinical Biochem*;39(9):907–912

Cam, M.E, Hazar-Yavuz AN, Yildiza S, Ertas B, Adakul BA, Taskin T, Alan S, Kabasakal L. (2019). The methanolic extract of *Thymus praecox* subsp. *skorpilii* var. *skorpilii* restores glucose homeostasis, ameliorates insulin resistance and improves pancreatic β-cell function on streptozotocin/nicotinamide-induced type 2 diabetic rats *Journal Ethnopharmacol;* 231 :29–38.

Dotzert, M. S., McDonald, M. W., Murray, M. R., Noble, E. G., and Melling, C. W. J.(2017). Effect of combined exercise versus aerobic-only training on skeletalmuscle lipid metabolism in a rodent model of type 1 diabetes. *Canadian Journal of Diabetes,*42(4), 404–411.

Einarson, T. R., Acs, A., Ludwig, C., & Panton, U. H. (2018). Prevalence ofcardiovascular disease in type 2 diabetes: A systematic literature review of scientific evidence from across the world in 2007-2017.*CardiovascularDiabetology*,17(1) , 83

Eleazu CO, Eleazu KC, Chukwuma S, Essien UN: Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. *J. Diabetes Metab. Disord.* 12, 60 (2013).

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*. 82: 70-77.

Ellman, G.L., Courtney, K.D., Anders, V.J.R., Featherstone, R.M., 1961. A new rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*; 7: 88–95.

Englehardt, A., 1970. Measurement of alkaline phosphatase. *Aerztl. Labor*, 16: 42-43.

Ewis S. A. and Abdel-Rahman, M. S. (1995). "Effect of metformin on glutathione and magnesium in normal and streptozotocin-induced diabetic rats," *Journal of Applied Toxicology*, 15(5):387–390.

Ezaki, O. (1990. The insulin-like effects of sodium selenate in rat adipocytes. *Journal of Biological Chemistry*;265: 1124–1128.

Farina, M., Brandao, R., Lara, F.S., Soares, F.A.A., Souza, D.O. and Rocha, J.B.T. (2003). Mechanisms of the inhibitory effects of selenium and mercury on the activity of δ-aminolevulinate dehydratase from mouse liver, kidney and brain. *Toxicology Letter*; 139:55–66.

Fotino, C., Dal Ben, D. and Adinolfi, E. (2018). Emerging roles of purinergic signaling in diabetes. *Med. Chem. (Los Angeles)*, 14 (5): 428-438,

Ganjali, S., Dallinga-Thie, G. M., Simental-Mendía, L. E., Banach, M., Pirro, M.,

and Sahebkar, A. (2017). HDL functionality in type 1 diabetes. *Atherosclerosis*,267:99–109.

Ghosh, S. , Bhattacharyya, S., Rashid, K. and Sil, P. C. (2015) "Curcumin protects rat liver from streptozotocin-induced diabetic pathophysiology by counteracting reactive oxygen species and inhibiting the activation of p53 and MAPKs mediated stress response pathways," *Toxicology Reports,* 2:. 365–376.

Giacco F and Brownlee M (2010) Oxidative stress and diabetic complications. *Circ Res* 107: 1058-1070.

Giannini, E. G, Testa R, Savarino V (2005). Liver enzyme alteration: A guide for clinicians. *CMAJ*,172: 367-379.

Goldberg D.M. and Spooner RJ (1983) in Methods of Enzymatic Analysis (Bergmeyen, H.V. Ed.) 3rd edn. *Verlog Chemie, Deerfield Beach,* Fl.3: 258-265,

Habig, W. H., Pabst, M. J. and Jakoby, W.B. (1974). Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*; 249: 7130–7139.

Hickman, I. J. and Macdonald, G. A. (2007). Impact of diabetes on the severity of liver disease. *American Journal of Medicine*, 120: 829 - 834.

Jaiswal D, Rai PK, Mehta S, Chatterji S, Shukla S, Rai DK, Sharma G, Sharma B and Watal G. (2013). Role of drumstick leaves (*Moringaoleifera*) in regulation of diabetes-induced oxidative stress. *Asian PacJ Trop Med*, 6: 426-432.

Jendrassik L and Grof P. (1938). In vitro determination of total and direct bilirubin. *Biochemica.*; 297:81.

Kade, I.J., Borges, V.C., Savegnago L., Ibukun, E.O., Zeni, G., Nogueira, C.W. and Rocha, J.B.T. (2009b). Effect of oral administration of diphenyl diselenide on antioxidant status, and activity of delta aminolevulinic acid dehydratase and isoforms of lactate dehydrogenase, in streptozotocin-induced diabetic rats. *Cell Biology Toxicology,* 25:415–424.

Kade, I.J., Nogueira, C.W. and Rocha, J.B.T. (2009a). Diphenyl diselenide and streptozotocin did not alter cerebral glutamatergic and cholinergic systems but modulate antioxidant status and sodium pump in diabetic rats, *Brain Research*, 1284: 202–211.

Kaleem, M., Asif, M., Ahmed, Q. U. and Bano, B. (2006). Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. *Singapore Medical Journal*;47:670–675.

Karigidi K. O., Akintimehin E. S., Omoboyowa D. A., Adetuyi F. O., Olaiya C. O. (2020). Effect of *Curculigo pilosa* supplemented diet on blood sugar, lipid metabolism, hepatic oxidative stress and carbohydrate metabolism enzymes in streptozotocin-induced diabetic rats. *Journal of Diabetes and Metabolic Disorders*, 19: 1173-1184

Karigidi K.O. and Olaiya C.O. (2020). Antidiabetic activity of corn steep liquor extract of *Curculigo pilosa* and its solvent fractions in streptozotocin-induced diabetic rats, *Journal of Traditional and Complementary Medicine* 10: 555-564

Kedare, S.B. and Singh, R.P., (2011). Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* 48 (4), 412–422.

Kumawat M, Sharma TK and Singh, I. (2013). Antioxidant enzymes and lipid peroxidation in type 2 diabetes mellitus

patients with and without nephropathy. *North American Journal of Medical Sciences*;5(3):213–219.

Lowry, O.H., Rosenbrough,, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with Folin-phenol reagent. *Journal of Biological Chemistry,* 193:265–275.

Mahmoud, A. M., Ahmed, O. M., Ashour, M. B. and Abdel-Moneim, A. (2015).“*In vivo* and *in vitro* anti-diabetic effects of citrusfla-vonoids; a study on the mechanism of action,”International Journal of Diabetes in Developing Countries, vol. 35, no. 3,pp. 250–263.

Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem.; 247:3170-3175.

Mugesh, G., Du Mont, W.W. and Sies, H. (2001a). Chemistry of biologically important synthetic organoselenium compounds. *Chemical Review;* 101: 2125–2179.

N.Stefanello,L.Pereira,R.Schmatz,S.Passamonti,G.Facco,D.Zanini,F.H.Abdalla,J.Vieira,V.Castro,V.Oliveira,M.Rocha,J.Kasten,V.Morsch,M.R.Schetinger,(2015).Chlorogenic acid caffeine and coffee reverse damages in liver, kidney and pancreas parameters of diabetic rats, *J.Diab.HealthPhot.*108:214–228.

Newsome PN, Cramb R, Davison SM, Dillon JF, Foulerton M. (2018). Guidelines on the management of abnormal liver blood tests. *Gut* 67: 6-19.

Nicholson, J. P., Wolmarans, M. R. and Park, G. R. (2000). The role of albumin in critical illness. *British Journal of Anaesthesia*; 85: 599–610.

Nogueira, C.W., Zeni, .G. and Rocha, J.B.T. (2004). Organoselenium and organotellurium compounds: toxicology and pharmacology, *Chemical Review;* 104:6255–6286.

Oche, O., Sani, I., Chiaka, N.G., Samuel, N.U. & Samuel, A. (2014) Pancreatic islet regeneration and some liver biochemical parameters of leaf extracts of Vitex doniana in normal and streptozotocin-induced diabetic albino rats. *Asian Pac J Trop Biomed;* 4(2): 124-130.

Ohkawa, H., Ohishi N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry,* 95:351–358.

Okoduwa S.I.R., Umar I.A., Ibrahim S., Bello F., Habila N. (2015). Age-dependent alteration of antioxidant defense system in hypertensive and type-2 diabetic patients. *J. Diabetes Metab. Disord.*;14: 32.

Oyedemi, S.O., Yakubu, M. T. and Afolayan, A. J. (2011). Antidiabetic activities of aqueous leaves extract of Leonotis leonurus in streptozotocin induced diabetic rats. *Journal of Medicinal Plant Research*; 5: 119–125.

Parmar, K. S., Singh, G. K., Gupta, G. P., Pathak, T., and Nayak, S. (2016). Evaluation of De Ritis ratio in liver-associated diseases. *International Journal of Medical Science and Public Health*, 5(9), 1782–1788.

Pia V. R. Bingbing W., Yixian, L. and Weiping, H. (2016). Pancreatic regulation of glucose homeostasis. *Exp Mol Med;* 48(3):219.

Reitman, S. and Frankel, S. A. (1957). Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Pathol.;*28:56e63.