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Effect of Diphenyl Diselenide on Blood Glucose Level and Hepatic Indices in Alloxan Induced Diabetic Wistar Rats

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Abstract

Diphenyl diselenide (DPDS) is an organoselenium compound that exhibits multi-pharmacological activities owing to its glutathione peroxidase (Gpx) mimicry. Herein, the effect of DPDS on blood sugar and hepatic indices in alloxan-induced diabetic wistar rats was investigated. Twenty albino rats were distributed into four groups: A was the normal control and recieved distilled water only, B was the negative control and recieved alloxan, C was the tested group and received alloxan with DPDS treatment, and D was the positive control and recieved alloxan with glibenclamide (standard andtidiabetic drug). Rats were induced with alloxan, and treated for 14 days. Animals weight and blood glucose level were measured, and on the last day, animals were sacrificed and blood was collected for the liver function analysis. Result revealed that alloxan administration led to a marked ($p<0.05$) fall in weights, but weight loss was reversed upon DPDS treatment. In addition, there was a profound ($p<0.05$) increase in blood glucose level of alloxan-treated rats. Nonetheless, treatment with DPDS exerted marked ($P< 0.05$) decrease in blood glucose level across day 7 and 14. Furthermore, the activities of serum hepatic enzymes, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and γ-glutamyl transferase (GGT) were markedly ($p<0.05$)

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increased with concomitant reduction in the levels of total protein, albumin and globulin, but be that as it may, treatment with DPDS restored normalcy to the hepatic abnormalities and this effect was comparable to the standard drug. DPDS could therefore be suggested for future development of novel drug for diabetes and diabetes related complications.

Keywords: Diphenyl diselenide, Alloxan, Diabetes, Hepatic indices, Blood sugar, Albino rats

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in both insulin secretion and/or insulin action. Hyperglycemia, the primary clinical manifestation of diabetes mellitus, is associated with non-enzymatic glycation of proteins and free radicals' generation (Stitt et al., 2008). These processes can cause protein cross-linking and damage in other biomolecules such as enzymes, lipids and DNA; contributing to the overall complications of diabetes mellitus (Maiese et al 2008).

Organoselenium compounds are a new class of emerging potent antioxidants (Arteel and Sies, 2001). Basically, their rational and synthesis was aimed at mimicking the native glutathione peroxidase enzyme in their reduction of hydroperoxidase at the expense of the ubiquitous antioxidant, glutathiones. The pharmacological effect of organoseleniums strictly depends on their GPx mimic. The Gpx is an important selenoprotein, an enzyme that catalyzes the reduction of a variety of hydroperoxides (ROOH and H2O2) using GSH as a reductant. The selenol group (-SeH) of a reduced selenocysteine molecule is oxidized by the hydroperoxides to generate a selenenic acid. The tripeptide GSH then reacts with the selenenic acid, resulting in the corresponding water and selenenyl sulfide. A second molecule of GSH attacks the sulfur in the latter species, producing disulfide and regenerating the selenol to complete the catalytic cycle (Flohe, 1989). Since glutathione peroxidase catalyzes the reduction of a wide variety of hydroperoxides and together with GSH constitutes a powerful cellular defense system against so called oxidative stress, considerable efforts have been made to find compounds capable of imitating the enzymatic properties of glutathione peroxidase. This becomes crucial considering some shortcomings of the administration of native GPx with therapeutic objectives, including instability and poor availability. Consequently, the high molecular weight of native GPx limits its

therapeutic application. Therefore, considerable efforts have been made to find organoselenium compounds capable of imitating the enzymatic properties of glutathione peroxidase and free of these aforementioned shortcomings (Kade et al.,2012). Several research groups have developed a number of small molecules, including substituted diselenides, N-Se heterocycles, and other type of organoselenium compounds with glutathione peroxidase-like activity (Bhabak and Mugesh 2010). Synthetic organoselenium compounds with glutathione peroxidase mimic such as ebselen and diphenyl diselenide, two compounds that have GPx-like activity (Wilson *et al.,* 1989) and have been found to exert antioxidant and protective effects in different in vitro and in vivo models of toxicity (Nogueira and Rocha, 2010; 2011).

Diphenyl diselenide (DPDS) is the simplest of the diaryl diselenides and has glutathione peroxidase-like activity (Xing et al., 2022), exhibits antioxidant, hepatoprotective (Meotti et al., 2003), antiulcer and anti-inflammatory properties (Nogueira *et al.,* 2008; Maciel *et al.,* 2000). DPDS possesses antioxidant activity, confirmed in several in vitro and in vivo systems, and thus has a protective effect against hepatic (Banda et al., 2018), renal and gastric injuries, in addition to its neuroprotective activity (Rosa et al., 2007).

The pharmacological properties of organoselenium-diphenyl diselenide (DPDS) has been linked majorly to their antioxidant actions that greatly rely on its mimicry of GPx (Brenneisen et al., 2005). However, there is dearth of experimentally guided information on the effect of DPDS in diabetic condition. Hence this work is designed to investigate the effect of DPDS on blood glucose level and hepatic indices in alloxan-induced diabetic *Wistar* rats.

MATERIALS AND METHODS

Chemicals and reagents

Alloxan, Diphenyl diselenide, Glibenclamide were gotten from Sigma Aldrich, Steinheim, Germany. All other chemicals which are of analytical grade were obtained from standard commercial suppliers.

Experimental Animals

Twenty (20) male albino rats were obtained from the Animal House of the Department of Biochemistry, Federal University, Wukari, Nigeria. The rats were weighing between 100 to

150g and were randomly distributed into five membered four groups, housed in cages with a 12 hours light–dark cycle.

Induction of Diabetes

Alloxan was used to induce diabetes in rats in three of the groups. 150mg/kg body weight of alloxan was administered intraperitoneally to the animals. After 3 days of alloxan treatment blood samples were collected from animals through the tail vein and blood glucose level was estimated using Accu-check Active® (Roche Diagnostics) Glucometer. Rats with blood glucose levels above 250 mg/dl were considered diabetic and selected for the study. Blood samples was collected from three rats from each group after treatment with standard drug and diphenyl diselenide and was used to carry out liver function test.

Experimental Design

20 rats were grouped according to body weight into five membered four groups.

Group A: Normal control animals (Non alloxan induced) were fed with a standard diet and given water for 14 days.

Group B: Negative control animals induced with 150 mg/kg body weight of alloxan intra peritoneally without treatment.

Group C: Positive control animals induced with 150mg/kg body weight of alloxan intra peritoneally and treated with 5 mg/kg body weight standard drug (Glibenclamide) intra peritoneally for 14 days.

Group D: Diabetic treated animals were induced with 150 mg/kg body weight of alloxan intra peritoneally and were treated with 10mg/kg body weight DPDS intra peritoneally for 14 days.

Determination of Blood Glucose Level

The rats were fed daily for 14 days the fasting blood glucose level of the rats was determined using accu-check active glucometer. The tail vein was punctured and blood from the tail was made to drop on the strip which was then inserted in the glucometer to obtain blood glucose concentration in mg/dl for each rat in all the groups at the following intervals of days: 0, 3, 7 and 14 while the feed was withdrawn from the rats a night before checking the blood glucose to obtain fasting blood glucose.

Estimation of Body Weight

The weight of all the rats were taken prior to induction of diabetes using an electronic weighing balance. The weight of all the rats were also taken after diabetes induction and subsequently at seven days' interval.

Collection of Blood Samples for Analysis

After fourteen (14) days of treatment, rats were fastened for twelve (12) hours after the last treatment and then anesthetized using chloroform. Whole blood was collected from the heart via cardiac puncture using sterile syringes and needles. The blood samples were collected into plain bottles and then corked immediately. The serum was separated by the centrifugation of the blood at 3000rpm/10 minutes and was used for the estimation of liver function test.

Determination of Hepatic Parameters

Serum was separated from the clotted blood by centrifugation at 3000 rpm for 10 minutes. Serum samples were immediately subjected to biochemical analysis of liver function: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Gamma-glutamyl transferase (GGT), Total protein (TP), Albumin (ALB) and Globulin using uv/visible Spectrophotometer. The activities of the assays were determined by the procedure described in the Randox reagent kit.

Statistical Analysis

The mean ± SD of all values was calculated and changes observed between the treatment group and the control was subjected to analysis of variance (ANOVA) using SPSS version 23. Difference between groups was considered significant at $p<0.05$.

RESULTS

Effect of DPDS on Body weight in Alloxan-induced Diabetic Rats

Figure 1 reveals that alloxan administration led to a marked ($p< 0.05$) fall in body weight in alloxan induced untreated diabetic rats across 7^{th} and 14^{th} day compared to day 0. But this anomaly was reversed upon treatment with DPDS resulting in significant increase in weight observed across 7^{th} and 14^{th} day of treatment ($p < 0.05$).

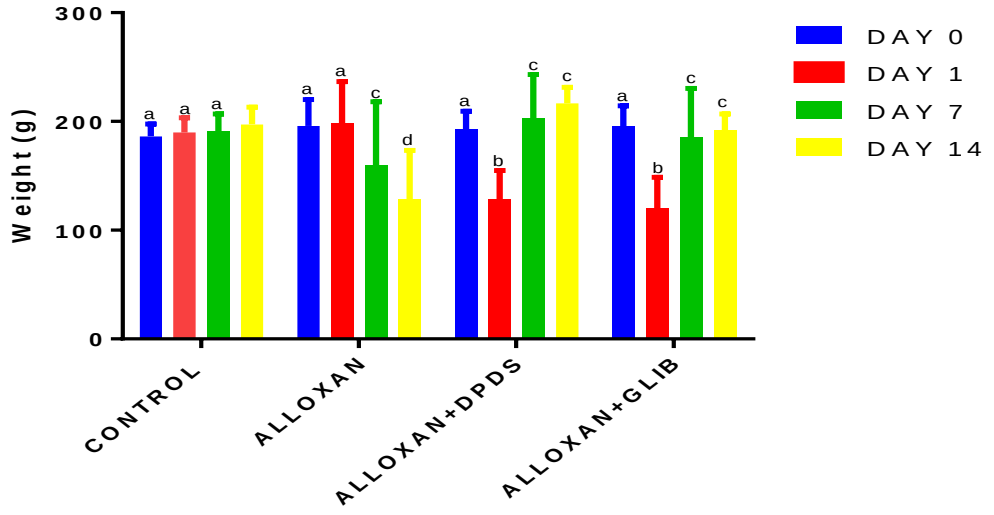

Figure 1: Effect of DPDS on Body weight in Alloxan-induced Diabetic Rats. Each value represents mean ± SD, n=3, mean values with different superscripts are significantly different (P<0.05) among days.

Effect of DPDS on Blood Glucose Levels in Alloxan-induced Diabetic Rats

There was profound (p>0.05) increase in blood glucose level of alloxan induced untreated rats and DPDS treated rats on the first day compared to the normal control rats (Figure 2). However, treatment with DPDS exert significant (p<0.05) decrease in glucose level across day 7 and day 14 after diabetes induction.

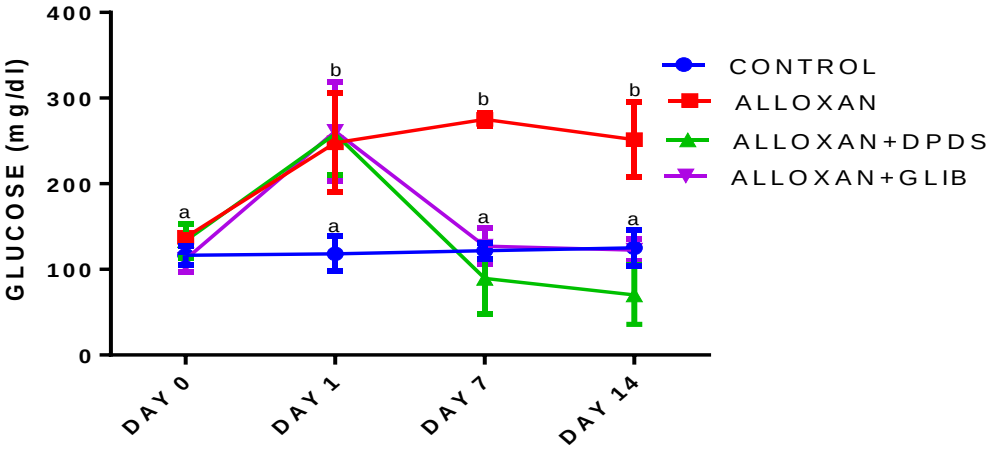

Figure 2: Effect of DPDS on Blood Glucose Levels in Alloxan-induced Diabetic Rats. Each value represents mean ± SD, n=3, mean values with different superscripts are significantly different (P<0.05) among days.

Effect of DPDS on Liver enzymes in Alloxan-induced Diabetic Rats

Significant ($p<0.05$) increase in activities of ALP, AST, GGT, ALT in the liver of untreated alloxan induced diabetic rats was observed, when compared with that of the normal control rats (Figure 3). Furthermore, treatment with DPDS significantly mitigated and in some cases reversed the trends of these alloxan treatment related changes in the biochemical parameters towards the control values.

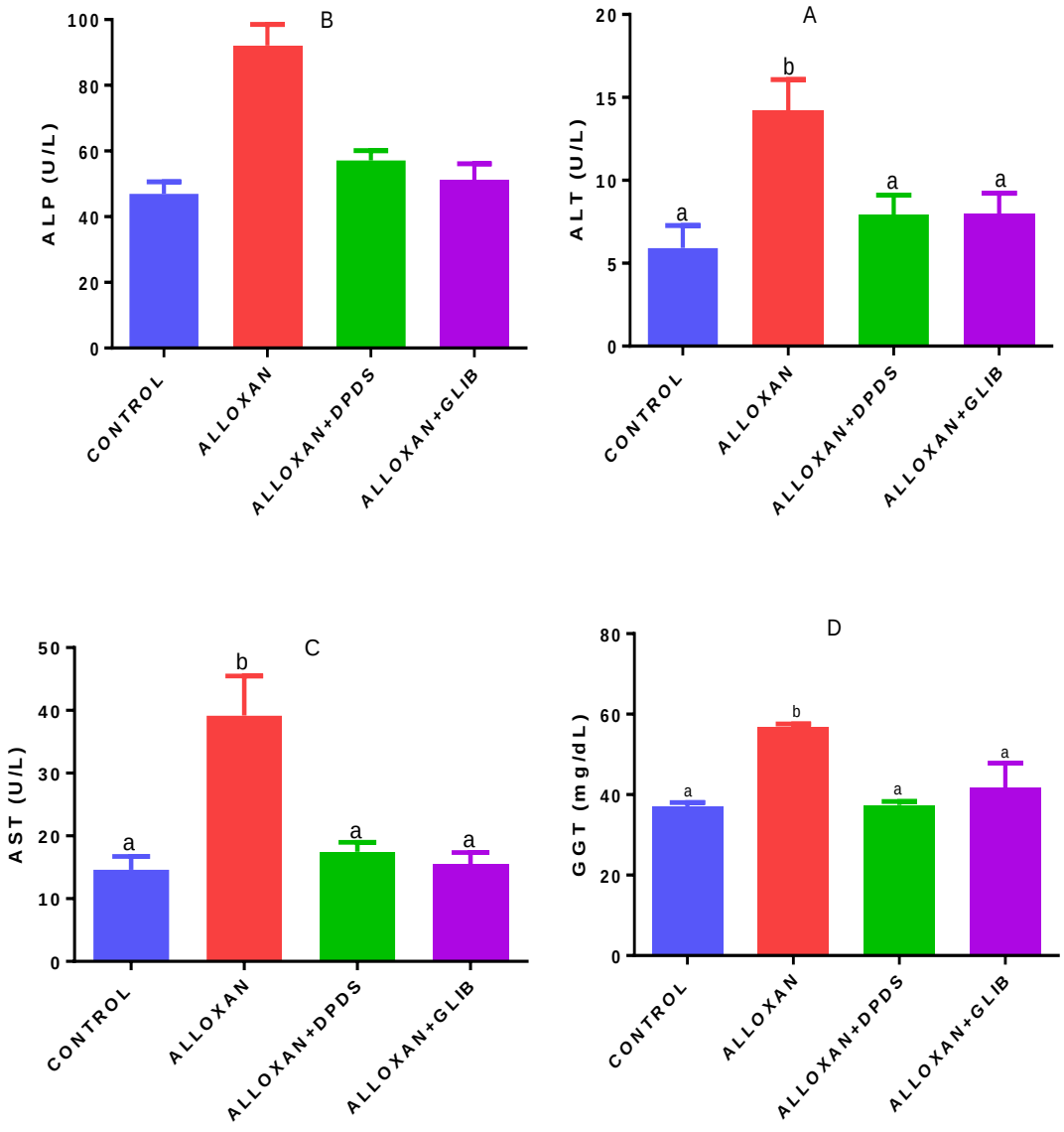

Figure 3: Effect of DPDS on Liver enzymes in Alloxan-induced Diabetic Rats. Each value represents mean ± SD, n=3, mean values with different superscripts are significantly different ($P<0.05$).

The Effect of DPDS on serum total protein (panel E), albumin (panel F) and globulin (panel G) in Alloxan-induced Diabetic Rats.

Figure 4 reveals that there was concomitant reduction ($p<0.05$) in the total levels of protein (panel E), albumin (panel F) and globulin (panel G) in the serum of untreated alloxan induced diabetic rats. However, on treatment with DPDS, elevated levels of total protein (panel E), albumin (panel F) and globulin (panel G) in the serum of DPDS treated alloxan induced diabetic rats were observed and this effect was comparable to the standard drug.

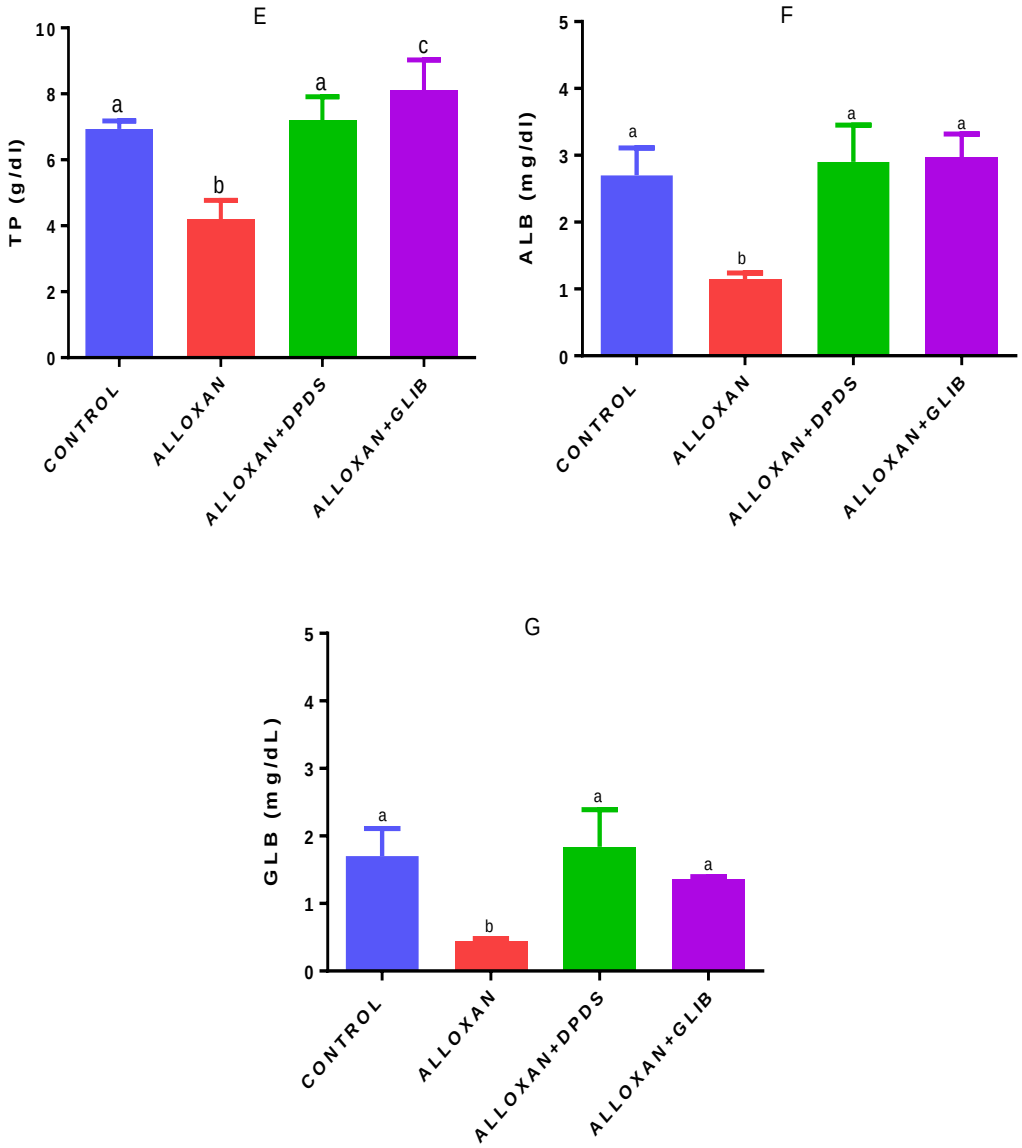

Figure 4: The Effect of DPDS on serum total protein (panel E), albumin (panel F) and globulin (panel G) in Alloxan-induced Diabetic Rats. Each value represents mean ± SD, n=3, mean values with different superscripts are significantly different ($P<0.05$).

DISCUSSION

Over the past few decades, many animal models for studying diabetes mellitus have been created, allowing researchers to assess the effectiveness of chemical substances, including plant extracts and their derivatives, as anti-diabetic agent(s). Chemicals like alloxan and streptozotocin, as well as surgical and genetic alterations, can all be used to cause diabetes in animals (Mannan *et al.*, 2014). Alloxan is one of the most effective and often used diabetogenic substances. Alloxan is a urea derivative that selectively necroses the pancreatic beta-cells, causing Type 1 diabetes in experimental mice (Maroyi, 2011). In addition to having a necrotic effect on the pancreas, it also has a negative impact on the other organs of the experimental animals, altering blood glucose levels and hepatic markers of organ function, which ultimately results in lowering these organs' normal function indices. According to studies, the pharmacology of synthetic organoselenium compounds suggests that they can be employed as immunomodulators, neuroprotectors, enzyme inhibitors, antioxidants, and anti-tumor and anti-infectious agents. Diphenyl diselenide has antioxidant activity, which has been demonstrated in numerous in vitro and in vivo systems. As a result, in addition to its neuroprotective effects, this compound also protects against hepatic, renal, and gastric injuries (Rosa *et al.*, 2007).

The impact of DPDS on body weight in alloxan-induced diabetic rats is depicted in Figure 1. It demonstrates that alloxan injection caused a significant ($p > 0.05$) decrease in body weight in alloxan-induced diabetic rats that were left untreated on days 7 and 14 compared to day 0. However, therapy with DPDS rectified this abnormality, leading to a substantial gain in weight that was seen between the 7th and 14th day of treatment ($p > 0.05$). It's possible that alloxan has specific cytotoxic effects on pancreatic beta cells, killing them off, which causes a significant (p0.05) decrease in the body weights of rats. However, injection of DPDS caused rats' body weight to grow and their blood glucose levels to drop in a significant ($p > 0.05$) manner. Additionally, the membrane lipids found in subcellular organelles are extremely vulnerable to damage from free radicals. When free radicals interact with polyunsaturated lipids, oxidative stress results, a highly destructive chain reaction that has both direct and indirect negative effects (Ale *et al.*, 2020).

Figure 2 depicts the effect of DPDS on blood glucose levels in rats with diabetes brought on by alloxan. On the first day, alloxan-induced rats treated with DPDS and untreated rats showed a significant ($p>0.05$) rise in blood glucose levels compared to the normal control

rats. However, administration of DPDS caused a significant ($p > 0.05$) drop in glucose levels on days 7 and 14 following the onset of diabetes. This result demonstrates that DPDS provided an antioxidant protective effect against oxidative assaults brought on by the alloxan, lowering glucose levels following the induction of diabetes.

Additionally, the effect of DPDS on liver enzymes in rats with diabetes caused by alloxan was assessed. When compared to the normal control rats, a significant ($p > 0.05$) rise in the liver activity of ALP, AST, GGT, and ALT was seen in the untreated alloxan-induced diabetic rats (Figure 3). The tendency of these alloxan treatment related alterations in the biochemical parameters towards the control values were also greatly attenuated and, in some cases, reverted by treatment with DPDS. The examination and diagnosis of diseases, as well as to some extent the toxicity of chemical compounds (Afolayan *et al.*, 2009), depend greatly on the evaluation of the activity of numerous enzymes in tissues and body fluids. Since the enzymes are cytosolic in origin and any damage to the plasma membrane will consequently cause their leakage to the external milieu, in this case the serum, an increase in the activities of the liver enzymes ALP, AST, GGT, and ALT suggest permeability changes as a result of damage by oxidative products of alloxan in figure 3. Additionally, a rise in ALP activity can be a sign of membrane integrity loss and cellular membrane peroxidation. As ALT participates in gluconeogenesis and insulin inhibits its transcription, it has been suggested that elevated activity is indicative of impaired insulin signaling rather than hepatocyte damage. The administration of the DPDS counteracted this rise in liver enzymes, reducing the compound's toxicity from the alloxan and resulting in a fall in liver enzyme activity. The hepatoprotective and antioxidant properties of DPDS may be responsible for the decrease.

Figure 4 depicts the impact of DPDS on blood total protein (panel E), albumin (panel F), and globulin (panel G). In the serum of untreated alloxan-induced diabetic rats, there was a concurrent decrease ($p0.05$) in the total amounts of protein (panel E), albumin (panel F), and globulin (panel G). However, after receiving DPDS treatment, alloxan-induced diabetic rats were shown to have increased serum levels of total protein (panel E), albumin (panel F), and globulin (panel G), with this impact being comparable to that of the usual medication.

The protein components of the cell, albumin, total protein, and globulin, which make up the liver's functional capacity, can also be utilized to measure it (Galet *et al.*, 2001). A useful

tool for determining the extent of liver damage is serum total protein, which serves as a marker of the liver's synthetic function. An increase in the rate of amino acid conversion to glucose and a decrease in ribosomal protein synthesis as a result of insulin deprivation may be the causes of the decrease in total protein levels in alloxan-induced diabetic rats (Imo *et al.,* 2014). Total protein, globulin, and albumin levels were decreased by alloxan in this study (figure 4), which may be related to the liver's decreased ability to synthesize proteins due to hepatocellular injury, increased catabolism, or excessive excretion from the body that may exceed the rate of synthesis (Lakmichi *et al*., 2011). The amoleriative action of DPDS on the toxic alterations caused by alloxan in the animals may be the cause of the higher levels of total protein, albumin, and globulin in the blood of treated alloxan-induced diabetic rats.

CONCLUSION

The results of this investigation showed that DPDS dramatically reduced alloxan-induced liver abnormalities and blood sugar in diabetic rats, and that this impact was comparable to that of the usual medication. Therefore, it is worthwhile to suggest that DPDS might be a good candidate for the design of an antidiabetic drugs in the future.

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