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Research Article

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Antidiabetic and Antioxidant Activity of *Zanthoxylum armatum* DC. on Type 2 Diabetic Rats

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Abstract: *Zanthoxylum armatum* DC, a medicinal plant species in India, is an exceptionally nutritious vegetable with a variety of potential uses in treating rheumatism, venomous bites, and microbial infections. In the present study, we aimed to investigated the antidiabetic and antioxidant effects of aqueous extracts of *Z. armatum* Stem Bark (*Za*-Aq) in High Fat Diet (HFD) low dose streptozotocin (STZ)-induced type 2 diabetic wistar albino rats and the objectives the were achieved through examine of blood glucose, Oral Glucose Tolerance Test (OGTT), Insulin Tolerance Test (ITT), and the levels of lipid peroxides and antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase and reduced glutathione in the liver tissues of control and experimental groups. Diabetic rats were treated with 200, 400 and 800 mg/kg BW *Za*-Aq for 45 days and the antidiabetic effects of the extract were evaluated by measuring changes in biochemical parameters in the serum and liver tissue. Oral administration of *Za*-Aq to diabetic rats for 45 days significantly reduced the levels of blood glucose, OGTT, ITT and lipid peroxidation, but increased the activities of antioxidant enzymes, like superoxide dismutase, reduced glutathione and glutathione peroxidase. The progression of diabetes was significantly reduced after *Za*-Aq treatment. In conclusion, *Za*-Aq exerts protective effects against HFD-STZ-induced diabetes. The *Za*-Aq supplementation is useful in controlling the blood glucose level and is beneficial in preventing diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats; therefore, it could be useful for prevention or early treatment of diabetes mellitus active constituents may be isolated from the extract for evaluation in future clinical studies. To best of our knowledge there has been no previous comprehensive study to evaluate antidiabetic activity of stem bark from *Z. armatum* DC and present study adds the protective effect of *Zanthoxylum armatum* DC stem bark as effective antidiabetic and antioxidant agent.

Keywords: *Zanthoxylum armatum* DC., Antidiabetic, Antioxidants, Metformin, High Fat Diet and STZ

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1. INTRODUCTION

Diabetes mellitus is a chronic heterogenic metabolic disorder characterized by hyperglycemia and glycosuria due to multiple aetiologies with profound consequences. It is a major socioeconomic challenge to the people of both developing and developed countries. (WHO). Diabetes and its associated complications are the major health problem in this modern world. Both types I and type II comprise the abnormalities of insulin action, such as insensitivity and resistance.[1] Diabetes mellitus is associated with long-term complications such as retinopathy, nephropathy, neuropathy, and angiopathy.[2] Furthermore, DM is considered a major risk factor for cardiovascular disorders, namely, ischaemic heart disease, cerebral stroke, and peripheral artery disease, leading to increased mortality of patients with diabetes. [3] Type 2 diabetes (T2D) is the most common and clinically important metabolic disorder, which has become a global pandemic in recent decades and a major healthcare burden worldwide.[4] In 2013, there were an estimated 382 million patients with diabetes globally. Concerningly, the T2D incidence continues to increase. It is projected that there will be >590 million patients diagnosed with this condition by 2035.[5] Changing lifestyle and food habits lead to several diseases, especially obesity, hypertension, diabetes, cardiovascular diseases, etc.; an extreme adipose tissue expansion due to an increase in nutrient intake and insufficient energetic expenditure is considered as Obesity.[6] This could cause chronic low-grade systemic, and local inflammation that leads to the emergence of insulin resistance-linked diabetes mellitus, even though the mechanism is unclear.[7] In addition, insulin resistance and hyperinsulinemia can contribute to the development of obesity. Reactive oxygen species (ROS) are atoms or molecules having unpaired electrons, highly unstable, and readily react with nearby atoms and molecules, resulting in tissue damage and also cause of many diseases such as diabetes, cardiovascular diseases, ageing, and cancer.[8] Diabetes mellitus conditions also increase the production of reactive oxygen species (ROS), which are responsible for tissue damage by oxidation of glucose and nonenzymatic protein glycosylation. The lack of insulin secretion and insulin resistance is mainly due to the oxidative damage of beta cells of islets of langerhans caused by obesity. High levels of reactive oxygen species (ROS) are intricately linked to obesity and associated pathologies, notably insulin resistance and type 2 diabetes.[9] Under diabetic conditions, various tissues produce ROS, and several sources of ROS in cells are non enzymatic glycosylation reaction, the electron transport chain in mitochondria, and membrane-bound NADPH oxidase.[10] In diabetic animals, glycation reaction is observed in various tissues and organs, and various kinds of glycated proteins such as glycosylated haemoglobin, albumin, and lens crystalline are produced in a nonenzymatic manner through the glycation reaction. Mitigation of mitochondrial ROS production and oxidative stress may be a possible therapeutic target in type 2 diabetes.[11]Therefore, several hypoglycaemic agents have been used to treat diabetes mellitus but show serious adverse side effects, such as liver problems, lactic acidosis, and diarrhoea.[12]There are several oral antidiabetic drugs available in the market with varying mechanisms of action for insulin stimulation. There is a remarkable revival of interest in using natural sources in treating diabetes due to the side effects of prolonged consumption of therapeutic drugs. Meanwhile, according to Shazhni et al. (2018), secondary metabolites from plants such as phenolic, alkaloids, and glycosides are the ones that are implicated as having antidiabetic effects.[13]*Zanthoxylum armatum* DC. selected for the present study belongs to the family Rutaceae, commonly known as prickly ash, timur, etc.; it is used to treat toothache, fever, and cholera. Its seeds are used as spices, and the essential oil is extracted. It is reported for its antibacterial, antifertility, antiseptic, *in vitro* disinfectant and antidiabetic activity.[14]The leaves, fruits, seeds, and bark of *Z. armatum* DC. possess various medicinal properties and have been used traditionally in several diseases such as carminative, antipyretic, appetizer, stomachic, dyspepsia, and toothache.[15] Hence the study aimed to evaluate the antidiabetic and antioxidant activity of aqueous extract of *Z.armatum* DC. in high-fat diet low dose STZ induced type II diabetic rats.

2. MATERIALS AND METHODS

Collection of plant material and extract preparation: *Zanthoxylum armatum* DC. Stem bark was collected in the Jammu and Kashmir, India. The selected plant was authenticated by Dr. Vijai K Agnihotri, Scientist, NPP Division, CSIR-IHBT, Palampur, HP, India. The stem bark was shade dried and ground well, and the powder was used for further studies. For the aqueous extract preparation, one part of plant powder was mixed with six-part of plant materials taken in medium flame and stirred well until the volume reduced to one third after the content was filtered, the filtrate was evaporated to dryness, and the paste formed of the extract was used for the further experimental studies.

2.1. Animals used in research

Male Wister rats of 160-200 g body weight were acquired and maintained at the PG and Research Department of Biochemistry, SrimadAndavan Arts and Science College (Autonomous), Affiliated to Bharathidasan University, Thiruvanaikovil, Tiruchirappalli, Tamilnadu - 620005, India. Fed on healthy pellet diet (Biogen, Bangalore, India) and water *adlibitum*. The report of the study was accepted by SrimadAndavan Arts and Science College's Institutional Ethical Committee (SAC/IAEC/BC/2017/May RP-001)

2.2. Induction of diabetes

Healthy wistar albino rats were grouped into six rats subjected to HFD-induced antidiabetic studies. For the induction of type 2 diabetes, animals were treated with HFD for 2 weeks before Streptozotocin IP (40mg/ kg BW). Diabetes induction was confirmed using blood glucose after 5 days of STZ induction.

Group - I: Normal control,
Group - II: Diabetic control
Group III: Diabetic rats treated with AEZA 200 mg/kg BW
Group IV: Diabetic rats treated with AEZA 400 mg/kg BW
Group V: Diabetic rats treated with AEZA 800 mg/kg BW
Group VI: Diabetic rats treated with Metformin 100 mg/kg BW

The extract and the standard antidiabetic drug metformin were administered orally by gavage to each rat as a single daily dose for 45 days.

2.3. Biochemical Analysis

Blood samples were collected by the tail vein method, and blood glucose[16] levels were measured immediately using a

glucometer for the 0, 15, 30 & 45 days, respectively. OGTT was measured on the 15th day after treatment, and ITT was measured on the 25th day. After the experimental period, all the animals were sacrificed under anaesthetic conditions, and blood and tissue samples were collected and used for further biochemical studies. Blood Glucose was taken during the experimental period. Antioxidant assays such as Lipid Peroxidation assay[17], Superoxide dismutase, enzyme activity[18], reduced glutathione assay[19] and Glutathione Peroxidase activity[20] were analyzed using the standard protocol.

3. STATISTICAL ANALYSIS

The data of all the parameters were analyzed using Analysis of variance (ANOVA). One-way ANOVA followed by Dunnet's test was performed in which the diabetic control was compared with all the other groups, and the values are expressed in Mean ± SEM. A P-value less than 0.05 were considered to be statistically significant.

4. RESULTS

4.1. Effect of Za-Aq on blood glucose level in normal and STZ induced diabetes treated rats

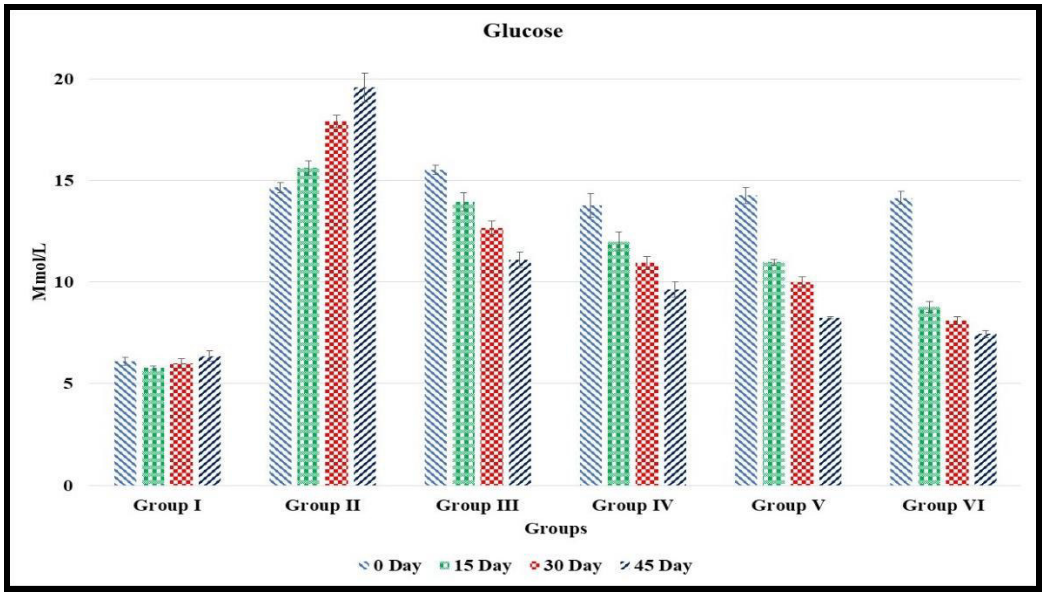
The biochemical parameters of glycemic control in the animals have been summarized in Figure 1. The intraperitoneal administration of Streptozotocin (STZ) resulted in a nearly fourfold increase in the fasting blood glucose levels in the male/female diabetic Wistar rats. The blood glucose level was measured at different time intervals during the research exertion, viz. on the very first day of induction of diabetes, in the middle of the study, i.e., on 15, 30th days, and at the end of the experiment, i.e., on the 45th day. It was observed that with the gradual increase in the dose of the *Za*-Aq, the blood glucose level was improvised. At the end of 45 days' period, Za-*Aq* treated diabetic animals showed a significant reduction of blood glucose levels nearly to the normal level compared with the diabetic animals ($p < 0.05$)

4.2. Effect of Za-Aq on OGTT and ITT in normal and STZ induced (treated) diabetic rats during 60 min (1 h)

The results from the research exertion indicate that the of Za-*Aq* (200, 400, and 800 mg/kg body weight) and Metformin (100 mg/kg) reduced the OGTT (Figure 2) and ITT Figure 3) levels (significant hyperglycemia due to administration of glucose load of 2 g/kg bw to a significant level ($p < 0.05$) after 1 h of oral administration as compared to the diabetic control group.

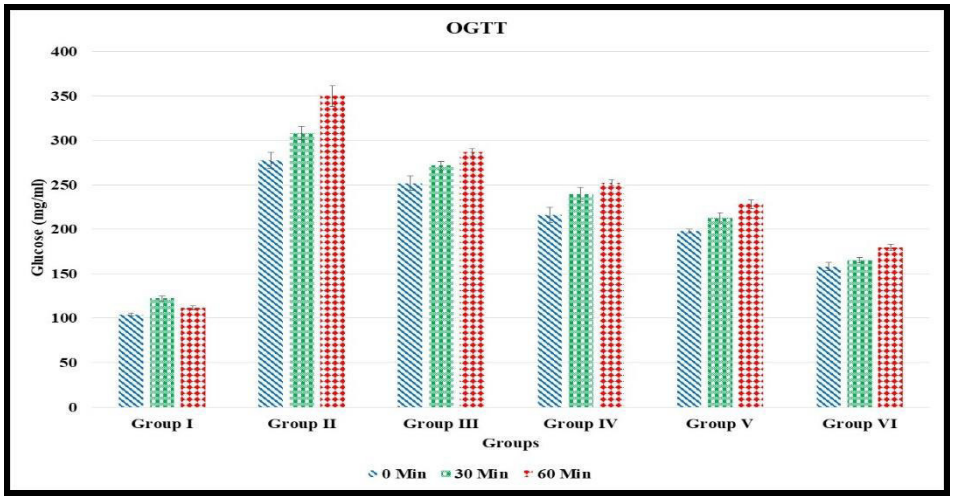
4.3. Effect of Za-Aq on oxidative stress parameters in normal and STZ induced diabetic (treated) rats

The results clearly illustrate the effect of Za-*Aq* on the antioxidant enzymes. The enhanced level of LPO (Figure 4) was reversed to near normal after administration of Za-*Aq* after administering 800 mg/kg body weight of Za-*Aq*. A marked reduction was noted in the level of superoxide dismutase (SOD) (Figure 5), Glutathione Peroxidase (GSH-Px) (Figure 6), and reduced glutathione (GSH) (Figure 7) in the STZ induced diabetic rats. Administration of Za-*Aq* at different doses for the 45 days to STZ induced diabetic rats significantly ($p < 0.05$) increased SOD, and GSH-Px levels, with the maximum effect seen at 800 mg/kg body weight. It is pertinent to note that the Za-*Aq* was equipped with the antioxidant effect in a dose-dependent manner (Figure 4).



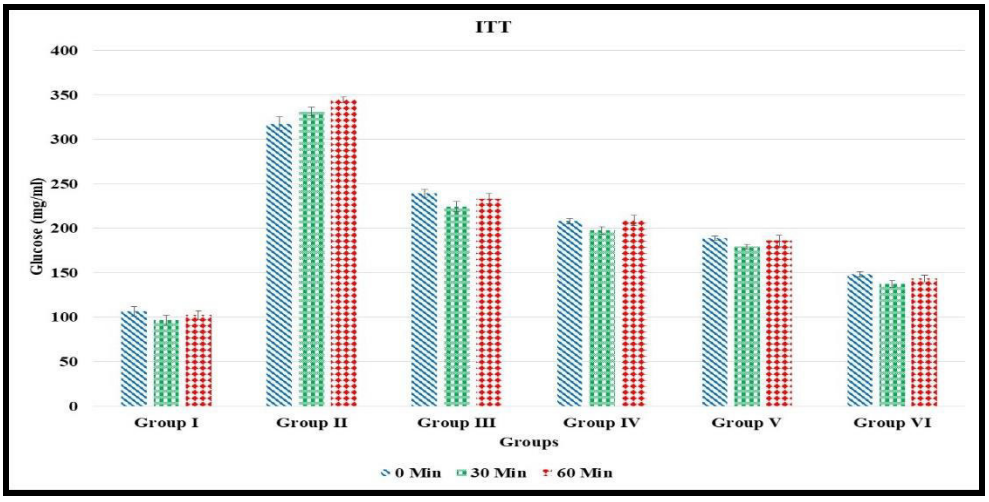
The values are Mean ± SEM (n=6) (P<0.05)

Fig 1: ***Effect of Za-Aq on blood glucose level in normal and STZ induced diabetes treated rats***



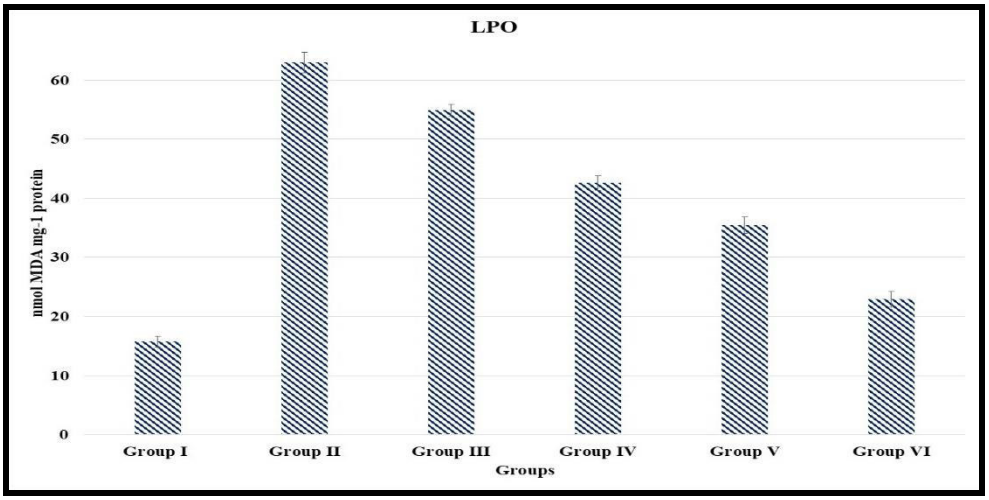
The values are Mean ± SEM (n=6) (P<0.05)

Fig 2: *Effect of Za-Aq on OGTT in normal and STZ induced (treated) diabetic rats during 60 min (1 h)*



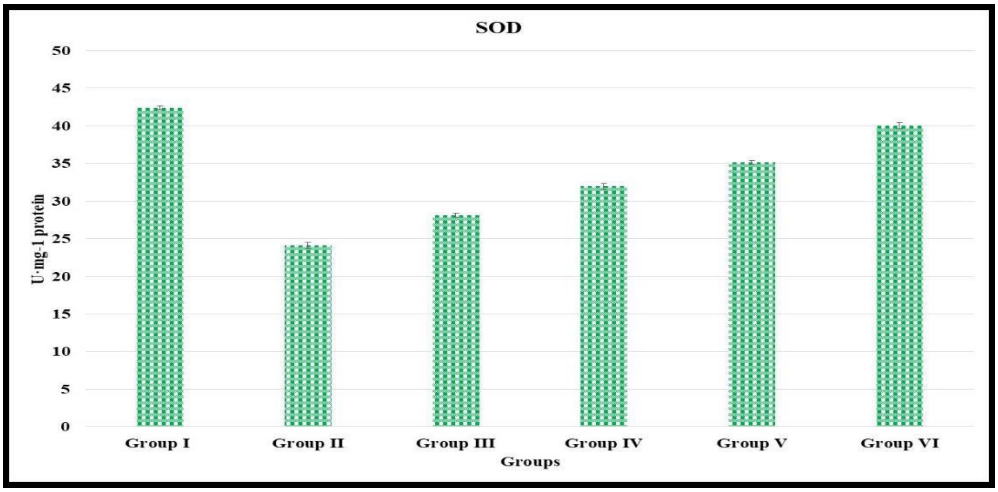
The values are Mean ± SEM (n=6) (P<0.05)

Fig 3: *Effect of Za-Aq on ITT in normal and STZ induced (treated) diabetic rats during 60 min (1 h)*



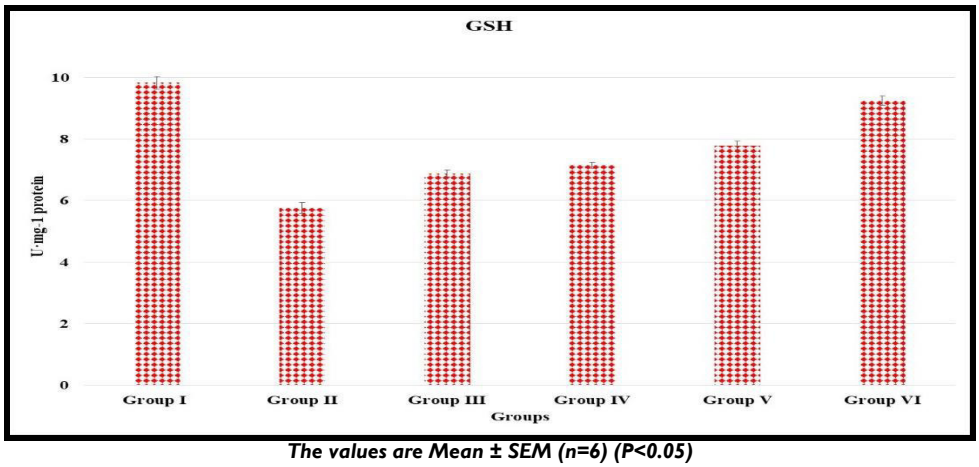
The values are Mean ± SEM (n=6) (P<0.05)

Fig 4: *Effect of Za-Aq on LPO in normal and STZ induced diabetic (treated) rats*



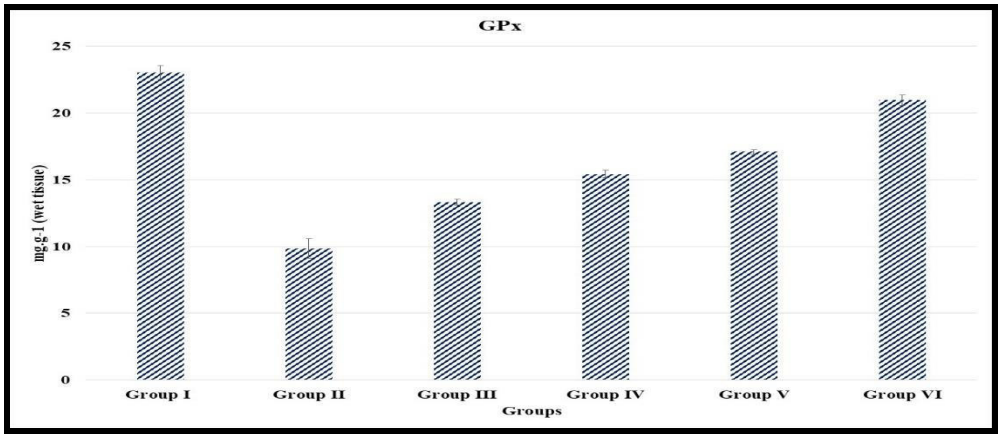
The values are Mean ± SEM (n=6) (P<0.05)

Fig 5: ***Effect of Za-Aq on SOD in normal and STZ induced diabetic (treated) rats***



The values are Mean ± SEM (n=6) (P<0.05)

Fig 6: ***Effect of Za-Aq on GSH in normal and STZ induced diabetic (treated) rats***



The values are Mean ± SEM (n=6) (P<0.05)

Fig 7: ***Effect of Za-Aq on GPx in normal and STZ induced diabetic (treated) rats***

5. DISCUSSION

An acute toxicity study of the Aqueous extract demonstrated that different doses of *Zanthoxylum armatum* DC were non-toxic throughout the experiment. The lethality was zero in the groups receiving the different doses of *Zanthoxylum armatum* DC. Stem Bark extract. In diabetic rats treated with *Za-Aq*, a substantial drop in blood glucose and stabilization of plasma insulin levels were found. This may be related to the effect of the extract on the pancreatic secretion of insulin from regenerated cells or its ability to release bound insulin from regenerated -cells by blocking ATP-sensitive K+ channels, similar to glibenclamide. [21] According to Yuan et al. (2001), hyperinsulinemia brought on by insulin resistance in peripheral tissues compromises the structural integrity of -cells.[22] In our work, the

immunohistochemistry examination revealed that diabetic rats had impaired and degranulated -cells and a decrease in -cells that stained positively for insulin. However, therapy with *Za-Aq* retained the -cell mass in diabetic rats. The substantial increase in insulin-immunoreactive expression in diabetic rats treated with *Za-Aq* supported the protective effect of *Za-Aq* in the reversal of HFD-STZ-induced pancreatic -cell damage. Coumarins are cited as possible hypoglycemic agents with insulin-sensitizing properties.[23,24]*Za-Aq* includes significant amounts of furocoumarin and ficusin, which may account for its insulin-sensitizing action. In addition, ficusin's antioxidant, antilipidemic, and antidiabetic effects on GLUT4 translocation and PPAR expression in rats with type 2 diabetes have been described.[25] OGTT and ITT were done on the 15th and 25th days, respectively. The *in vivo* peripheral insulin action and insulin resistance in animals can be assed by OGTT; it is a straightforward and generally established approach.[26] In our investigation, *Za-Aq* enhanced the glucose absorption into peripheral tissues of diabetic rats in a dose-dependent manner. ITT is useful to measure insulin sensitivity by exogenous infusion of insulin. *Za-Aq* (250 and 500 mg/kg) reduced the blood glucose significantly at 30 and 60 min, suggesting improved insulin sensitivity possibly by improving one or more defects, namely insulin receptor, insulin receptor substrate, glucose transporters, or enzymes involved in phosphorylation of glucose.[27,28] These results from ITT and OGTT tests indicated that the *Za-Aq* administration lowered the glucose level by the mechanism of insulin-sensitized glucose absorption. Menaka et al. (2010) have also reported that the comparable mechanism of insulin-sensitized glucose absorption has been seen in ITT and OGTT of *Sida rhomboidea* extract on the C57BL/6J mice model.[29] The results (Figure 4) demonstrated that STZ-induced diabetic rats had enhanced lipid peroxidation (LPO). Studies have shown that diabetic rats had increased lipid peroxidation in their liver, kidneys, and brain.[30] This may be due to the comparatively large content of peroxidizable fatty acids in the tissues. In the present investigation, a rise in the levels of LPO was observed, and these levels were dramatically reduced after *Za*-Aq and metformin administration (Figure 4). This suggests that plant extract inhibits oxidative damage due to the antiperoxidative impact of Za- Aq's. This may accord with prior research indicating that Cassia auriculata flowers.[31] STZ-induced rats had significantly (p0.05) lower levels of superoxide dismutase (SOD)(Figure 5), reduced glutathione (GSH) (Figure 6), and glutathione peroxidase (GPx) (Figure 7). When treated with *Za*-Aq, these unfavourable alterations were reversed to values close to normal. It is widely known that SOD and GPx play a crucial role in protecting tissues against the production of free radicals.[31] The antioxidant status of tissues has been proven to be a significant component in developing diabetes complications.[32] As a cofactor or substrate for some enzymes, reduced glutathione plays a significant function in detoxification and metabolism. As antioxidants shield tissue from oxidative stress, it is a common marker of free radical damage. Enzymatic antioxidants such as SOD are considered the main enzymes since they eliminate ROS directly.[33] SOD is a crucial defensive enzyme that scavenges O_2 anion from H_2O_2 and, as a result, reduces the toxicity of this radical and other free radical formed from secondary reactions.[34] Antioxidant enzymes such as SOD are hindered by nonenzymatic glycosylation and oxidation in diabetes mellitus.[35] As previously reported, SOD activity decreased in diabetic rats in the present investigation, which alloxan-generated reactive oxygen species may have caused.[36] The *Za*-Aq had reversed the activities of these enzymatic antioxidants, which may have resulted from reduced oxidative stress, as indicated by a drop in LPO. Glutathione (GSH) is the most abundant non-protein thiol in living organisms and plays a crucial function in coordinating the antioxidant defence activities of the body. Decreased GSH levels contribute to the development of diabetes complications.[37] Reduced glutathione, predominantly generated in the liver, is an essential nonenzymatic antioxidant in the antioxidative defence mechanism. The substantial depletion of GSH observed in the tissue of alloxan diabetic mellitus rats may result from two antioxidant enzymes, GPx and GST, using this substance as their substrate.[38] GPx catalyzes the reaction between hydroperoxides and reduced glutathione to produce glutathione disulfide (GSSG) and the hydroperoxide's reduction product. In our investigation, a decrease in the activity of these enzymes in STZ-induced animals and a return to near normalcy in *Za*-Aq treated rats indicate that the extract neutralized alloxan-induced oxidative stress.[39] Administration of *Za*-Aq to STZ-induced diabetic rats effectively and dose-dependently restored the impaired level of antioxidant enzymes. The altered lipid profile, glycosylation of proteins, oxidative stress, and chronic hyperglycemia exacerbates the elevated risk of cardiovascular disease. As diabetes is a metabolic condition, it is also characterized by an altered lipid profile and elevated glucose levels. Therefore, the possible mechanism of action of *Za*-Aq in regulating blood glucose could be the augmentation of insulin secretion by pancreatic -cells. In addition, the process of lipid profile correction may be a result of enhanced glycogenesis in the liver.

6. CONCLUSION

According to the data reported in this study, *Zanthoxylum armatum* DC. Stem Bark extract protects -cells against ROS-mediated death by increasing the levels of antioxidant enzymes and reducing hyperglycemia in High Fat Diety low dose of STZ-induced diabetics, which could be caused by the release of insulin from residual recovered -cells in the pancreas. The aforementioned research supports future usage of aqueous extract of *Zanthoxylum armatum* DC as an important source of natural antioxidants with antidiabetic potential and as a viable food additive or functional food.

7. AUTHOR CONTRUBUTION STATEMENT

Keerthana Kesavan performed this study, collected the data and drafted this manuscript. Hariharan Govindasamy collected the data and performed this study. Sridharan Gurunagarajan contributed data and analysed the data. Jothi Gnanasekaran conceived and designed this study and given inputs to design this manuscript. All authors read and approve the final version of the manuscript.

8. FUNDING ACKNOWLEGEMENT

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

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