The Glucose Lowering Effect of *Zornia gibbosa* Span Extracts in Diabetic Rats

Diyabetik Sıçanlarda *Zornia gibbosa* Span Ekstrelerinin Glukoz Düşürücü Etkisi

🆔 Mallikarjuna Rao TALLURI[1*], 🆔 Rajananda Swamy TADI[2], 🆔 Ganga Rao BATTU[2], 🆔 Mohammad ZUBAIR[3]

[1]Anacipher Clinical Research Organisation, Department Bio-Analytical, Telangana, India
[2]Andhra University, College of Pharmaceutical Sciences, Department of Pharmacognosy and Phytochemistry, Andra Pradesh, India
[3]University of Tabuk, Faculty of Medicine, Department of Medical Microbiology, Tabuk, Kingdom of Saudi Arabia

ABSTRACT

Objectives: Diabetes mellitus is a chronic, lifelong condition that affects our body physiology. Untreated diabetes mellitus causes diseases such as diabetic retinopathy, diabetic nephropathy and diabetic neuropathy, auto immune diseases, polyuria, polydipsia, loss of weight, and cardiovascular diseases. The use of medications for the treatment of diabetes mellitus causes adverse effects with long-term use, and sometimes leads to death. Today, researchers are working on the discovery of new anti-diabetes drugs from plants with low or no adverse effects. From this point of view, the present work was conducted to evaluate the anti-diabetic activity of *Zornia gibbosa* Span.

Materials and Methods: This acute toxicity study was conducted for ethyl acetate and ethanol (70%v/v) extracts of *Z. gibbosa* as per OECD guidelines. The anti-diabetic activity of selected plant extracts were tested using alloxan-induced diabetes in a rat model.

Results: No mortality was observed in the administered doses of *Zornia gibbosa* Span extracts. The tested extracts significantly ($p \leq 0.01$) restored the physiologic changes that occurred due to the alloxan-induced diabetes mellitus. The hydroalcoholic extracts at 500 mg/kg body weight concentration showed more activity compared with other extracts at different concentrations along with standard drug (glibenclamide). *Zornia gibbosa* significantly decreased glucose concentrations and restored the altered enzymes levels caused by damage to different organs by diabetes.

Conclusion: The results of the present study indicate that *Z. gibbosa* has a significant anti-diabetic activity. Therefore, it may be capable of use as an alternate medicine along with allopathic medicine in the treatment of diabetes as well as its health problems.

Key words: *Zornia gibbosa* Span, diabetes mellitus, alloxan, glibenclamide

ÖZ

Amaç: Diyabet, vücudumuzun fizyolojisini etkileyen, yaşam boyu kronik bir hastalıktır. Tedavi edilmeyen diabetes mellitus, diyabetik retinopati, diyabetik nefropati ve diyabetik nöropati, otoimmün hastalıklar, ploüri, polidipsi, kilo kaybı ve kardiyovasküler hastalıklar gibi hastalıklara neden olur. Diabetes mellitus tedavisinde kullanılan ilaçlar, uzun süreli kullanımda yan etkilere neden olur, bazen ölüme yol açar. Günümüzde araştırmacılar, yan etkileri düşük veya hiç olmayan bitkilerden hareketle yeni anti-diyabetik ilaçların keşfedilmesi üzerinde çalışmaktadır. Bu açıdan, mevcut çalışmada, *Zornia gibbosa* Span'ın anti-diyabetik etkinliğinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Akut toksisite çalışması OECD kılavuzlarına göre *Z. gibbosa*'nın etil asetat ve etanol (%70 v/v) ekstreleri için yürütülmüştür. Seçilen bitki ekstrelerinin anti-diyabetik aktivitesi, sıçanlar üzeinde oluşturulan alloksan-nedenli diyabet modeli kullanılarak değerlendirilmiştir.

Bulgular: *Zornia gibbosa* Span ekstrelerinin uygulanan dozlarında herhangi bir ölüm görülmemiştir. Ekstreler, alloksan-nedenli diabetes mellitus nedeniyle oluşan fizyolojik değişiklikleri önemli ölçüde ($p \leq 0.01$) iyileştirmiştir. 500 mg/kg konsantrasyonundaki sulu-alkollü ekstreler, standart ilaç (glibenklamid) ile birlikte farklı konsantrasyonlardaki diğer ekstrelerle karşılaştırıldığında, daha yüksek aktivite göstermiştir. *Z. gibbosa* glukoz konsantrasyonunu önemli ölçüde düşürmüş ve diyabetin değişik organlara hasar vermesi nedeniyle değişen enzim düzeylerini iyileştirmiştir.

Sonuç: Bu çalışmanın sonuçları, *Z. gibbosa*'nın önemli anti-diyabetik etkiye sahip olduğunu göstermektedir. Bu nedenle, diyabet tedavisinde ve diyabetin neden olduğu diğer sağlık problemlerinde *Z. gibbosa*'nın allopatik tıp ile birlikte kullanılabileceği ortaya konulmuştur.

Anahtar kelimeler: *Zornia gibbosa* Span, şeker hastalığı, alloksan, glibenklamid

INTRODUCTION

Glucose is a simple sugar found in all foods and an essential nutrient that provides energy for the proper functioning of body cells. However, it cannot be delivered alone to cells and it needs insulin to aid its transport into the cells. Insulin is a hormone produced by specialized cells (β-cells) of the pancreas. Without insulin, cells become ravenous for glucose because carbohydrates are broken down in the small intestine and the glucose in digested food is then absorbed by the intestinal cells into the bloodstream, and is carried by the bloodstream to all cells in the body where it is used.[1,2] High blood sugar levels over a prolonged period causes diabetes mellitus (DM). DM is a chronic, lifelong condition that affects our body's ability to use the energy found in food. There are three major types of DM. One is type 1 DM, which results from the pancreas's failure to produce enough insulin. In type 2 DM, either the amount of insulin produced is not enough for the body's needs, or the body's cells are resistant to it, and finally the third is gestational diabetes, which occurs in pregnant women.[3-6]

Globally, an estimated 415 million people have DM.[7] In the last three years, 1.5 to 5 million deaths occurred per year due to DM. All three forms of DM increase the risk of long-term complications.[8,9] The main complication due to DM is damage in the blood vessels. Untreated DM affects primary organs of the body such as the eyes, kidneys, and nerves by causing diseases diabetic retinopathy, diabetic nephropathy and diabetic neuropathy, auto immune diseases, polyuria, polydipsia, loss of weight, and cardiovascular diseases. Low levels of insulin cause the liver to turn fatty acids into ketone bodies for fuel instead of glucose, which causes ketosis. This in turn decreases the blood pH levels, which causes severe dehydration, hypotension, and finally death. These evets mainly occur in type 1 DM.[1,10-12]

DM is a chronic disease, for which there is no cure, mainly for type 1 DM.[1] Blood sugar levels of patients with type 2 DM and gestational DM can be controlled with a healthy diet, exercise, weight loss, and use of appropriate medications, but there is no cure.[2] Blood glucose (sugar) levels of patients with type 1 DM are controlled by taking insulin. Patients with type 2 and gestational DM use oral medications such as biguanides (metformin),[13] sulfonylureas (tolbutamide, glibenclamide, glimepiride), and thiozolidinediones (pioglitazone, rosiglitazone). The use of these medications causes adverse effects with long-term use,[14,15] sometimes causing severe acute diseases and lead to death. The adverse effects are mainly rapid or shallow breathing; painful or difficult urination; anxiety; blurred vision; chest discomfort; depression; irregular, pounding, or racing heartbeat or pulse; behavior changes similar to being drunk, difficulty with concentrating, drowsiness; lack or loss of strength; and restless sleep. Therefore, safer and more effective anti-diabetic drugs are still urgently needed.[16]

Natural products, mainly herbal medicines, have been playing an important role in treating diabetes around the world for centuries particularly in Asia, India, and Africa.[17,18] Many researchers are working on the discovery of new anti-diabetes drugs and have reported many new plants and their extracts' anti-diabetic activity using advancements of novel technology.[19,20] These findings provide valuable leads for the development of new isolated compounds for the treatment of diabetes.[21] However, there are still many medicinal plants available to screen for their biologic activities for disease including diabetes. Many pharmaceutical companies and academic laboratories are engaged in the discovery of new targets, pathways, and treatments for diabetes to supplement the current chemotherapies.[22] From this point of view, the present work was conducted to evaluate the anti-diabetic activity of *Zornia gibbosa* (*Z. gibbosa)* based on its traditional use. *Z. gibbosa* is an annual herb belonging to the family fabaceae. It has around 70 species in the *Zornia* genus, commonly known as Nellujollusoppu, and it grows at high altitudes i.e. 450-2000 m around India. It has been used in folklore medicine for the treatment of different ailments.[23-25]

MATERIALS AND METHODS

Reagents and chemicals
All reagents used for the present study were of analytical grade. Diagnostic kits were purchased from Span Diagnostics Ltd, Gujarat, India. Alloxan monohydrate was from Sigma chemicals, St Louis, USA, and Glibenclamide from Avantis Pharma Ltd.

Plant material and preparation of extracts
The plant material, *Z. gibbosa,* was collected at Guntur, Andhra Pradesh, India. The authentication of the plant was performed by Rtd. Prof. M. Venkaih, Department of Botany, Andhra University, Visakhapatnam (AU/DP&P/BGR/72). The plant material aerial parts were separated and shade dried at room temperature and powdered. The powdered material was used for extraction separately with ethyl acetate and hydroalcoholic (Hyd. ext) using the maceration process. The extracted solvents were concentrated to dryness under vacuum using a rotavapour.

Selection of animals
Healthy male and female albino rats of weighing between 180-250 g aged 60-90 days were used for the study. The rats were housed under standard light and humidity and were supplied proper food and water ad libitum.

Acute toxicity studies
The acute toxicity study was conducted for ethyl acetate and ethanol (70% v/v) extracts of *Z. gibbosa* as per Organisation for Economic Co-operation and Development (OECD) guidelines 420 (OECD.2001) and regulations of the Institutional Animal Ethics Committee (Reg no. 516/01/A/CPCSEA). The male albino rats were divided into two groups of 6 animals. They were maintained for one week before the experiment under room temperature and allowed free access to water and diet. The animals were subjected to an acute toxicity study using each extract at a dose of 2000 mg/kg orally in 2 groups at regular time intervals, *i.e.,* 1, 2, 4, 8, 12 and 24 h. During this time, the animals were under observation to note different conditions such as skin changes, morbidity, aggressiveness, oral secretions, sensitivity to sound and pain, respiratory movements, and mortality.

Grouping of animals for glucose lowering test

The experimental design consisted of 48 rats divided into eight groups. Group 1 received 0.1 mL of normal saline; the blood glucose of the rats was elevated by the administration of 100 mg/kg body weight of alloxan monohydrate intraperitoneally after an overnight fast but with access to drinking water, except groups 1. The animals were then housed in a controlled facility and allowed to drink 5% glucose solution to overcome the hypoglycemia. The hyperglycemic state was confirmed by the measurement of fasting blood glucose concentration using a glucometer with blood collected by tail vein puncture. Rats with blood glucose ≥200 mg/dL after 72 h were considered diabetic and used for the research.[26,27] Group 2: normal rats treated with the ethanol (70% v/v) extract 500 mg/kg body weight to determine the effect of the extract on normal blood glucose levels. Group 3: diabetic untreated (animals were treated with 100 mg/kg body weight of alloxan monohydrate); group 4: diabetic animals treated with standard drug (5 mg/kg body weight of glibenclamide); group 5: diabetic animals treated with 250 mg/kg body weight of ethyl acetate extract orally; group 6: diabetic animals treated with 500 mg/kg body weight of ethyl acetate extract orally; group 7: diabetic animals treated with 250 mg/kg body weight of ethanol (70% v/v) extract orally, and group 8: diabetic animals orally treated with 500 mg/kg body weight of ethanol (70% v/v) extract of *Z. gibbosa.*

Treatment with extract and standard drug

Extracts of *Z. gibbosa* and glibenclamide (standard drug) were dissolved in 10 mL normal saline (0.9% NaCl) before oral administration. Respective doses of extract and standard drug were then administered to the rats once daily in the morning (09:00-10:00 AM) for twelve days and the blood glucose was checked every four days. On the seventeenth day, the rats were fasted for 12 h and euthanized. Blood samples were collected by carotid puncture into heparinized tubes, centrifuged at 1000 r/min for 5 min, and the clear serum supernatant was used immediately for the assessment of the lipid profile, and liver and kidney function tests.

Serum biochemical parameters

The biochemical parameters that were investigated includes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, and creatinine using Span diagnostics Ltd. kits, and serum electrolytes (K^+, Cl^-, Na^+) were determined using Randox diagnostic kits.

Plasma lipid profiles

The plasma total cholesterol, triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined using Randox diagnostic kits. The absorbance was determined and calculated using a fully-smart semi-automated analyzer, BS Biosciences.

Statistical analysis

Results are expressed in mean±standard error of mean using ordinary two-way analysis of variance using GraphPad Prism-6 software. The results of $p < 0.01$ were considered as significant.

RESULTS

Acute toxicity of the selected plant extracts was tested as per OECD guidelines. There were no behavior signs such as alertness, motor activity, breathlessness, restlessness, diarrhea, tremor, convulsion, and coma observed at the administered doses. The rats were physically active and no deaths were recorded in present study of extracts treated at 2000 mg/kg body weight. Therefore, the LD50 is greater than 2000 mg/kg body weight.

The glucose levels were decreased in all tested groups compared with the untreated diabetes group, which continued to have elevated levels until the animals were sacrificed. Among all doses of tested extracts, hyd. ext at 500 mg/kg body weight showed better activity in the reduction of elevated glucose levels (Figure 1) and retained the body weight of the rats (Figure 2) as the treatment moved forward, except in the diabetic untreated group. No hyperglycemia or hypoglycemia was observed in the rats treated only with the extracts.

ALT, AST, ALP, total bilirubin, total protein, creatinine levels were significantly elevated in the diabetic animals. The various doses of extracts and standard drug significantly ($p < 0.01$) restored the ameliorated levels of ALT, AST, ALP, total bilirubin, total protein, creatinine levels. Normal levels of ALT and ALP levels showed a normoglycemic condition. The hydroalcoholic extract at 500 mg/kg body weight showed better activity in the increase of reduced ALT and creatinine levels as well as the reduction of increased AST, ALP, albumin and total protein levels (Figure 3, 4).

The electrolyte levels (sodium, chloride, and potassium) were varied in the diabetes-induced groups, extracts of *Z. gibbosa* significantly adjusted the electrolyte levels at the tested doses, as much as standard drug (Figure 5).

LDL, TG, and cholesterol levels were increased, whereas HDL levels were decreased in the diabetic animals. The extracts of *Z. gibbosa* and standard drug significantly decreased levels of LDL, TG, and cholesterol, and increased the HDL levels ($p < 0.01$) (Figure 6).

DISCUSSION

DM is caused by failure to maintain a stable level of blood glucose in the face of normal fluctuations of supply and demand. The secretary product of pancreatic β-cells, insulin, is central in the pathophysiology of DM.[28] Type 1 DM, or insulin-dependent DM, results from an absolute deficiency of insulin due to autoimmunologic destruction of the insulin-producing pancreatic β-cells.[29] In type 2 DM, or non-insulin-dependent DM, muscle and fat cells are 'resistant' to the actions of insulin and compensatory mechanisms that are activated in the β-cells to secrete more insulin are not sufficient to maintain blood glucose levels within a normal physiologic range.[30,31]

DM is characterized by chronic hyperglycaemia and it leads to the development of different physiologic changes in the body, and finally causes diseases.[32-35] In the present study, alloxan induction was used to cause DM in the animals. It leads to
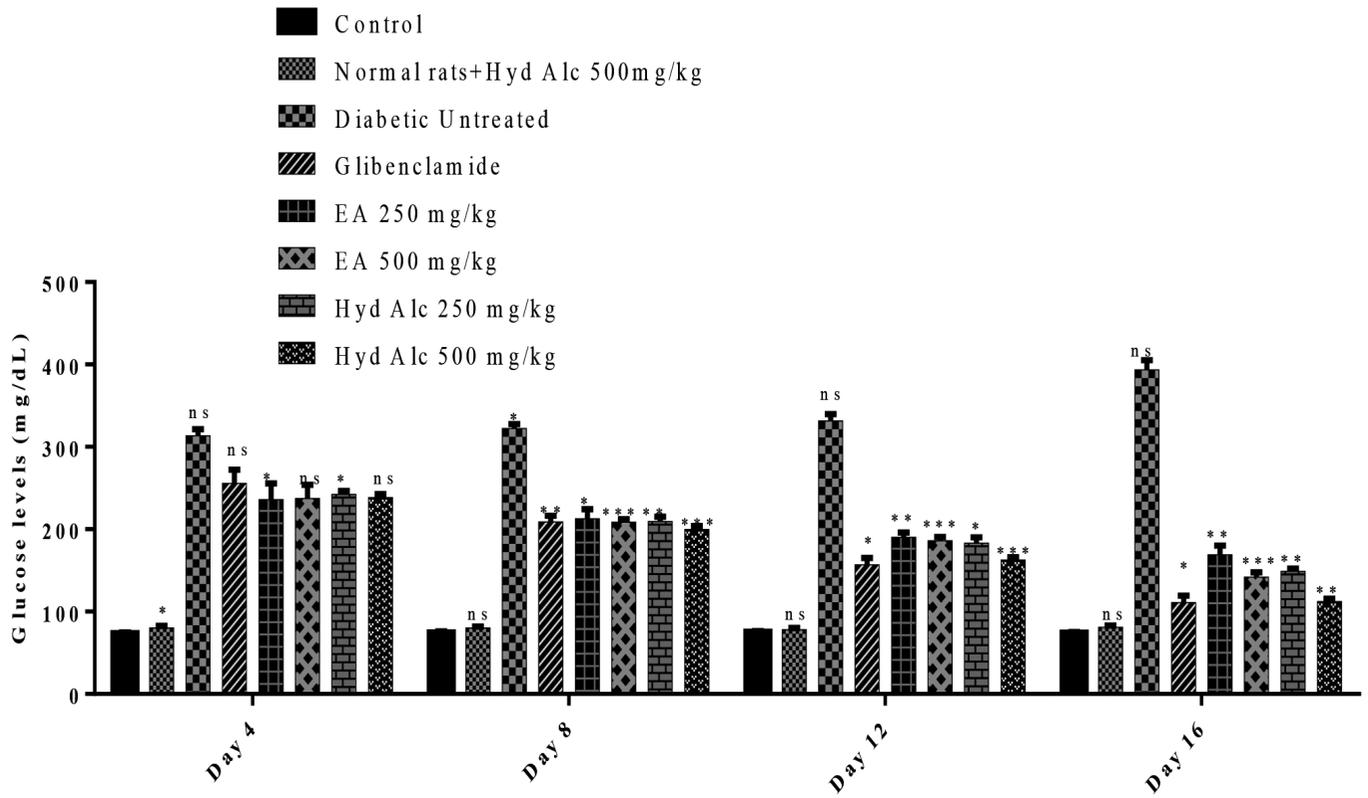
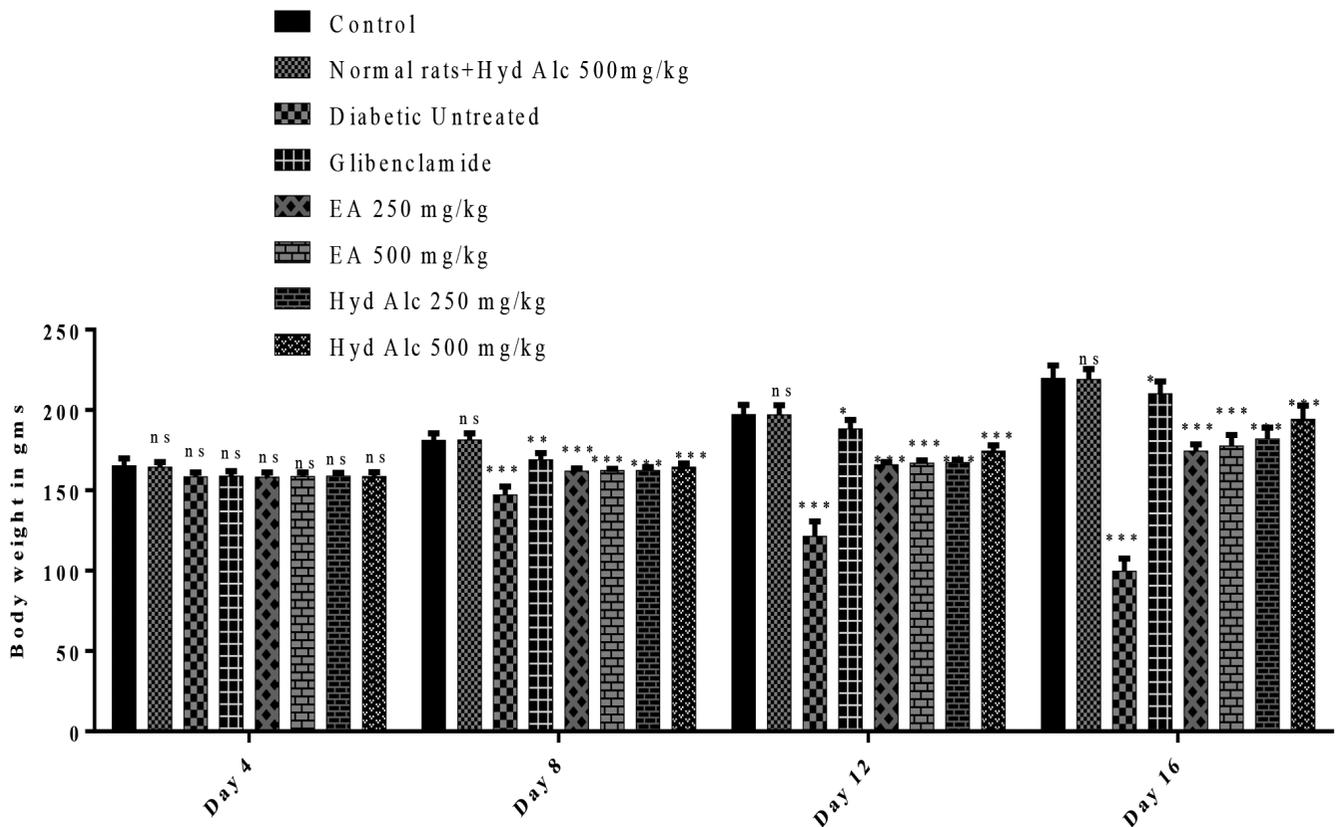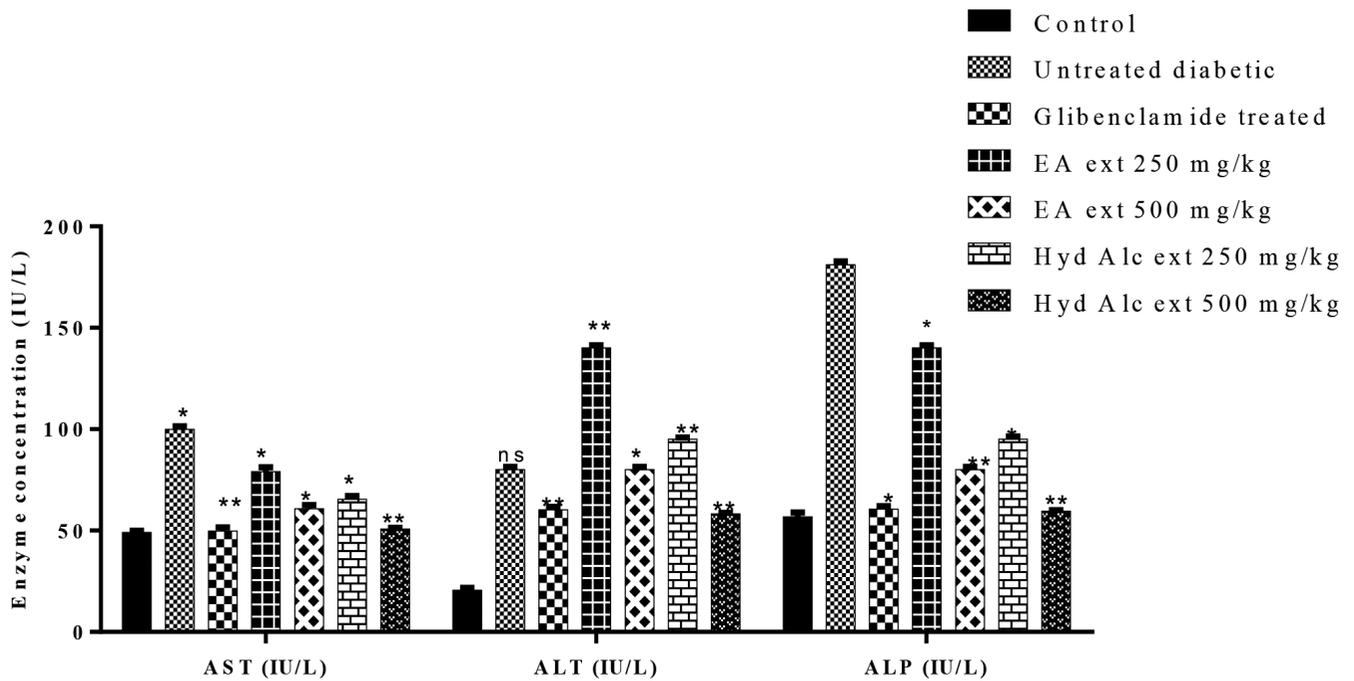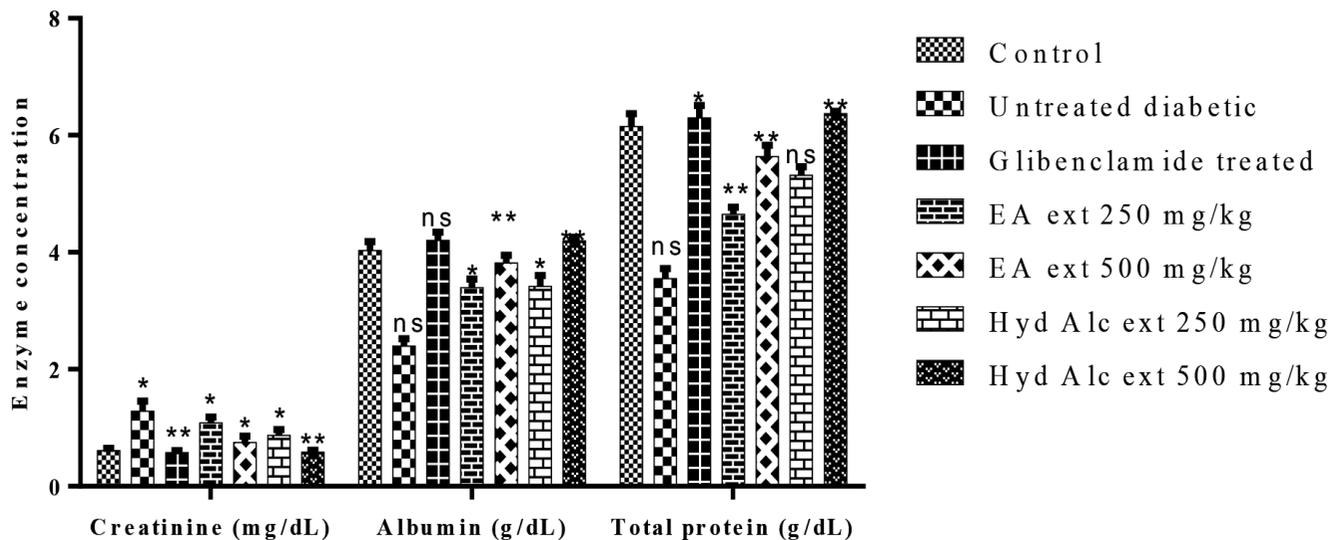**Figure 1.** Glucose levels of rats in different groups

Figure 2. Body weight of the rats in different groups

Figure 3. Effect of *Z. gibbosa* extracts on AST, ALT, ALP levels in alloxan-induced diabetic rats

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

Figure 4. Effect of *Z. gibbosa* extracts on creatinine, albumin and total protein levels in alloxan-induced diabetic rats

variations in their body physiologic condition[36,37] by causing necrosis to the pancreatic β-cells,[38-40] this leads to a reduction in insulin production and finally altering enzymatic levels in different organ's functions in the body such as the kidneys and liver (Figure 3 to Figure 6). The tested extracts of *Z. gibbosa* showed a significant reduction of the increased blood glucose levels (Figure 1). The reduction of glucose levels by *Z. gibbosa* may have been through protection of the β-cells from undergoing necrosis.[1,2] The weight loss also observed after diabetic induction was probably due to excessive breakdown of

tissue proteins and lipid for energy to maintain the body organs' function, but after treatment with the extracts of *Z. gibbosa*, the animals started to regain their body weight (Figure 2). The gained body weight may be due to improved metabolic activities with normal levels of glucose in the body.

Insulin helps glucose uptake in muscle and fat and inhibits hepatic glucose production. Insulin also stimulates cell growth, differentiation, and promotes the storage of substrates in fat and muscle by stimulating lipogenesis, glycogen, and protein synthesis, and inhibiting lipolysis, glycogenolysis, and protein

breakdown. Insulin deficiency (type 1 DM) or resistance (type 2 DM) results in profound deregulation of these processes, and produces elevations in fasting and postprandial glucose and lipid levels.[41-43] In the present study, elevations in electrolytes, albumin, and creatinine levels were also observed in the alloxan-induced diabetic rats and *Z. gibbosa* extracts significantly reinstated altered electrolytes, albumin, and creatinine levels in the diabetic rats (Figure 4, 5). This means that *Z. gibbosa* extracts have the ability to protect nephron function and increase electrolyte absorption in renal tubules.[44]

The enzyme levels of AST, ALT, ALP, and bilirubin indicate the functioning of the liver in the body[45,46] and alterations in these enzymes were observed in the alloxan-induced diabetic rats,

which indicates that DM affects the functions of organs.[47] *Z. gibbosa* extracts significantly revised the reduced or increased enzymes levels of the liver and its function (Figure 3) due to DM, possibly through regeneration of damaged functions due to DM.

DM causes diabetic dyslipidemia, i.e. low-density cholesterol levels (LDL) and increases TG and high-density cholesterol levels (HDL), which increases the risk for heart disease and stroke.[48,49] Variations in these levels (lipid profile) were observed in the present experiment and the variations were restored in the *Z. gibbosa* extract-treated groups compared with the diabetic untreated group (Figure 6). This might be due to reduced hepatosynthesis of triglycerols or reduced
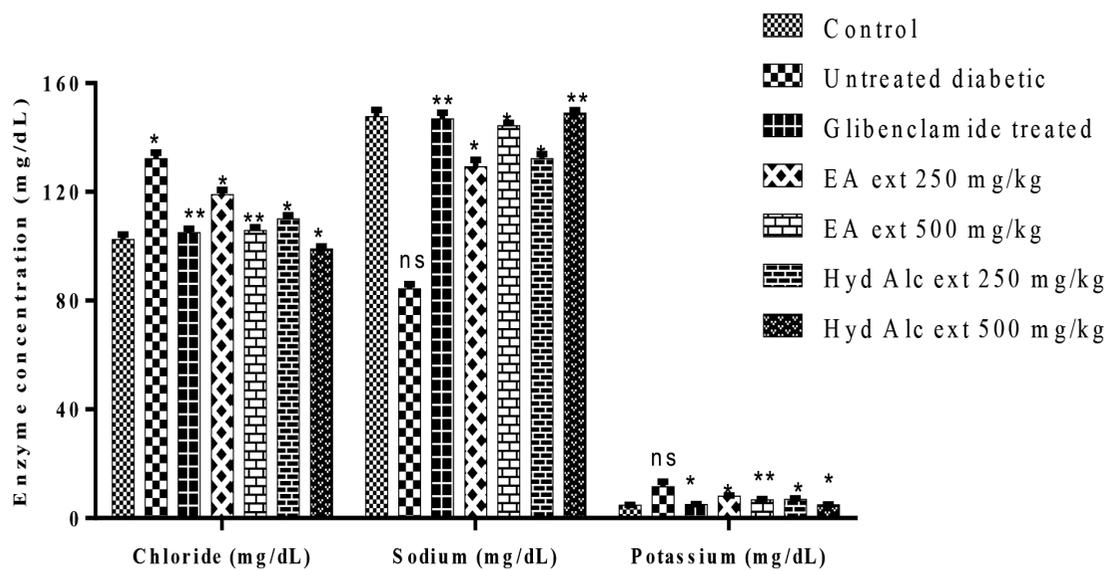

Figure 5. Effect of *Z. gibbosa* extracts on chloride, sodium and potassium levels in alloxan-induce diabetic rats
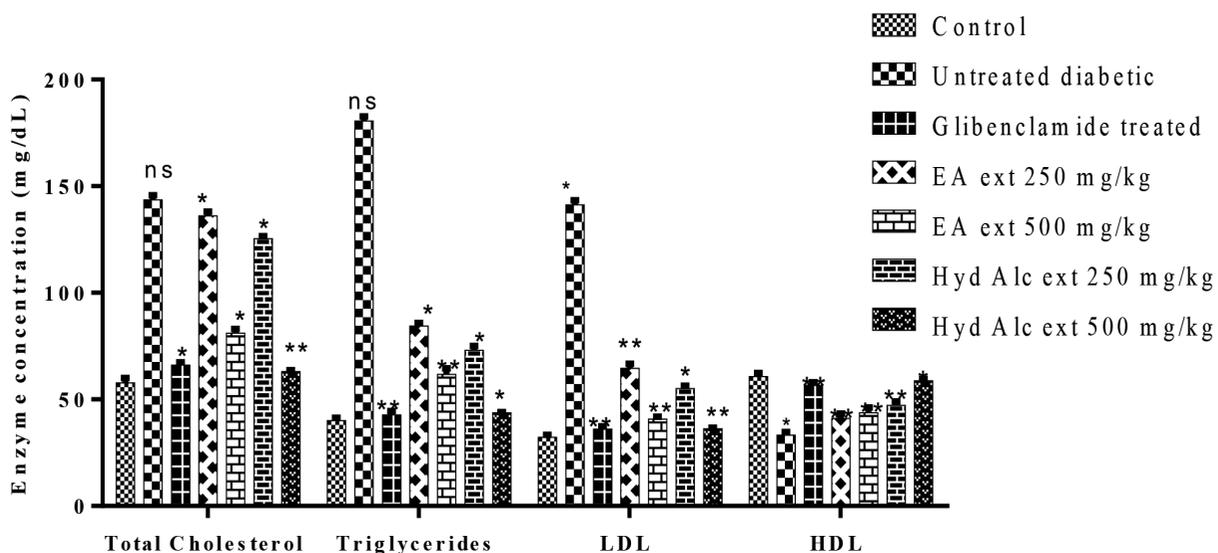

Figure 6. Effect of *Z. gibbosa* extracts in total cholesterol, triglycerides, LDL and HDL levels in alloxan-induced diabetic rats
LDL: Low-density lipoprotein, HDL: High-density lipoprotein

lipolysis because deficiency of insulin enhanced the hydrolysis of triacylglycerols.[50,51] The increased HDL levels in *Z. gibbosa* extract-treated groups indicates that these have the ability to suppress the enzymes' action responsible for LDL formation (3-hydroxy-3-methylglutaryl coenzyme A reductase) in the diabetic condition.[52] From the above results, it can be summarized that, the tested extracts of *Z. gibbosa* have the ability to restore physiologic changes that occur due to the diabetes-like reduction in glucose concentrations, gaining of body weight, and failure of organs such as the kidneys and liver.

CONCLUSIONS

DM is a well-known chronic disorder around the world with various late complications such as retinopathy, neuropathy, and nephropathy. *Z. gibbosa* has significant antidiabetic activity (glucose lowering). Therefore, it can be used as an adjuvant medicine along with allopathic medicine in the treatment of diabetes as well as for its late complications. A further study is underway in our laboratory to isolate the active principle and to study the mechanism of its action.

ACKNOWLEDGEMENTS

Conflict of Interest: No conflict of interest was declared by the authors.

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