Glucose lowering effect of Zornia gibbosa Span extracts in diabetic rats

Diyabetik sıçanlarda *Zornia gibbosa Span* özütlerinin glikoz düşürücü etkisi

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Short title: **Anti-Diabetic activity of *Zornia gibbosa Span* Extracts**

Kısa başlık: *Zornia gibbosa Span* özütünün anti diyabetik etkinliği

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ABSTRACT

Purpose: Diabetes mellitus is a chronic disease, lifelong condition that affects our body physiology. The untreated Diabetes mellitus causes diseases like diabetic retinopathy, diabetic nephropathy and diabetic neuropathy, auto immune diseases, Ployuria, Polydipsia, loss of weight and cardiovascular diseases. The use of medications for treatment of diabetes mellitus causes the side effects on long term use, sometimes leads to death. Today, researchers are working in the discovery of new anti-diabetes drugs from plants with low or no side effects. In this point of view, the present work was carried out to evaluate the anti-diabetic activity of *Zornia gibbosa* Span.

Methods: The 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 rats model. **Results:** There was no mortality observed in the administered doses of *Zornia gibbosa Span* extracts. The tested extracts significantly (P≤0.01) restored the physiological changes occurred due to the alloxan-induced diabetes mellitus. The hydroalcoholic extracts at 500 mg/kg body weight concentration showed more activity compared to other extracts at different concentrations along with standard drug (Glibenclamide). *Zornia gibbosa* has significantly decreased the glucose concentration and restored the altered enzymes levels by different organs damage by diabetes. **Conclusion:** The results of present study indicates that, *Z. gibbosa* has significant anti-diabetic activity. Therefore, it be capable of be use as an alternate medicine along with allopathic medicine in treatment of diabetes as well as its health problems.

Key Words: *Zornia gibbosa,* Diabetes mellitus, Alloxan, Glibenclamide.

ÖZET

Amaç: Diyabet, vücudumuzun fizyolojisini etkileyen, kronik bir hastalıktır. Tedavi edilmeyen Diabetes mellitus, diyabetik retinopati, diyabetik nefropati ve diyabetik nöropati, oto bağışıklık hastalıkları, Ployuria, Polidipsia, 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 yeni anti diyabet ilaçları keşfediyorlar. Bu açıdan, mevcut çalışma, Zornia gibbosa Span'ın anti-diyabetik etkinliğini değerlendirmek için gerçekleştirildi. **Yöntemler:** Akut toksisite çalışması OECD kılavuzlarına göre Z. gibobosa'nın etil asetat ve etanol (% 70 v / v) ekstreleri için yürütülmüştür. Seçilen bitki ekstraktlarının anti diyabetik aktivitesi, sıçanlar modelinde alloksanla indüklenen diyabet kullanılarak test edildi. **Bulgular:** Uygulanan Zornia gibbosa Span ekstraklarının dozlarında herhangi bir ölüm görülmedi. Test edilen özütler, alloksana bağlı diabetes mellitus nedeniyle oluşan fizyolojik değişiklikleri önemli ölçüde (P≤0.01) restore etti. 500 mg / kg vücut ağırlığı konsantrasyonundaki hidroalkolik özler, standart ilaç (Glibenclamide) ile birlikte farklı konsantrasyonlarda diğer özlerle karşılaştırıldığında daha fazla aktivite gösterdi. Zornia gibobosa glikoz konsantrasyonunu önemli ölçüde düşürdü ve diyabetin değişik organlara hasar vermesi nedeniyle enzim düzeylerini değiştirdi. **Sonuç:** Bu çalışmanın sonuçları, Z. gibbosa'nın önemli anti-diyabetik etkinliğe sahip olduğunu göstermektedir. Bu nedenle, diabet tedavisinde ve sağlık problemlerinde allopathic tıp ile birlikte alternatif bir ilaç olarak kullanılabilir.

Anahtar Kelimeler: *Zornia gibbosa Span*, Şeker hastalığı, Alloksan'dan, Glibenklamid.

INTRODUCTION

Glucose is a simple sugar found in all food and an essential nutrient that provides energy for the proper functioning of the body cells. But it cannot alone delivered to the cells and it needs insulin to aid its transport into the cells. Insulin is a hormone produced by specialized cells (Beta 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 the cells in the body where it is utilized [1, 2]. The high blood sugar level over a prolonged period is Diabetes mellitus (DM). DM is a chronic disease, 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-results from the pancreas's failure to produce enough insulin. Two is type 2 DM-either the amount produced is not enough for the body's needs, or the body's cells are resistant to it and finally third is gestational diabetes-occurs in pregnant women [3-6].

Globally, an estimated 415 million people are suffering with DM [7]. In last three years, 1.5 to 5 million deaths was 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. The untreated DM effects the primary organs of the body like eyes, kidney and nerves by causing diseases Diabetic retinopathy, Diabetic nephropathy and Diabetic neuropathy, auto immune diseases, Ployuria, Polydipsia, loss of weight and cardiovascular diseases. The low levels of insulin causes the liver to turn fatty acids to ketone bodies for fuel instead of glucose causes the ketosis. It decreases the blood pH

levels causes' severe dehydration, hypotension and finally death. It occurs mainly in type 1 DM [1, 10-12].

DM is a chronic disease, for which there is no cure, mainly for type 1 DM [1]. Type 2 DM and gestational DM peoples' blood sugar levels will be control with a healthy diet, exercise, weight loss, and use of appropriate medications, but there is no cure [2]. Type 1 DM people control the blood glucose (Sugar) level with taking insulin. Type 2 and gestational DM suffering people are using oral medications like biguanides (Metformin)[13], sulfonylureas (tolbutamide, glibenclamide, glimepiride) and thiozolidinediones (pioglitazone, rosiglitazone). The use of these medications causes the side effects on long term use [14, 15], sometimes causes severe acute diseases are leads to death. The side effects are mainly fast or shallow breathing, painful or difficult urination, Anxiety, blurred vision, chest discomfort, depression, irregular, pounding, or racing heartbeat or pulse, Behavior change 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 medicine have been playing an important role in treating diabetes around the world for centuries particularly in Asia, India and Africa countries [17, 18]. Many researchers are working in the discovery of new anti-diabetes drugs and reported many new plants, their extracts' anti-diabetic activity using advancements of novel technology [19, 20]. These findings provide us valuable leads to develop new isolated compounds in the treatment of diabetes [21]. But still there is many medicinal plants are available to screen for their biological activities 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]. In this point of view, the present work was carried out to evaluate the anti-diabetic activity of *Zornia gibbosa* (*Z. gibbosa)* based on its traditional use. *Z. gibbosa* is an annual herb belongs to family fabaceae having around 70 species in *Zornia* genus, commonly called as Nellujollusoppu and it grows on high altitudes i.e. 450-2000m around the India. It have been using in folklore medicine [23-25] for treatment of different ailments.

MATERIALS AND METHODS

Reagents and chemicals

All the reagents used for the present study were of analytical grades. 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 done 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 maceration process. The extracted solvents were concentrated to dryness under vacuum using rotavapour.

Selection of animals

Healthy albino rats of either sex weighing between 180-250 g aged 60-90 days were used for the study. The rats were taken care of at standard light and humidity by supplying proper food and water.

Acute toxicity studies

The acute toxicity study was conducted for ethyl acetate and ethanol (70%v/v) extracts of *Z. gibbosa* as per OECD guidelines 420 (OECD.2001) and regulations of the Institutional Animal Ethics Committee (Regd no. 516/01/A/CPCSEA).The albino rats of single sex, were selected in to two groups of consisting 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 for acute toxicity study using each extract at a dose of 2000 mg/kg orally in 2 groups at regular intervals of time, *i.e.*, 1, 2, 4, 8, 12 and 24 h. During this time, the animals were under observation to note different conditions like skin changes, morbidity, aggressiveness, oral secretions, sensitivity to sound and pain, respiratory movements and finally their mortality.

Grouping of animals for glucose lowering test

The experimental design consisted of 48rats divided into VIII groups. Group I- received 0.1 mL of normal saline; The blood glucose of 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 I and II. 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 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 II- Normal rats treated with the ethanol (70%v/v) extract 500mg/kg body weight to know the extract effect on normal blood glucose levels. Group III-Diabetic untreated (Animals were treated with 100mg/kg body weight of alloxan monohydrate); Group IV- Diabetic animals treated with standard drug (5 mg/kg body weight of glibenclamide); Group V- Diabetic animals treated with 250mg/kg body weight of ethyl acetate extract orally; Group VI- Diabetic animals treated with 500mg/kg body weight of ethyl acetate extract orally; Group VII- Diabetic animals treated with 250mg/kg body weight of ethanol (70%v/v) extract orally and Group VIII- Diabetic animals treated with 500mg/kg body weight of ethanol (70%v/v) extract orally 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 rats once daily at morning time (9-10 AM) for twelve days and the blood glucose was checked after 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 freshly for the assessment of lipid profile, liver, and kidney function tests.

Serum biochemical parameters

The biochemical parameters that were investigated include: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin,

total protein, 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 (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) were determined using Randox diagnostic kits. The absorbance was determined and calculated using fully smart semiautomated analyzer, BS Biosciences.

Statistical Analysis

Results are expressed in mean±SEM using ordinary Two-way ANOVA using Graph pad Prism-6 software. The results of $P<0.01$ were considered as significant.

RESULTS

Acute toxicity of the selected plant extracts were tested as per OECD guidelines. There was no behavior signs such as alertness, motor activity, breathlessness, restlessness, diarrhea, tremor, convulsion and coma were observed at the administered doses. The rats were physically active and no death was 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 to untreated diabetes group that continued to elevate the levels until the animals sacrificed. Among all doses of tested extracts, hyd. ext at 500 mg/kg body weight showed better activity in reduction of elevated glucose levels (Figure 1) and retain the body weight of rats (Figure 2) as the treatment move forward except in the diabetic untreated group. There was no

hyperglycemia or hypoglycemia nature observed in the rats treated with the only 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 were significantly ($P < 0.01$) restored the ameliorated levels of ALT, AST, ALP, total bilirubin, total protein, creatinine levels. The normal levels of ALT and ALP levels shows normoglycemic condition. The hydroalcoholic extract at 500mg/kg body weight showed the better activity in the increase of reduced ALT and creatinin levels as well as reduction of increased AST, ALP, Albumin and Total protein levels (Figure 3 and Figure 4)

The electrolytes level (Sodium, Chloride and Potassium) were varies in diabetic induced groups, extracts of the *Z. gibbosa* were significantly adjust varied electrolytes level at tested doses as much as standard drug (Figure 5).

LDL, TAG and cholesterol levels were increased while HDL level was decreased in the diabetic animals. The extracts of *Z. gibbosa* and standard drug were significantly ($P < 0.01$) decreased the levels of LDL, TAG and cholesterol, increased the HDL level (Figure 6).

DISCUSSION

DM caused by failure to maintain a stable level of blood glucose in the face of the normal fluctuations of supply and demand. The secretary product of pancreatic β-cells insulin is central in pathophysiology of DM [28]. Type I DM or insulin-dependent DM is results from an absolute deficiency of insulin due to autoimmunological destruction of the insulin producing pancreatic β –cell [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 -cell to secrete more insulin are not sufficient to maintain blood glucose levels within a normal physiological range [30, 31].

DM is characterized by chronic hyperglycaemia and it leads to the development of different physiological changes in the body and finally causes diseases [32-35]. In the present study, Alloxan induction causes the DM in animals, it leads to variations in their body physiological condition [36, 37] by causing the necrosis to the pancreatic β-cells [38-40], this leads to the reduction in the insulin production and finally altering enzymatic levels in different organs functions in the body like kidney, liver etc (Figure 3 to Figure 6). The tested extracts of *Z. gibbosa* showed the significant reduction of the increased blood glucose levels (Figure 1). The reduction of the glucose levels of the *Z. gibbosa* was may be by protecting the β- cells from undergoing necrosis [1, 2]. The weight loss also observed after diabetic induction, probably due to excessive breakdown of the tissues protein and lipid for the energy to maintain body organs function, but after treatment with the extracts of *Z. gibbosa* the animals started to gain the body weight (Figure 2). The gained body weight may be due to the improved metabolic activities by normal levels of glucose in the body.

Insulin helps glucose uptake in muscle and fat and inhibits the 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 I DM) or resistance (Type II DM) results in profound deregulation of these processes, and produce elevations in fasting and postprandial glucose and lipid

levels [41-43]. In the present study also the elevations in electrolytes, Albumin and creatinine levels observed in alloxan induced diabetic rats and *Z. gibbosa* extracts reinstate altered electrolytes, Albumin and creatinine levels in the diabetic rats significantly (Figure 4 and Figure 5). That means *Z. gibbosa* extracts have the ability to protect the nephrons function and increase the electrolytes absorption in renal tubules[44]. The enzyme levels of AST, ALT, ALP, Biluribin indicates the functioning of liver in the body [45, 46] and the alterations in these enzymes were observed in the alloxan-induced diabetic rats, it indicates that DM effects the functions of organs [47]. *Z. gibbosa* extracts significantly revised the reduced or increased enzymes levels of liver and its function (Figure 3) due to DM may be by regeneration of damaged functions due to DM.

DM causes the diabetic dyslipidemia, i.e. low density cholesterol levels (LDL) and raise triglyceride (TG) and high density cholesterol levels (HDL), which increases the risk for heart disease and stroke [48, 49]. The variations in these levels (Lipid profile) was observed in the present experiment and the variations were restored in the *Z. gibbosa* extracts treated groups compared to the diabetic untreated group (Figure 6). This might be due to the reduced hepatosynthesis of triglycerols or reduced lipolysis because deficiency of insulin enhanced the hydrolysis of troacylglycerol [50, 51]. The increased HDL levels in *Z. gibbosa* extracts 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 diabetic condition [52]. From the above results, it can be summarized that, the tested extracts of *Z. gibbosa* have the ability in the restore of physiological changes occurred due to the diabetes like reduction in the glucose concentration, gaining of body weight, failure of organs like kidney and liver.

CONCLUSION

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

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