

Anti-Diabetic Activity Of Leaves Of *Zizyphus nummularia* By Dexamethasone Induced Diabetic Rat Model

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Abstract: The hypoglycemic and hypolipidemic effect of Ethanolic and aqueous extract of *Zizyphus nummularia* (250mg/kg & 500mg/kg) was evaluated by dexamethasone-induced diabetic rats. Animals were induced for diabetes with dexamethasone (10 mg/kg of body weight- s.c.) and treated orally with Ethanolic and aqueous extract of *Zizyphus nummularia*. The extracts showed significant ($p < 0.01$ & $p < 0.05$) anti-hyperglycemic and hypolipidemic activity as compared to diabetic control. The extract shows beneficial effects on blood glucose. It also reduces the elevated biochemical parameters such as triglycerides (TGL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and total cholesterol (TC), increased the reduced level of high density lipoprotein (HDL) and maintains body weight. The histological slides shows normal architecture with extracts treated groups compared to diabetic control. Thus both extracts could serve as good oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

Key words: Hypoglycaemic, Hypolipidemic, *Zizyphus nummularia*, Dexamethasone, Glibenclamide.

INTRODUCTION

According to WHO, the prevalence of diabetes is likely to increase by 35% by the year of 2025 currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 79.4 million by 2025, making it the country with the highest number of diabetics in the world^{1,2}. Diabetes is a serious metabolic disorder with micro and macrovascular complication that results in significant morbidity and mortality³. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries⁴. Modern medicines like Biguanides, Sulphonylureas and Thiozolidinediones are available for the treatment of diabetes. But they also have undesired effects associated with their uses⁵. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability⁶. Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity^{7,8}. Medicinal Plants are a rich source of natural products.

Medicinal plants and their products have been widely used for treatment of diabetic populace all around the world with less known scientific basis of their functioning^{9,10}. Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-diabetic activity. The plant *Zizyphus nummularia* belonging to family *Rhamnaceae* and commonly known as Aja-priya in Sanskrit, Jhar Beri in Hindi, Korgodi in Tamil and Nelaregu in Telugu^{11,12}. The plant used for anthelmintic, blood purification and digestion¹³. As per the literature review plant having hypoglycemic and hypolipidemic property, this is not scientifically documented. In the present study, we reported hypoglycemic and hypolipidemic potentials of *Zizyphus nummularia* in dexamethasone diabetic rat model.

MATERIALS AND METHODS

Collection and authentication of the plant material

The plant *Zizyphus nummularia* had been collected from the field of thoppur forest, Dharmapuri, TamilNadu, India. The plant was identified and authenticated by the botanist Mr. A Balsubramanian, Executive Director ABS botanical garden, Salem, Tamil Nadu.

Extract preparation

The fresh leaves of *Zizyphus nummularia* were collected and dried under shade, sliced into small pieces and ground into powder with mechanical grinder. The powder was passed through sieve no.30 and stored in a container. The dried powder of leaves of *Zizyphus nummularia* was defatted with petroleum ether in a Soxhlet apparatus by hot percolation. The defatted powder material (marc) thus obtained was further extracted with Ethanol (95% v/v) and aqueous extract prepared by maceration. The solvent was removed by distillation under reduces pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. Finally both extract subjected to different phytochemical screening¹⁴.

Experimental Animals

All the experiments were carried out using Swiss Albino mice (25-30 g) and Wister rats (150-200 g). The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2 °C and relative humidity of 30–70%. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed.

Acute oral toxicity studies

The acute toxicity study was carried out as per OECD 425 Guidelines. Mortality in each group within 24 h was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The Ethanolic and aqueous extract of *Zizyphus nummularia* had good margin of safety and did not shown any lethal effects on the animals up to the doses of 5000mg/kg. Hence the LD50 of *Zizyphus nummularia* was considered as 5000mg/kg. Studies were carried out with 1/10 of the LD50 as effective dose 250mg/kg and double the dose of effective dose 500 mg/kg.

Dexamethasone induced diabetic model¹⁵

In the experiment a total of 42 overnight fasted rats were used. The 36 rats were rendered diabetic by the aqueous solution of Dexamethasone (10mg/kg, s.c.). The animals divided into seven groups of six rats each. Group I normal control received 5% CMC, Group II served as Diabetic control, Group III served as standard treated with 5 mg/kg of Glibenclamide, Group IV & V treated with 250 and 500 mg/kg of Ethanolic extracts and Group VI & VII administered with 250 & 500 mg/kg of aqueous extracts. Treatment was continued for 11 consecutive days along with dexamethasone except normal control. The blood samples were collected from the retro orbital of each rat under mild ether anesthesia on 11th day and serum separated by centrifugation of blood at 4000 rpm for 10mins. Blood Samples were subjected to glucose measurement and separated serum was used for the estimation biochemical parameters of TGL, HDL, LDL, VLDL and TC by a semi auto analyzer. Finally the animal was sacrificed, pancreas was isolated and examined (Sharma SR et al., 1997; Dash GK et al., 2005).

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Dunnett's method of multiple comparisons was employed using Graphpad Instat 3.0 software. A probability value of $p < 0.01$ & $p < 0.05$ was considered to be statistically significant.

RESULT

Preliminary phytochemical screening

The preliminary phytochemical analysis of both the extracts of *Zizyphus nummularia* shows presence of flavonoids, saponins, alkaloids, mucilage, tannins and phenolic compounds.

Acute toxicity

Acute oral toxicity studies following OECD guidelines-425, up and down procedure, showed that both the extracts upto 5000mg/kg are non-toxic and safe.

Body weight

The table.1 shows the body weight of the normal and treated groups significantly differ from diabetic control on 11th day. The treated groups animal body weight maintained throughout the experiment compare to diabetic control except.

Blood glucose level

The standard (Glibenclamide (5mg/kg) and Ethanolic and aqueous extract (250 & 500mg/kg) treated groups, the peak values of blood sugar significantly decreased to 116.66, 153.50, 142.83, 153.83 and 146.66 mg/dl simultaneously on the 11th day (**Table 2 & Figure 1**). Thus, the Ethanolic extract 500mg/kg was found to be more significant ($p < 0.01$) as standard drug in lowering blood glucose level compare to diabetic control.

Biochemical parameters

Table 3 & Figure 2 shows extract have significantly reversed the diabetes-induced hyperlipidemia Compared to diabetic control. A significant percentage reduction of total cholesterol level, LDL, TGL and VLDL in extracts treated was significant to diabetic group. However HDL level increased with extracts and GLB group respectively.

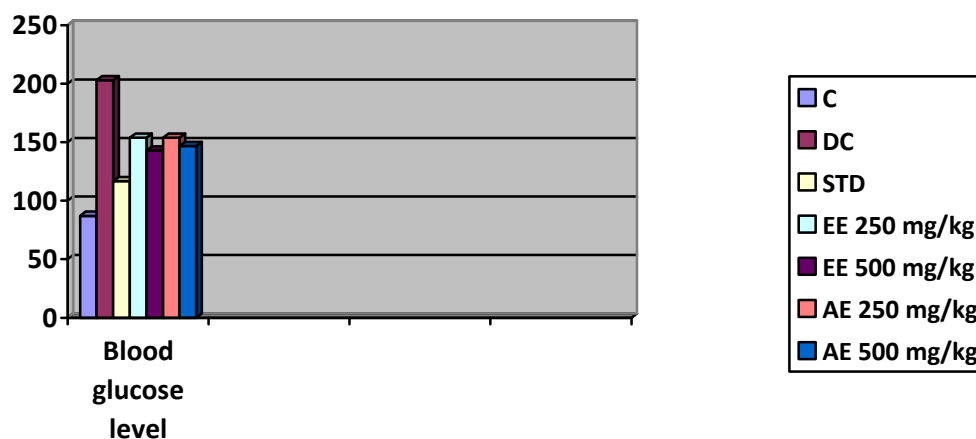
Table 1 Effect of Ethanolic and Aqueous extracts of dried leaves of *Zizyphus nummularia*(Burm.f.)Wight&Arn on body weight by Dexamethasone induced rats.

S. No	Treatment	Initial body wt. in gm	3 rd day	6 th day	9 th day	11 th day
1	Normal control	108.33±8.16	109.16±10.20	112.5±10.36	112.5±6.89	113.33±8.16
2	Diabetic control	103.33±4.08	80±3.34	74.17±3.76	75.83±3.76	69.16±5.84
3	Standard treated glibenclamide (5mg/kg)	105 ^{ns} ±6.32	81.66±4.08	90±3.16	94.17±3.76	100.83±3.76
4	Dexa+Alcoholic ext.250 mg/kg	103.33±4.08	80±3.16	83.05±4.88	85±4.47	87.5±5.24
5	Dexa+Alcoholic ext. 500 mg/kg	106.66±8.05	81.83±4.26	88.83±5.84	90.83±4.91	94.16±3.76
6	Dexa+Aqueous ext.250 mg/kg	106.66±8.75	80.16±3.52	85.83±3.76	86.67±5.16	95.83±5.84
7	Dexa+Aqueous ext.500 mg/kg	105.83±10.22	84.17±5.84	90±6.89	94.16±2.03	100±3.16

Table 2 Effect of Ethanolic and Aqueous extracts of leaves of *Zizyphus nummularia*(Burm.f.)Wight&Arn, on blood glucose level on Dexamethasone induced rats after 11 days.

S. No	Groups	Blood glucose concentration (mg/dl)
1	Normal control	87±2.352
2	Diabetic control	203±3.011
3	Standard treated glibenclamide (5mg/kg)	116.66±2.04*
4	Ethanol Ext. 250 mg/kg	153.50±3.31*
5	Ethanol Ext. 500 mg/kg	142.83±2.41*
6	Aqueous Ext.250 mg/kg	153.83±2.54*
7	Aqueous Ext.500 mg/kg	146.66±2.62*

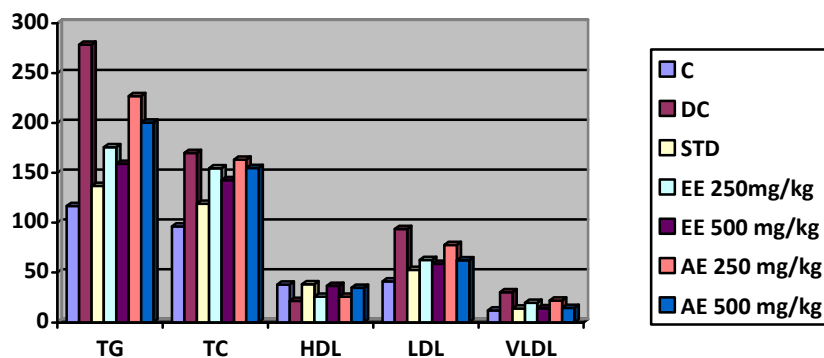
The values are mean±SEM, n=6 when compared with diabetic control *p<0.01

Figure 1 Effect of Ethanolic and Aqueous extracts of leaves of *Zizyphus nummularia* (Burm.f.)Wight&Arn, on blood glucose level on Dexamethasone induced rats after 11 days.**Table 3- Effect of Ethanolic and Aqueous extracts of dried leaves of *Zizyphus nummularia* (Burm.f.)Wight&Arn on biochemical parameters after 11 days treatment by dexamethasone induced rats.**

S. No	Groups	Serum Triglyceride concentration (mg/dl)	Serum Total cholesterol concentration (mg/dl)	HDL mg/dl	LDL mg/dl	VLDL mg/dl
1	Normal control	116.74±2.67	96.36±4.96	37.83±0.72	41.00±2.80	11.96±0.72
2	Diabetic control	278.41±10.16	169.81±10.11	21.5±1.4	93.50±2.38	30.33±1.22
3	Standard treated glibenclamide (5mg/kg)	136.88±24.93*	118.86±11.42*	38.33±6.72*	52.67±1.82*	13.98±1.12*
4	Ethanol ext. 250 mg/kg	175.52±7.77*	154.40±17.69*	25.83±1.4*	62.58±0.85*	19.73±0.95*
5	Ethanol ext. 500 mg/kg	159.00±8.33*	142.56±10.33*	36.67±1.26*	58.67±1.52*	14.10±1.42*
6	Aqueous ext.250 mg/kg	226.69±8.36*	163.05±10.15*	25.67±1.16*	77.76±4.37	21.83±0.60*
7	Aqueous ext.500 mg/kg	200.41±11.05*	154.87±10.51*	34.83±0.94*	62.33±1.44*	14.55±1.55*

The values are mean±SEM, n=6 when compared with diabetic control *p<0.05

Figure 2 Effect of Ethanolic and Aqueous extracts of dried leaves of *Zizyphus nummularia*(Burm.f.)Wight&Arn on biochemical parameters after 11 days treatment by dexamethasone induced rats.



Histopathological studies

Normal control

Section shows pancreas with normal architecture. The islets are composed of normal acini. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. There is no evidence of inflammation or necrosis.

Diabetic control

Section shows pancreas with engorged and congested blood vessels. The islets show patchy necrosis. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. There is mild inflammation composed of lymphocytes.

Standard

Section shows pancreas with normal architecture. The islets are composed of normal acini. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

Ethanolic extract 250 mg/kg

Section shows pancreas. There is a granulomatous infiltrate of lymphocytes within the stroma. The acinar cells are normal.

Ethanolic extract 500 mg/kg

Section shows pancreas with normal architecture. The islets are composed of normal acini. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. There is an infiltrate of lymphocytes and a few plasma cells within the stroma.

Aqueous extract 250 mg/kg

Section shows pancreas with patchy necrosis. The islets are composed of normal acini. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei. There is an infiltrate of lymphocytes and a few plasma cells within the stroma.

Aqueous extract 500 mg/kg

Section shows pancreas with normal architecture. The islets are composed of normal acini. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

Histopathology of rat pancreas

Figure 3 Diabetic control

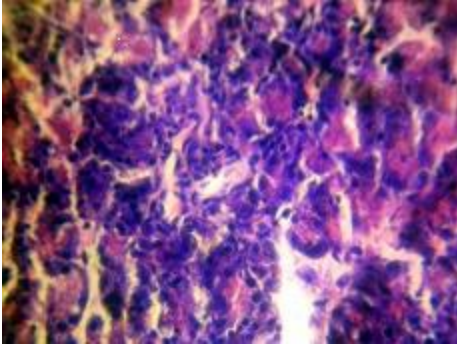


Figure 6 Ethanolic extract 250mg/kg

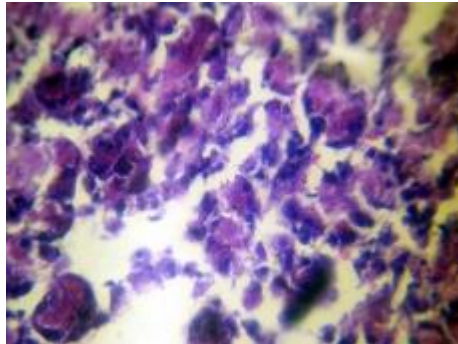


Figure 4 Normal control

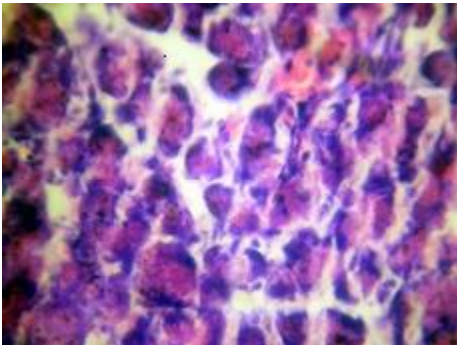


Figure 7 Ethanolic extract 500mg/kg

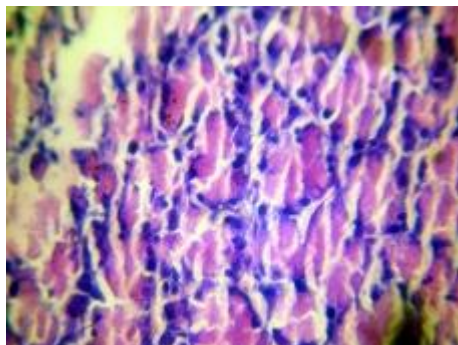


Figure 5 Standard

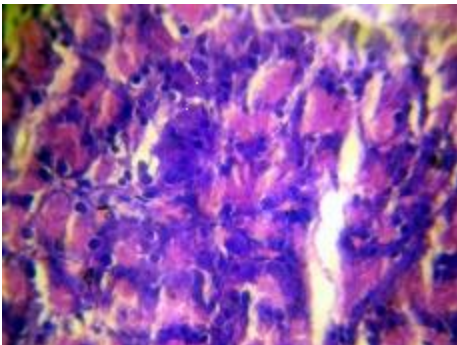


Figure 8 Aqueous extract 250 mg/kg

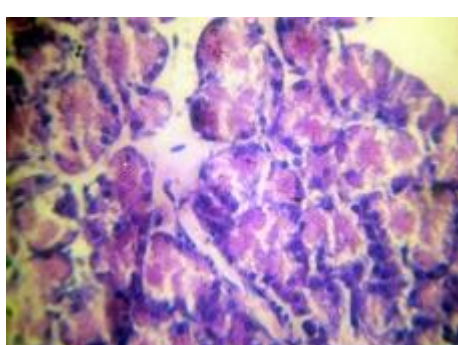
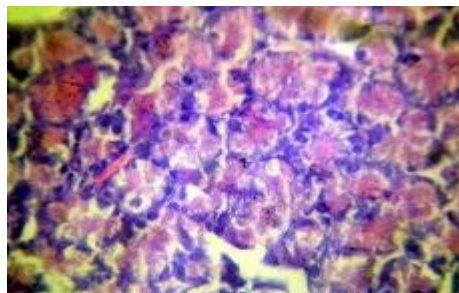


Figure 9 Aqueous extract 500mg/kg



DISCUSSION

Glucocorticoids are widely used therapeutic tools, particularly in treatment for anti-inflammatory and immunomodulatory purposes. Side effects of glucocorticoid treatment include steroid diabetes^{16,17}. Glucocorticoid-induced hyperglycemia is partially due to increased hepatic glucose production and insulin resistance of peripheral tissues. Moreover, glucocorticoids are known to inhibit insulin secretion^{18,19}. The underlying mechanism involves increased α_2 -adrenoceptor signaling²⁰, increased Kv channel activity²¹ and impaired glucose metabolism^{22,23}. Although reduced insulin secretion during glucocorticoid treatment can be overcome by blocking adrenoceptor signaling or by inhibition of Kv channels, compelling evidence suggests that the proper functioning of β -cells also depends on cell survival²⁴. Accordingly, a reduction of β -cell mass in long-standing glucocorticoid therapy may contribute to the consecutive development of steroid diabetes. Lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. High level of TC and LDL are major coronary risk factors. Hence, measurements of biochemical parameters are necessary to prevent cardiac complications in diabetes condition. In this study, *Zizyphus nummularia* extracts showed significant reduction in TC, TG, LDL, VLDL levels and increased level of HDL in diabetic model rats. However, the increased HDL (cardioprotective lipid) Therefore, *Zizyphus nummularia* has potential role to prevent formation of atherosclerosis and coronary heart disease. Again the histological studies revealed extracts protect the pancreas and it may be interfere with α_2 adreno-receptor this may be influenced on the level of insulin to maintain the normal glucose level. Several authors reported that secondary metabolites, such as saponins, flavonoids, phenolic compounds, and triterpenoids have anti-hyperglycemic and hypolipidemic activity²⁵⁻²⁷. Hence, the hypolipidemic properties of *Zizyphus nummularia* may be due to different types of active secondary metabolites, each with a single or diverse range of biological activities.

CONCLUSION

The present study demonstrated that both extracts of *Zizyphus nummularia* could be useful in management of diabetes associated with abnormalities in lipid profiles. Further study need to be isolate, identify the active compounds and find out the possible mechanism of actions.

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