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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF LEAVES OF *ZIZYPHUS NUMMULARIA (Burm.f.)Wight&Arn.*

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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 Alloxan-induced diabetic rats. Animals were induced for diabetes with Alloxan (150 mg/kg of body weight- i.p.) and treated orally with Ethanolic and aqueous extract of *Zizyphus nummularia.* The extracts showed significant ($p<0.01$) 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), total cholesterol (TC), increased the reduced level of high density lipoprotein (HDL) and maintain 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, Hypolipidemi, *Zizyphus nummularia*, Alloxan, 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 (King H *et al*., 1998) (Boyle JP *et al*., 2001). Diabetes is a serious metabolic disorder with micro and macrovascular complication that results in significant morbidity and mortality (Rang HP *et al*., 1991). Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (Sharma AK, 1993). Modern medicines like Biguanides, Sulphonylureas and Thiozolidinediones are available for the treatment of diabetes. But they also have undesired effects associated with their uses (Fowler MJ, 2007). Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability (Valiathan MS, 1988). Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity (Dineshkumar B *et al*., 2009; Grover JK *et al*., 2002). 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 (Patwardhan B *et al*., 2004; Said O *et al*., 2007). Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-diabetic activity. The plant *Zizyphus nummularia* belonging to

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family *Rhamnaceae* and commonly known as Aja-priya in Sanskrit, Jhar Beri in Hindi, Korgodi in Tamil and Nelaregu in Telugu (Kaul RN, 1963) (Ghosh RC, 1977). The plant used for anthelmintic, blood purification and digestion (Gupta RK, 1975). 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 *Ziziphus nummularia* in diabetic rat model.

MATERIALS AND METHODS

Preparation of extracts

The fresh leaves of *Zizyphus nummularia* were collected and dried under shade and ground into powder with mechanical grinder. The powder was passed through sieve no.30. The extract was prepared by continuous hot extraction using ethanol and water as a solvent.

Preliminary phytochemical screening

The extracts of *Zizyphus nummularia* was screened for the presence of various phytoconstituents like steroids, alkaloids, flavonoids, saponin, mucilage, tannin and phenolic compounds (Kokate CK, 1986).

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^{o}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.

Alloxan induced anti-diabetic study

In the experiment a total of 42 overnight fasted rats were used. The 36 rats were rendered diabetic by the intraperitoneal injection of Alloxan (150 mg/kg) 48hrs after Alloxan injection, the animals which did not developed hyperglycemia i.e. glucose level > 200mg/dL, were rejected and replaced with new animals. Immediately after confirmation of diabetes rats were classified 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 14 consecutive days with once a day dose. The blood samples were collected from the retro orbital of each rat under mild ether anesthesia on 0th, 3rd, 6th, 9th and 14th 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 $p<0.05$ was considered to be statistically significant.

RESULT

Preliminary phytochemical screening

The preliminary phytochemical analysis of Ethanolic extract 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 Figure.1 shows the body weight of the normal and treated groups significantly differ from diabetic control on 14th day. The treated groups animal body weight maintained throughout the experiment compare to diabetic control.

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 130, 155, 133, 157 and 144 mg/dL simultaneously on the 14th day (Table 2). 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

Figure 3 shows extracts has 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.

Histopathological Studies

The histological changes in the pancreas were shown in Figure 4-10. On histological studies, the diabetic control shown different morphology of islets of pancreas, islets are disrupted and acini tissue is dissolved. In standard and extracts groups shows normal architecture of pancreas and no specific abnormalities were observed except 250mg/kg of Ethanolic and aqueous extracts. The 250mg/kg of both extracts revealed depletion of cells in islets but architecture is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

Figure 1. Effect of Alcoholic and aqueous extracts of dried leaves of *Zizyphus nummularia(Burm.f.)Wight&Arn* on Body weight by Alloxan induced rats

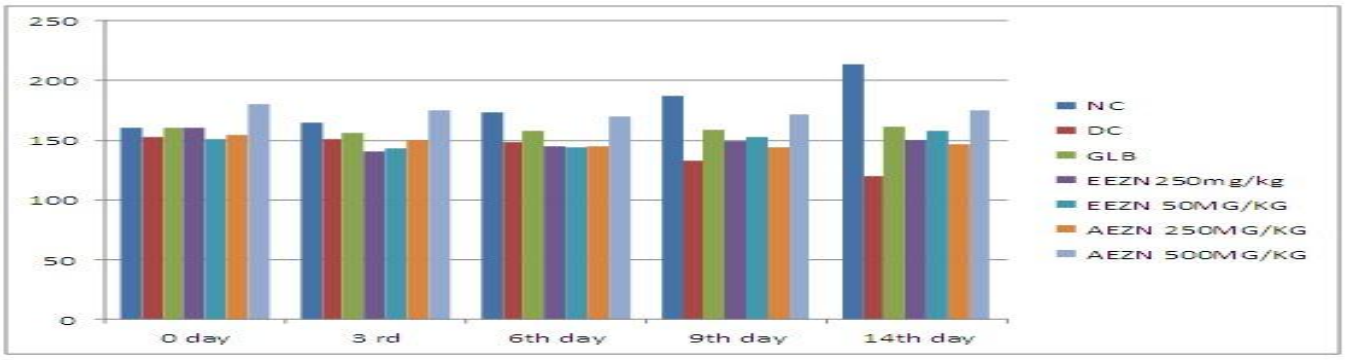


Figure 2. Effect of Ethanolic and aqueous extracts of leaves of *Zizyphus nummularia (Burm.f) Wight&Arn,* blood glucose level on Alloxan induced rats

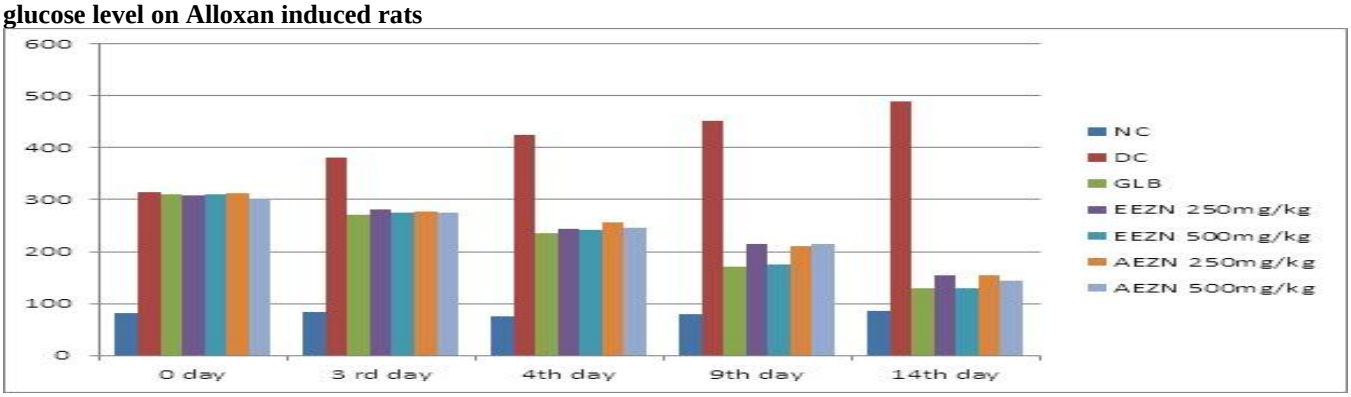


Figure 3. Effect of Ethanolic and aqueous extracts of dried leaves of *Zizyphus nummularia (Burm.f) Wight&Arn italic)* on biochemical parameters after 14 days treatment by Alloxan induced rats

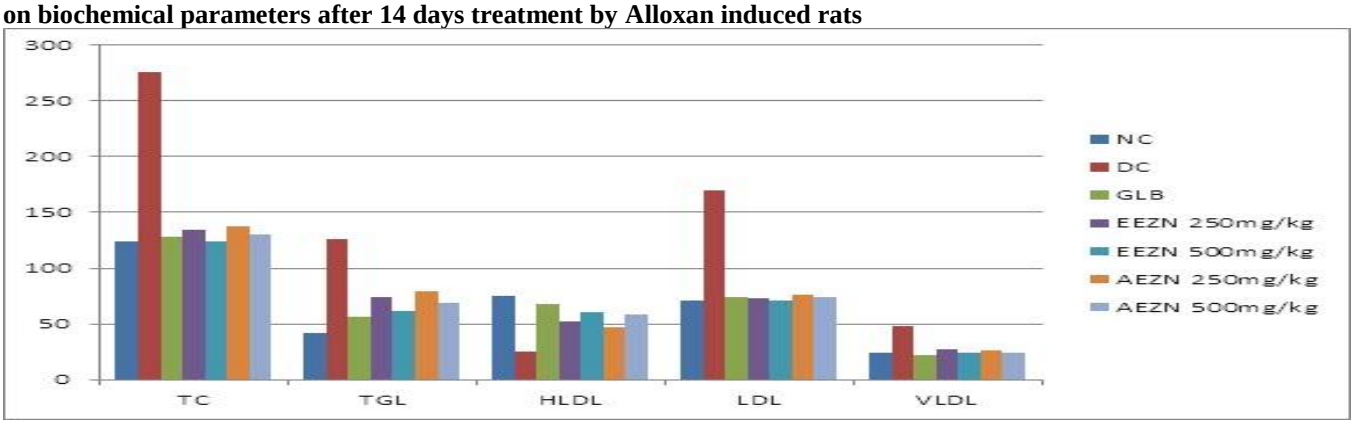


Figure 4. Normal control

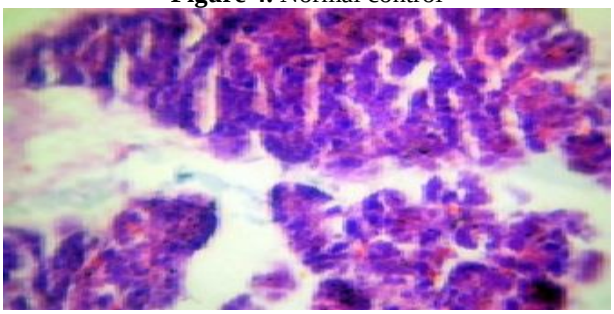


Figure 5. Diabetic control

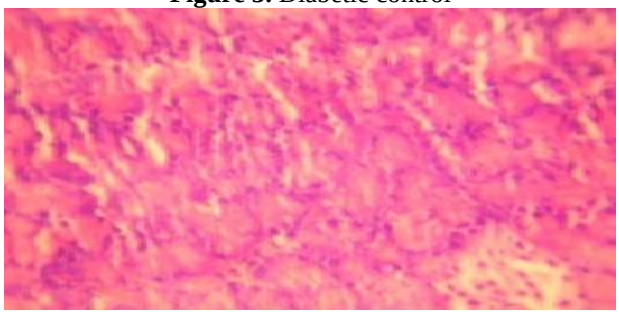


Figure 6. Standard

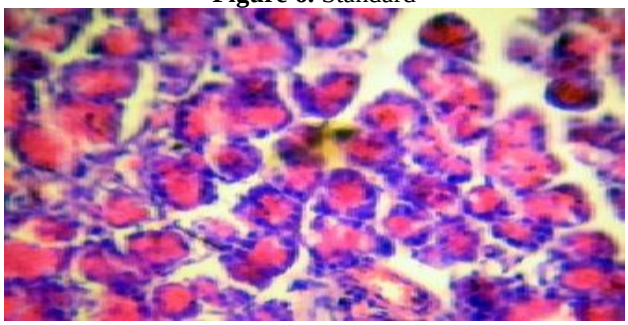


Figure 7. Ethanolic. Ext 250mg/kg

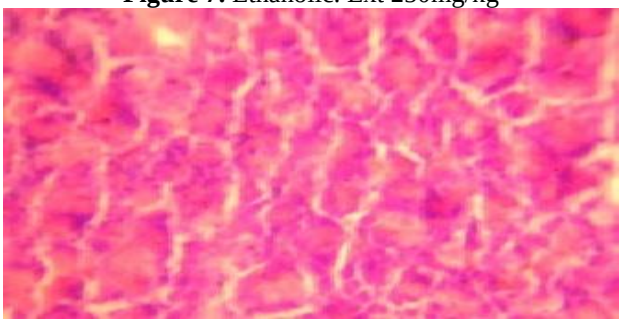


Figure 8. Ethanolic.Ext 500mg/kg

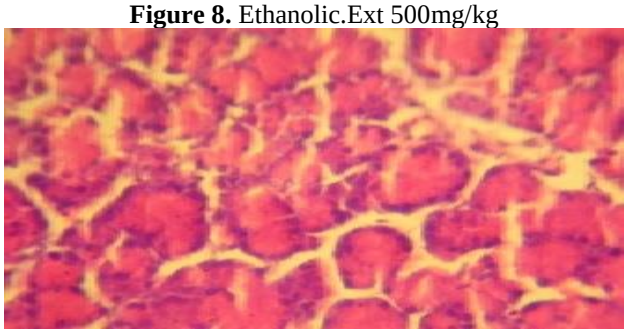


Figure 9. Aqueous.Ext 250mg/kg

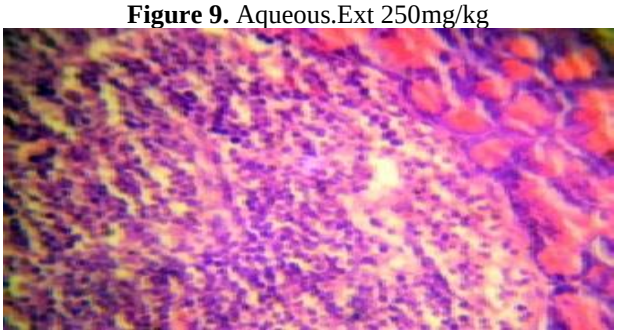
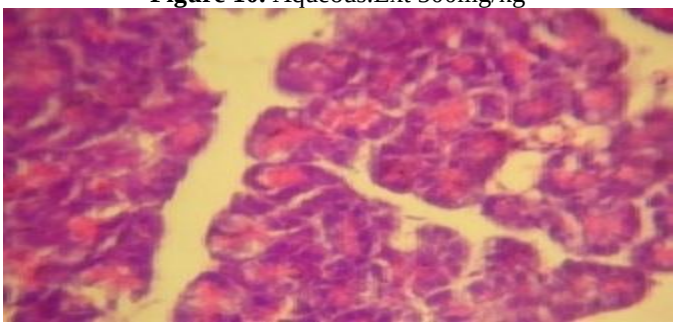


Figure 10. Aqueous.Ext 500mg/kg



DISCUSSION

The present study was undertaken to investigate the antidiabetic and hypolipidemic effects of *Zizyphus nummularia.* Alloxan produce highly reactive hydroxyl radicals the actions of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of β-cells and thus increase the blood sugar (Szkudelski T, 2001; Yadav SB *et al*., 2000). 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 (Temme Eh *et al*., 2002). 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)

level by *Zizyphus nummularia* was comparable to the standard drug Glibenclamide. Therefore, *Zizyphus nummularia* has potential role to prevent formation of atherosclerosis and coronary heart disease. Again the histological studies revealed extracts reverse the damage of pancreas and 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 activity (Leontowicz H *et al*., 2002; Kimura Y *et al*., 1989; Ogawa H *et al*., 2005). Hence, the hypolipidemic properties of Ougenina Oojeinensis 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 formulation.

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