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Anti-Diabetic activity of hydro-alcoholic root extract of *Echinops echinatus* and its beneficial effects on nephropathy in experimental rats

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ABSTRACT

Anti-diabetic activity of 70% Hydro-alcoholic root extract of *Echinops echinatus* at a dose of (100& 200 mg/kg body weight p.o) was investigated in diabetes Nephropathic rats. α- Amylase inhibitory activity of the *Echinops echinatus* was determined against Diastase and has shown 72% inhibitory effect of amylase at conc of 25 mg/ml. The preliminary phytochemical screening of hydro-alcoholic root extract revealed the presence of alkaloids, carbohydrates, steroids, glycosides, flavonoids, phenols, tannins, aminoacids and absence of saponins and terpenoids. In acute toxicity study (423 Guideline), there was no mortality up to a dose of 2000mg/kg body weight p.o, thus considered as maximum tolerated dose. When treated with 70% Hydro-alcoholic root extract of *Echinops echinatus* at a dose of 100 and 200 mg/kg b.w p.o. Group IV and group V has produced significant regeneration of cells in diabetic nephropathic rats under histopathological studies. The effect was more pronounced in 200 mg/kg b.w than 100mg/kg body weight & showed Nephroprotective as obvious by significant reinstatement levels of Oral glucose tolerance test, blood glucose levels in biological parameters serum urea, serum creatinine &serum lipid profile (Triglycerides, Total cholesterol, HDL, LDL, VLDL)The purpose of the study was to evaluate the antidiabetic activity of hydroalcoholic extract on kidneys in experimental rats by using α- amylase & Alloxan induced diabetic rats. Results showed that the 70% Hydro-alcoholic root extract of *Echinops Echinatus* exhibited significant and dose-dependent Anti-diabetic activity in all the diabetic common rats. Annova tests for biological parameters(blood glucose level, Serum urea, serum creatinine, HDL, LDL, VLDL) was performed by using Dunnet's t- tests and was found to be significant $p<0.0001$; $p<0.01$; $p<0.05$. Results of our study suggest that 70% Hydro-alcoholic root extract of *Echinops echinatus* possess Anti-diabetic activity which may be due to the presence of flavonoids in the extract as it has anti-secretory, cytoprotective and antioxidant properties, anti-diabetic, anti-hyperglycemic, anti-inflammatory, hypoglycemic, diuretic, anti-bacterial, anti-fungal, antispasmodic, antipyretic and analgesic.

1. INTRODUCTION

Echinops echinatus (family: Asteraceae) known as Brahmadandi is an indigenous herb found in India. It is a commonly occurring weed present in many regions of the country. It is composition of many traditional remedies. *Echinops echinatus* has occupied a pivotal position in Indian culture and folk medicine. It has been used in almost all the traditional system of medicine, such as in Ayurveda, Unani and Siddha1 Traditionally the plant is reported to be used as asthmatic,abortification, aphrodisiac, antipyretic, analgesic, ophthalmic, chronic fever, inflammation and used in brain disorders . Till date no work has been reported on the anti-diabetic with nephropathy on medicinal value of *Echinops echinatus.* .Therefore the present study is the first attempt to assess the anti-diabetic activity of hydro alcoholic root extract of *Echinops echinatus* and its beneficial effects on Nephropathy

2. MATERIALS AND METHODS

Collection and Authentication of Plant material: The roots of *Echinops echinatus* were collected from Pochampad village, Nizambad Dist. The whole plant and the roots were authenticated by an expert Botanist P.Venu (Additional Director) at Botanical survey of India bearing Survey No. BSI/DRC/2013-2014/Tech./969 at Attapur road, Hyderabad.

Extraction and Phytochemical Screening: The collected Roots of *Echinops echinatus* was washed with distilled water from dust and shade dried at room temperature for about 15-18 days and the shade dried leaves were crushed mechanically to get a coarse powder. The powdered roots (200gms) was successively extracted with 340ml of Ethanol and 145 ml of distilled water in 7:3 ratio by Soxhalation method For further concentration of the extract it was evaporated on water bath at 50˚c which gave a sticky residue. It was stored in an airtight container in a

refrigerator. The concentrated extract was weighed. Standard methods were used for preliminary phytochemical screening to know the nature of phytoconstituents present.

Chemicals: Ethanol, α-amylase, potassium dihydrogen orthophosphate, dipotassium hydrogen phosphate anhydrous, starch, sodium potassium tartarate tetrahydrate, Sodium hydroxide, Dinitro Salicylic acid, Glucose.

Drugs: Standard drug – Sitagliptin [35 mg/kg body weight p.o], Inducing drug –Alloxan monohydrate [120mg/kg body weight i.p].

Experimental Animals: The experimental protocol was approved by Institutional Animal Ethics Committee, Central Animal House (Registration No. 769/2011/CPCSEA), SICRA Labs Pvt. Ltd., Hyderabad, India. Female Albino Wistar rats (180–200 g) were used for Acute Toxicity study and male wistar rats weighing between (180-200 g) were used for evaluating antidiabetic activity. The animals were maintained under standard laboratory conditions, 12-hr light/ dark cycle at appropriate room temperature of 25°C temperature. All animals were acclimatized to laboratory environment for at least one week and they were given standard pellet diet and water ad libitum before the commencement of experiment.

Evaluation of In-vitro model to Study the Inhibition of Carbohydrate Digesting Enzymes:

α- amylase inhibitory activity of Hydro-alcoholic root extract of *Echinops echinatus*: The inhibitory activity of different concentration of root extract (0-50mg/ml) was determined against α-amylase by the following method. The enzyme(0.5%) was prepared in Phosphate buffer (pH6.8).Breifly,500µl of different concentration of root extract and 500µl of 0.1M Phosphate buffer (P^h6.8) containing α – amylase were incubated at 25^{o}c for 10 minutes. After pre incubation, 500µl of a 1%starch solution in 0.1M Phosphate buffer (P^h 6.8) was added to each tube and further incubated at 25^{O}C for 10minutes. The reaction was stopped by addition of 1ml of dinitro salicylic acid reagent. The same was performed for Control where extract was replaced with buffer. The test tubes were placed in a boiling water bath at 45^{O}C for 10minutes and cooled. To each tube, 10ml of distilled water was added and the absorbance was measured spectrophotometrically at 540nm.

Acute Toxicity Studies: Animals were fasted prior to dosing, food but not water was withheld overnight. Following the period of fasting, the animals were weighed and the test substance was administered orally. After the substance was administered, food was withheld for a further 3-4 hrs. As a dose was administered in fractions over a period, it was necessary to provide the animals with food and water depending on the length of the period.

Three animals were used for each step. The dose level used as the starting dose was selected from one of the four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level was that which was most likely to produce mortality in some of the dosed animals. After the administration of the test samples, the animals were observed for behavioral changes for 4 hours and 48 hours for mortality rate, if any.

Oral Glucose Tolerance Test: Animals were fasted for 24 hours before experimentation but they were allowed free access to water. Fasted rats were divided into 5 groups of six rats each.

Group.1: Normal rats received Normal Saline (p.o).

Group.2: Control rats received Glucose (2g/kg b.w. p.o.) in Normal saline.

Group.3: Standard drug: Rats received standard (Sitagliptin 35mg/kg p.o) + Glucose (2g/kg b.w. p.o.) in Normal saline.

Group.4: Treatment.1: Rats received Effective dose 1 (100mg/kg p.o) + Glucose (2g/kg b.w. p.o.) in Normal saline.

Group.5: Treatment.2: Rats were received Effective dose 2(200mg/kg p.o) + Glucose (2g/kg b.w. p.o.) in Normal saline.

The rats of all groups were given glucose (2g/kg body weight, per orally) 30 min after administration of the drug. Blood samples were collected from the tail vein at every 0, 30, 60, 90 and 120 min after the glucose loading. The amount of blood glucose level was estimated for both control and drug treated groups by tail tipping method using Gluco-chek sensor Glucometer.

Evaluation of *In vivo* model for assessing oral Anti diabetic activity:

Effect of Hydro-alcoholic root extract of *Echinops echinatus* on Alloxan monohydrate Induced Diabetes: 30 Albino rats of either sex (150-200gms) were used into 5 groups with 6 rats each and grouped as follows:

Table.1.Gropuing of animals and treatment pattern

Groups	Inducing Agent	Treatment
Group 1: Normal (n=6)	-	Rats were given Normal saline p.o.
Group 2: Control (n=6)	Alloxan monohydrate **(120mg/kgb.w,i.p)**	Rats were given Normal saline p.o.
Group 3: Standard(n=6)		Treated with Standard drug Sitagliptin (35mg/kg b.w) in Normal saline p.o
Group 4: Extract I (n=6)		Treated with 70% Hydro alcoholic extract of *Echinops ehinatus*(100mg/kg b.w) in Normal saline p.o.
Group 5: Extract II (n=6)		Treated with 70% Hydro alcoholic extract of *Echinops echinatus* (200mg/kg b.w) in Normal saline p.o.

The normal and diabetic control groups were given 1ml normal saline, p.o. Animals in the Group 2 to 5 were made diabetic by inducing Alloxan monohydrate at a dose of 120mg/kg of body weight, i.p, with Normal saline. Animals in the third group were treated with the Standard drug i.e., Sitagliptin at a dose of 35mg/kg.b.w, p.o, and fourth group animals (T1) were given hydro alcoholic root extract of *Echinops echinatus* at a dose of 200mg/kg of body weight while fifth group animals (T2) were treated with hydro alcoholic root extract of *Echinops echinatus* at a dose of 200mg/kg.bw, p.o, for 21 days.

Biochemical estimations: Body weights and blood glucose levels were checked at every seven days intervals throughout the duration of experiment. The blood glucose levels were determined by tail tipping method using Gluco check sensor Glucometer. On the 21st day blood from all groups was collected by retro-orbital puncture under mild anesthesia, Serum was separated quickly for estimating Serum urea, Serum creatinine, Triglycerides, Total Cholesterol, High density lipoprotein, Low density lipoprotein andVery high density lipoprotein.

Histopathological studies: On the last day of the experiment, animals were decapitated and pancreas and kidneys were removed and was fixed in 10%neutral formalin. Then they were embedded in paraffin and 3 µ thick sections slides were prepared. The sections were stained with Haematoxylin and Eosin stain and were examined under light microscope.

Statistical Analysis: The data was represented as Mean ± SEM. The data of anti-ulcer activity of 70% Hydro-alcoholic root extract of *Echinops Echinatus* was analysed by one way ANOVA followed by Dunnett‟s *t*-test and the whole analysis was carried out using Graph Pad Prism 6.0 version ©1992-2004. „P‟ value were considered significant when *P<0.05, **P<0.01, *** P<0.001 when the test and reference were compared with the control group.

3. RESULTS AND DISCUSSION

Percentage yield of total extract: 200gm of *Echinops echinatus* plant powder in dry form was taken and to that 340ml of ethanol and 145 ml of distilled water i.e., 70% ethanol and 30% distilled water was added in a Soxhalet apparatus, after evaporation in a water bath 9 gram of extract has been found and the percentage yield of extract was found to be 4.5%.

Preliminary Phytochemical screening of *Echinops echinatus* extract: The results of preliminary phytochemical screening of hydro-alcoholic root extract of Echinops echinatus revealed the Presence of alkaloids, carbohydrates, steroids, glycosides, flavonoids, phenols, tannins, proteins and aminoacids and absence of saponins and triterpenoids. The result are represented in Table 2.

Acute Toxicity Studies: There was no behavioral changes seen up to 4 hours and no mortality was observed up to the end of 48hrs even at the maximum tested dose level of 2000mg/kg per oral. Based on these results an Effective dose of 1/5th, 1/10th of maximum tolerated dose i.e., 100mg/kg and 200mg/kg body weight was taken.

Table.2. Preliminary Phytochemical screening of *Echinops echinatus* extract

Test	Inference	Test	Inference
Test For Alkaloids		**Test For Glycosides**	
Dragendroff's Test	+Ve	Legal Test	+Ve
Mayer's Test	+Ve	Balijet Test	+Ve
Wagner's Test	+Ve	Borntrager's Test	+Ve
Hager's Test	+Ve	**Saponin Test**	
Test For Carbohydrates		Foam Test	-Ve
Molisch's Test	+Ve	Haemolytic Test	-Ve
Fehling's Test	+Ve	**Test For Flavonoids**	
Benedict's Test	+Ve	Shinoda Test	+Ve
Barfoed's Test	+Ve	Sodium Hydroxide Test	+Ve
Test For Steroids		**Test For Triterpinoids**	-Ve
Libermann Burchard Test	+Ve	**Detection of Phenolics and Tannins**	
Salkowski Test	+Ve	Ferric Chloride Test	+Ve
Test For Proteins And Amino Acids		Dilute HNO_3 Test	+Ve
Biuret Test	+Ve		
Ninhydrin Test	+Ve		

Table.3. Acute Toxicity Studies

Behavioral Responses	
Stereotypy	Decreases
Irritability	No change
Fearfulness	Increases
Touch response	Increases
Analgesia	Increases
Spontaneous activity	Increases
Grooming	Increases
Restlessness	No change
Inclined plane test	No change
Body Temperature	No change
Neurological Responses	
Righting response	No change
Limb tone	No change
Grip strength	Increases
Twitching	No change
Abdominal tone	Increases
Pinneal reflex	No change
Corneal reflex	No change
Straub tail	Increases
Tremors	No change
Convulsions	No change
Catalepsy	No change
Autonomic Responses	
Writhing	Increases
Defecation	Increases
Urination	Increases
Piloerection	Increases
SMA	No change
Respiration	No change
Pupil size	No change
Cyanosis	No change
Heart rate	No change
Ataxia	Increases
Ptosis	No change
Salivation	No change

Effect of Hydro-alcoholic root extract of *Echinops echinatus* on α-Amylase inhibitory activity: The study on the α- amylase inhibitory activity of the *Echinops echinatus* was determined against Diastase (amylase).The extract caused a dose- dependent inhibition of amylase activity. The highest inhibition of amylase i.e. 72% was observed at Hydro-alcoholic root extract of Echinops echinatus concentration at 25mg/ml. The result is represented in figure 1.

Oral Glucose Tolerance test: For the oral glucose tolerance test, the glucose levels were estimated before drug treatment and at different intervals. In the Control group the glucose level was found to increase linearly from basal value of 113.16±2.982 mg/ml to 127.66±1.358 mg/ml in the first 30 minutes. After 60 min of glucose loading, the blood glucose was found to increase further to 143.5±1.839 and at 90th min glucose levels of 100±0.966 mg/ml was seen and at 120th glucose level got decreased to 92.33 mg/ml. Whereas, in the Glucose loaded animals treated with 100mg/kg,b.wt extract, only a little elevation in the blood glucose was seen and maximum glucose tolerance was observed i.e., 132.83±1.77 mg/ml at 60th minute and at 90th min glucose level got declined to 90.66mg/ml and at 120th min to 86.39 mg/ml. For 200mg/kg,b.wt maximum glucose tolerance was seen i.e., 129.75±1.982 at 60th minute which got decreased further at 90th min to 86.83 and 81mg/ml at 120th min.In standard Sitagliptin group the blood glucose level was found to be107 at 30 min and at 60th min the blood glucose level was found to increase further to 127 mg/ml and at 90th & 120th min the blood glucose level was decreased to 81.7 mg/ml and 74.16 mg/ml .The results have been tabulated in the Table 4 and represented in figure 2.

Table.4. Oral Glucose Tolerance test

Groups	0 Min	30 Min	60 Min	90 Min	120 Min
Normal	92 ± 1.065	89.1 ± 1.79	94±1.291	87 ± 1.06	92.33±1.14
Control	113.16±2.982	127.6±1.358	143.5 ± 1.839	100±0.966	94 ±2.00
Standard	81.24±1.402***	107 ±1.18*	127 ± 1.317	81.7±0.742**	74.16±1.49***
T1	97.16 ± 2.056	125.3 ±1.33	132.83±1.77**	90.66 ±0.881	86.39±1.67*
T2	85 ± 1.065**	122.36±1.29	129.75±1.982	86.83±1.249**	81±1.549*

Values are given as Mean ± SEM for five groups of six animals in each group. Standard drug treated rats and Extract treated rats were compared with diabetic control rats. ***P<0.0001, **P<0.01 , *P<0.05 is considered extremely Significant.

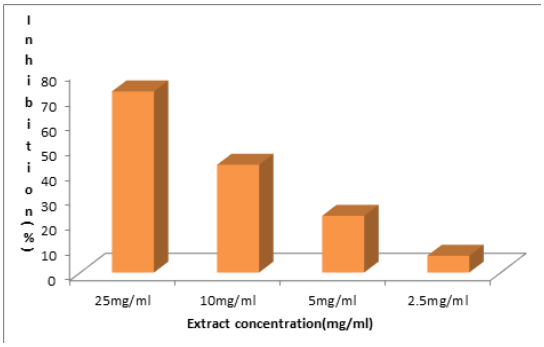

Figure.1.Amylase inhibitory activity of Hydro-alcoholic root extract of Echinops echinatus

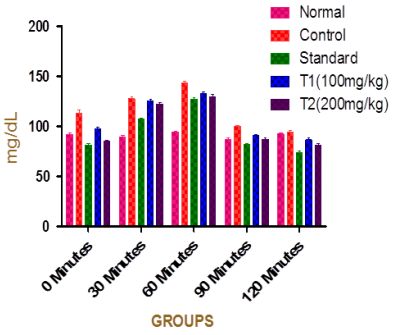

Figure.2. Oral Glucose Tolerance Test

ANOVA followed by dunnet's t – test.

*P<0.05, **P<0.001, ***P>0.0001 was considered Significant Comparing to Diabetic control group.

Anti-diabetic activity of Hydro-alcoholic root extract of *Echinops echinatus* on Blood glucose levels: Anti diabetic activity effect of hydro-alcoholic root extract on blood glucose level in diabetic rats is shown in Table 4. The blood glucose level of diabetic rats treated with standard Sitagliptin 35mg/kg body weight significantly reduced from 163 mg/dL to 140.66 mg/dL. The blood glucose level of diabetic rats in Group 4 treated with extract 100mg/kg b.w significantly reduced from 182.83mg/dL to 176.83mg/dL and significant reduction of glucose level is seen in Group 5 with extract 200mg/kg,b.w which is from 171.66mg/dL to 164.33mg/dL. These results are comparable with Negative control group from 1st day to 21st day.

Table 5 & figure 3 shows that prior to the extract administration, there was no significant difference between the blood glucose levels of the four diabetic group of animals. However, after 21 days, the blood glucose levels of the treated rats were significantly lower than the diabetic control. In contrast, the blood glucose level of the untreated diabetic rat remained elevated throughout the experimental period. The blood glucose level of the healthy (Group 1) remained unchanged during the course of the investigation.

Table.5.Blood Glucose levels (mg/dl) on 1st, 7th, 14th, & 21st day of the Treatment

Groups	1st day	7th day	14th day	21st day
Normal	88.3±1.28	85.5 ± 2.446	89.33±2.47	91.66±1.358
Control	181.33± 2.201	222 ± 2.176	262.5 ± 1.80	277.6 ± 2.76
Standard	163 ± 1.653*	189.83±1.302*	161.8±1.833**	140.66±1.856**
T1	182.8± 1.558	207.33±1.667	191.5 ± 2.72*	176.83±1.276*
T2	171.66±1.944*	199.6 ± 0.88*	179.6±2.404*	164.3 ± 1.687*

Values are given as Mean ± SEM for five groups of Six animals in each group. Standard drug treated rats and Extract treated rats were compared with diabetic contol rats. ***P<0.0001, **P<0.01 , *P<0.05 is considered extremely Significant.

The Effect of Hydro-alcoholic root extract of *Echinops echinatus* on, Serum Urea, Serum Creatinine: The Anti-diabetic activity of Hydro-alcoholic root extract of *Echinops echinatus* on Serum parameters of experimental animals was determined. The Serum Urea, Serum Creatinine levels of the treated Sitagliptin, T1, T2 diabetic rats, after 21 consecutive days of treatment, were significantly enhanced compared to the untreated diabetic rats. It was observed that there was an increase in Serum Urea and Serum creatinine levels in negative control group 2. The Serum insulin level was increased by standard Sitagliptin, T1 (100mg/kg) and T2 (200mg/kg body weight) compared with diabetic control group. After the treatment with test drug and standard Sitagliptin the Serum urea and Serum Creatinine levels got decreased when observed after 21 days treatment. The results of this study have been tabulated in Table 6 and represented in figure 4 and 5 (Serum Urea, Serum Creatinine).

Table.6.Effect of Hydro-alcoholic root extract of *Echinops echinatus* on Serum Biochemical Parameters after 3 weeks Treatment

Groups	Serum Urea	Serum Creatinine
Normal Group	18.26 ± 0.861	0.625 ± 0.0076
Control Group	40.05 ± 0.834	2.108 ± 0.0130
Standard sitagliptin (35mg/kg, body weight p.o)	22.98 ± 0.684***	0.815 ± 0.0076**
T1(100mg/kg)	30.45 ± 0.545	1.195 ± 0.016*
T2(200mg/kg)	25.75 ± 0.625*	0.891 ± 0.015**

Values are given as Mean ± SEM for five groups of six animals in each group.

Diabetic control rats were compared with normal control rats. Diabetic + *Echinops echinatus* (T1 + T2) and Diabetic + standard drug (Sitagliptin) treated rats were compared with Diabetic Control rats.

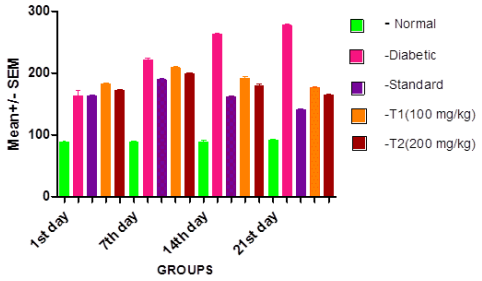

Figure.3.The Anti-diabetic activity of Hydro-alcoholic root extract of Echinops echinatus on Blood glucose levels (mg/dl) on 1st, 7th, 14th, & 21st day of the Treatment

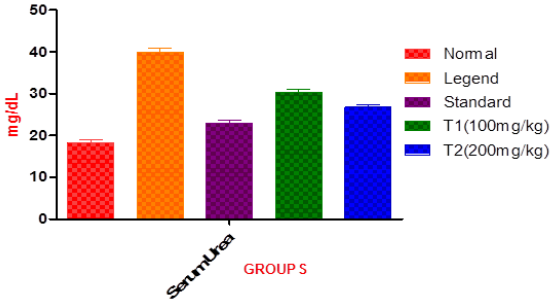

Figure.4.Biochemical estimation of serum urea

ANOVA Followed by Dunnet's t – test.

*P<0.05, **P<0.01, ***P<0.001 was Considerd Significant comparing to Diabetic Control Group.

The effect of hydro alcoholic root extract of *Echinops echinatus* on serum lipid profile in Alloxan Induced Diabetic Rats: The effect of Hydro alcoholic root extract of *Echinops echinatus* on Serum Lipid parameters parameters of experimental animals was determined on the 21st day after oral administration of Sitagliptin and Extract at doses of 100mg/kg, 200mg/kg body weight.

It was observed that the Alloxanised rats of group 2 showed increased levels of Total cholesterol level, Triglycerides level, LDL, and VLDL level where as HDL levels got decreased in Alloxanised rats.

Echinops echinatus extract at 100mg/kg b.w showed significant reduction from 119.5 to 100.82 (P<0.05) while *Echinops echinatus* at 200mg/kg b.w significantly decreased to 97.23(P<0.01), the total cholesterol levels were with high in Negative control group 270.83 (P<0.05) and significantly reduced in T1 (100mg/kg body weight) to 100.82 mg/dL. At 200mg/kg body weight the total cholesterol level got decreased to 124.16 mg/dL and in standard sitagliptin (35mg/kg body weight) group it showed high significant reduction i.e., to 108.83 mg/dL.

Significant reduction of Triglycerides, P<0.05 was seen with *Echinops echinatus* extract at 100mg/kg b.w and the values found were P<0.01 with *Echinops echinatus* at 200mg/kg b.w. whereas, high significant reduction P<0.01 was seen with Sitagliptin at 35mg/kg b.w. The results are tabulated in table 7 and represented in figure 6.

Effect of Hydro alcoholic Root Extract of Echinops echinatus on Serum Lipid Parameters after 3 weeks Treatment: Rats of Negative control group 2 showed impairment in normal lipid profile leading to increased levels of LDL, VLDL while HDL was decreased. HDL and VLDL were significantly reduced P<0.05 with Hydro-alcoholic root extract of *Echinops echinatus* at 100mg/kg body weight, but with Hydro-alcoholic root extract of *Echinops echinatus* at 200mg/kg b.w,and Sitagliptin at 35mg/kg body weight the values of LDL and VLDL was found to be P<0.01. Whereas, HDL levels were significantly increased with Hydro-alcoholic root extract of *Echinops echinatus* at 200mg/kg b.w,and Sitagliptin at 35mg/kg body weight to P<0.01 when compared to normal and untreated groups. Results are tabulated in Table 8 and represented in figure 7.

Table.7.Triglycerides & Total cholesterol

Groups	Triglycerides	Total Cholesterol
Normal	79.66 ± 2.290	119.06 ± 1.933
Control	119.5 ± 2.814	270.83 ± 1.493
Standard Sitagliptin(35mg/kg,b.w)	87.5 ± 0.763***	108.83 ± 2.442**
T1- *Echinops echinatus* extract (100mg/kg b.w)	100.82 ± 1.698*	142.5 ± 1.258
T2 - *Echinops echinatus* (200mg/kg b.w)	97.23 ± 1.014**	124.16 ± 1.014*

Table.8. Biochemical estimations of HDL, LDL, VLDL

Groups	HDL	LDL	VLDL
Normal	36.68 ± 0.743	63.69 ± 1.837	18.68 ± 0.435
Control	29.70 ± 0.583	122.19 ± 1.170	23.9 ± 0.562
Standard	51.02 ± 0.731***	65.41 ± 1.555***	15.915 ± 0.539***
T1(100mg/kg)	46.04 ± 1.001*	77.30 ± 1.018*	20.20 ± 0.356**
T2(200mg/kg)	42.05 ± 0.761**	71.16 ± 1.088*	18.25 ± 0.677***

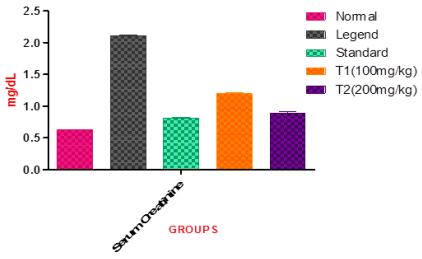

Figure.5.Biochemical estimation of serum creatinine

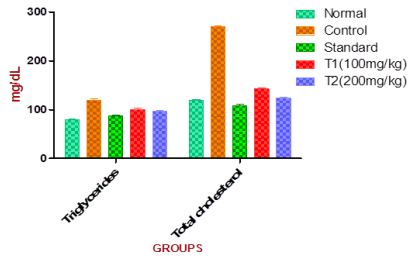

Figure.6.Biochemical estimations of triglycerides & total cholesterol

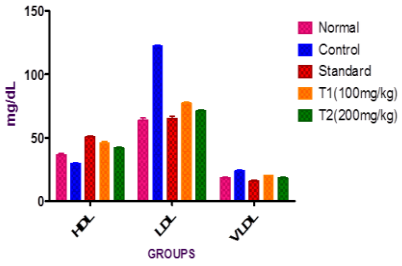

Figure.7.Biochemical estimations of HDL, LDL, VLDL

ANOVA Followed by Dunnet's t – test.

****P<0.01 was Considered Significant comparing to Diabetic Control Group.**

Histopathology of pancreas:

Group.1: Normal control showed normal acini and normal cellular population in the islets of Langerhans in Pancreas.

Group.2: Diabetic Control showed normal exocrine pancreas along with degeneration of islets of Langerhans and with infiltration of inflammatory cells.

Group.3: Sitagliptin treated group showed normal exocrine and endocrine pancreas with few islets cells and inflammatory cells.

Group.4: Echinops echinatus (100mg/kg b.w p.o) treated group showed Preserved islets and exocrine pancreas.

Group.5: Echinops echinatus (200mg/kg b.w p.o) treated group showed normal exocrine pancreas with few islet cells and few chronic inflammatory cells.

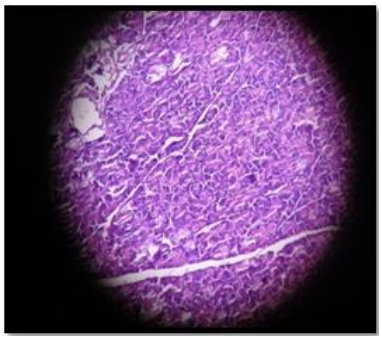

Figure.8.Normal Control Pancreas

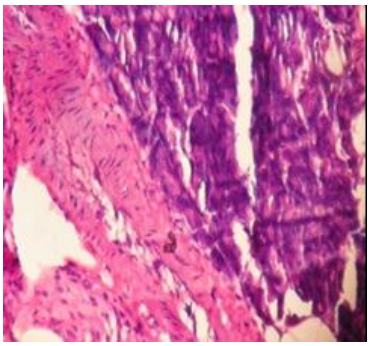

Figure.9.Diabetic Control Pancreas

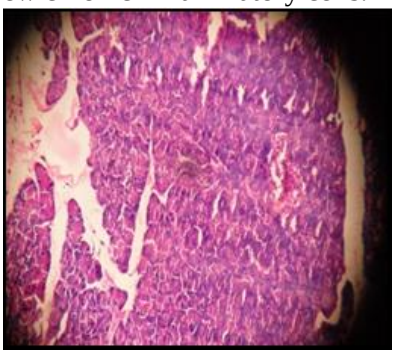

Figure.10.Sitagliptin Treated Pancreas

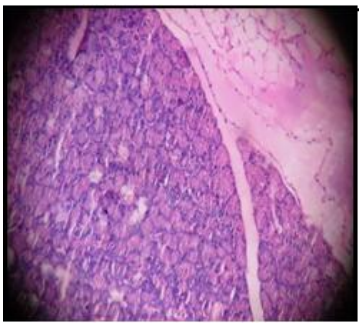

Figure.11.T1(100mg/kg) Treated Pancreas

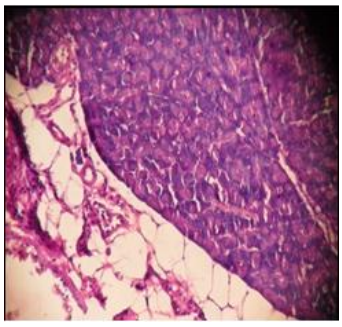

Figure.12.T2(200mg/kg) Treated Pancreas

Histopathology of kidneys

Group.1: Normal Control showed normal structure of glomeruli and proximal and distal convoluted tubules in Kidneys.

Group.2: Diabetic Control kidneys showed an increase in the Mesangial cell and matrix of glomeruli and hyanalization of arterioles.

Group.3: Sitagliptin treated group showed normal kidney structure which appeared more or less as normal.
Group.4: *Echinops echinatus* (100mg/kg body weight) treated group showed -Glomerulus shows some dilated loops (DL) and suffused RBCs.
Group.5: *Echinops echinatus* (200mg/kg body weight) treated group showed kidney with less increase in the Mesangial cell matrix of glomeruli and few hyanalization of arterioles.

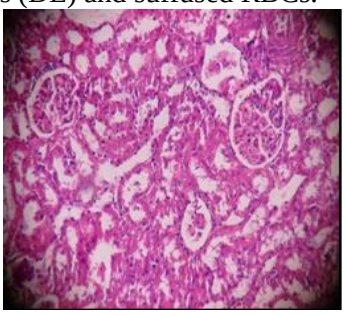

Figure.13.Normal Control Kidney

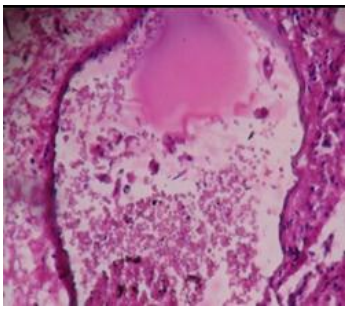

Figure.14.Diabetic Control Kidney

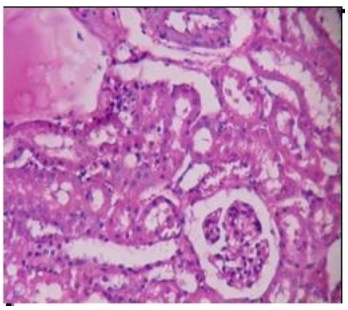

Figure.15.Sitagliptin Treated Kidney

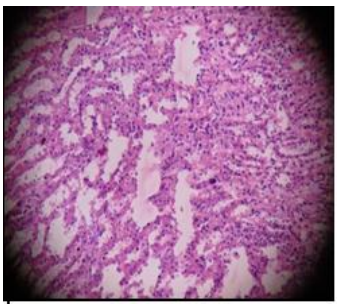

Figure.16.T1(100mg/kg)Treated Kidney

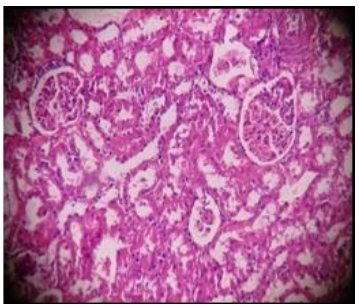

Figure.17.T2 (200mg/kg) Treated Kidney

Discussion: In recent times many traditionally used medicinally important plants were tested for their anti-diabetic potential activity by various investigators in experimental animals. Based on the same line, we have under taken a study on *Echinops echinatus* for its anti-diabetic property and its effect on Nephropathy. Preliminary phytochemical analysis of the Hydro-alcoholic root extract of *Echinops echinatus* showed that the plant has a rich possession of phytochemicals like alkaloids, flavonoids, carbohydrates, sterols, tannins, phenols, aminoacids, steroids.

Acute toxicity studies revealed the nontoxic nature of the hydro-alcoholic extract of *Echinops echinatus*. Neither lethality nor any profound toxic reactions was observed at a dose of 2000mg/kg body weight. This indirectly pronounces the safety profile on the plant extract. The Hydro-alcoholic extract showed significant improvement in oral glucose tolerance in glucose fed hyperglycemic normal rats. The effect was less significant when compared to standard drug Sitagliptin. The present study revealed that the in vivo model caused increased levels in Kidney parameters such as Serum urea, Serum creatinine with decreased levels and increased levels in Serum lipid profiles: Total cholesterol, Triglycerides, LDL, VLDL with decreased levels in HDL. However, there was significant decreased levels of Triglycerides, Total cholesterol, LDL, VLDL, Serum creatinine, Serum urea with increase in HDL levels. This may be due to the Hydro-alcoholic root extract of *Echinops echinatus* at 200mg/kg b.w p.o and Sitagliptin 35 mg/kg body weight. A significant increase in HDL levels in treated rats with Hydro-alcoholic extract of Echinops echinatus at 200mg/kg body weight was observed. Hence, it is considered that *Echinops echinatus* extract is not only anti-diabetic but also a Nephroprotective. The ultra-structure of alloxan diabetic pancreas showed considerable reduction in the islet langerhans and depleted islets. The diabetic rats showed pancreatic islet regeneration. The regenerative effect of the pancreatic cells by *Echinops echinatus* via exocrine cells of pancreas may enlighten the positive effects of these agents on the production of insulin. The goal of these studies was to evaluate the effect of *Echinops echinatus* (70% ethanolic extract) on development of kidney and pancreatic tissue damage or complications in alloxan – induced diabetic rats. Our data show that the *Echinops echinatus* extract was found to effectively improve the kidney and pancreas function and reduced the lesions associated with diabetic state in alloxan – diabetic rats.

4. CONCLUSION

In the light of the results, our study indicates that *Echinops echinatus* root extract have antidiabetic activity and had showed its effect on kidneys.

They have also improved conditions of Diabetes mellitus as indicated by parameters like serum urea, serum creatinine and lipid profiles along with triglycerides, total cholesterol, HDL, LDL and VLDL.

The number of functionality intact β-cells in the islet organs is of decisive importance in the development course and outcome of diabetes mellitus. In our studies damage to pancreas and kidneys in alloxan- treated diabetic control group and regeneration of β-cells and normal structure of glomeruli and proximal and distal convoluted tubules in Kidneys was observed

The outcome of this study was observed to be a possible potentiation of the glucose lowering effect of Sitagliptin, with a possible amelioration of pancreatic damage associated with alloxan administration and Renal function was apparently unaffected and would be of benefit while treating diabetes with Sitagliptin and *Echinops echinatus*. On the basis of above information and evidences, further investigations can be carried out.

REFERENCES

1. Zhang HF, and Li SM, Research progress of natural hypoglycemic agents.China Traditional Herbal Drugs, 31, 2004, 184–186.

2. Hongxiang H, George T, and Vay LW, Hypoglycemic herbs and their action mechanisms. Chinese Medicine, 4, 2009, 11-16.

3. Marles R, and Farnsworth R, Hypoglycemic Activity of Methanolic Stem Bark of *Adansonnia digitata* Extract on Blood Glucose Levels of Streptozocin-Induced Diabetic Wistar Rats. Phytomedicine, 2, 1995, 137–189.

4. Balandarin MF, Klocke JA, Wurtele ES, and Bollinger WH, Natural plant chemicals: sources of industrial and medicinal materials, 228, 1985, 1154-1160.

5. Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: A collaborative meta-analysis of 102 prospective studies, The Lancet, 375, (9733), 2010, 2215–22.

6. Cooke DW, Plotnick, Type 1 diabetes mellitus in pediatrics, Pediatr Rev, 29 (11), 2008, 374–84.