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ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF ROOTS OF *ECHINOPS ECHINATUS* ROXB.

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Abstract

Echinops echinatus Roxb. (Compositae), commonly known as Brahmadandi, is a pubescent annual herb. The study under taken to check physicochemical parameters of roots of *Echinops echinatus* Roxb. and quality control and standardization according to WHO guideline. The methanolic extract of *Echinops echinatus* root powder showed the presence of carbohydrates, steroids, terpenoid glycosides, phenolics and tannins. The DPPH radical scavenging, superoxide anion scavenging and reducing power of methanolic extract of EERE (100 mg/kg and 200mg/kg) shows significant ($p<0.001$) *In vitro* antioxidant activity in a higher dose than standard antioxidant. The methanolic extract of EERE (100 mg/kg and 200mg/kg) also shows significant activity against alloxan induced diabetes (120mg/kg) in rat evidence by significant effect to decrease blood glucose, when it was administered in normoglycemic rats and alloxan induced diabetes rats. Serum cholesterol, serum triglyceride, serum LDL, serum VLDL, serum alkaline phosphate were decreased significantly ($p<0.001$) by Metformin and both extract of *Echinops echinatus* with 15 days treatment and HDL level were increased by Metformin and both extract of *Echinops echinatus* and not significant change in SGOT, SGPT, serum creatinine, serum urea, serum bilirubin, total protein, serum albumin.

Keywords: Brahmadandi , LDL, VLDL, SGOT, SGPT, Antidiabetic, Anti-Oxidant.

Introduction

A study of ancient literature indicates that diabetes (madhumeha) was fairly well known and well conceived as an entity in India. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age. 'Madhumeha' is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, i.e. in sweat, mucus, breathe, blood, etc. The practical usage of juices of various plants achieved the lowering of blood glucose by 10-20% .

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades. In conventional therapy, Type 1 diabetes is treated with exogenous insulin and Type 2 with oral hypoglycemic agents (sulphonylureas, biguani - des etc). Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with antidiabetic activity. Since time immemorial, patients with non-insulin dependent diabetes have been treated orally in folk medicine, with a variety of plant extracts. In India, a number of plants are mentioned in ancient literature (Ayurveda) for the treatment of diabetic conditions.

Echinops echinatus Roxb.(Compositae), commonly known as Brahmadandi, is a pubescent annual herb of 1-3 ft height with branches widely spreading from the base. The species is found practically throughout India, Pakistan, Afghanistan etc.

According to an ethnomedicinal survey carried out Infusion of the root is given in seminal debility, impotence, hysteria and uses the suspension of root bark powder in milk (100 g / 250ml) for the treatment of diabetes.

The present study under taken to check physicochemical parameters of roots of *Echinops echinatus* Roxb. and quality control and standardization according to WHO guideline. . Quantitative estimation of major compound and compound responsible for significant *In vivo* antidiabetic activity was undertaken.

Materials and Methods

1. Plant material

Echinops echinatus Roxb. Is a pubescent annual herb of 1-3 ft height with branches widely spreading from the base. Roots are 30-50cm long with a diameter of 0.5-1cm. Outer surface is grayish brown in color with long longitudinal wrinkles and small rootlets in the lower region. The wood is smooth and yellowish white.

2. Preparation of plant extract

The roots were manually separated from the plant. Then, they were powdered and extracted using methanol by Soxhlet extraction. Later, methanol was evaporated off and dry the residue in vacuum oven to remove solvent completely from extract. Dry residue was weighed and dissolves in water. The extracts so prepared were subjected for further studies.

3. Animal used

Female albino rats of Wistar strain, weighing 190-290 gm were used for study. The animals were housed in a one rat per cage under well controlled conditions of temperature (25+1°C), humidity (55+5%) and 12h/12h light-dark cycle. Animals had access to standard pellet diet and water given ad libitum.

4. Physico-chemical parameters

- Evaluation of Physical parameters

(I.P. Vol.II, 1996; Harbone, 1998; WHO/QCMMPM guidelines, 1992)

Various physical parameters like moisture content, determination of foreign matter, determination of total ash, acid- insoluble ash, water-soluble ash, determination of alcohol soluble extractive, determination of water soluble extractive, determination of foaming index, determination of swellinf index etc. are done.

- Physicochemical analysis

  - Materials, Instruments and chemicals

Test tubes, soxhlet apparatus, desiccators, distillation apparatus, TLC chamber, sprayer, melting point apparatus, digital electronic weighing instrument, chromatographic paper, TLC plates, china dish, beakers, conical flasks were used. All the chemical used are of analytical grade reagents.

  - Successive solvent extraction

Method:

The powder of the dried stems, weighing about 50 gms was taken and extracted in Soxhlet apparatus with the solvents of increasing polarity as follow.

1. Petroleum ether → 2. Benzene → 3. Chloroform → 4. Acetone → 5.Ethanol (95%) → 6.Water

All the extracts were concentrated by distilling the solvents and the extracts were dried in an oven at 500°C. The marc finally macerated chloroform water for 24 hrs to obtain the aqueous extract. The consistency, colour, appearance of the extracts and their percentage yield were noted. The extracts were then subjected to various qualitative test using reported methods (Kokate, 1991; Khandelwal, 2001 Harbone, 1998), to determine the presence of various phytoconstituents such as alkaloids, glycosides, bitter principles, flavonoids, saponins, and coumarins.

➢ Thin Layer Chromatography

TLC studies were carried out for methanol extracts. The different solvent systems of different polarities were prepared and TLC studies were carried out to select the solvent system capable of showing better resolution.

✓ Preparation of extract:

About 500 gm of air dried powdered material was refluxed with methanol for 1 hr. then filter it and evaporate to dryness on water bath. Then collect the residue and dissolve 1 mg residues in 1 ml methanol.

✓ TLC procedure:

The TLC plate prepared with silica gel-G (activated) was the stationary phase having a thickness of about 0.5 mm. 20 µl each of test solution was applied on silica gel-G plate (5x15 cm).The TLC plate was developed in the saturated chromatographic chamber containing the solvent system:

For Methanol Extraxt:

Toluene: Ethyl acetate: glacial acetic acid: chloroform (10:1:0.1:2)

✓ After the development they were removed and observed in day light, UV lightand one of the plates was sprayed with 1% anisaldehyde in sulphuric acid. The later plate was heated at 110°C for 10 min.

5. *In vitro* Anti-Oxidant activity

- Estimation of DPPH free scavenging activity

150 ul DPPH solution was added to 3 ml methanol and absorbance was taken immediately at 516 nm for control reading.

Different volume level of extract were screened and made150 ul of each dose level by dilution with methanol.

150 ul DPPH was added to each test tube.

Absorbance was taken on UV-visible spectrometer shimadzu, UV-1700, Japan, after 15 min. at 516 nm using methanol as blank (yaushisakono, 1978; Ulyana et al.,2002)

The free radical scavenging activity was calculated usin the following equation:

$$\% \text{ Scavanging} = \frac{A_{control} - A_{test}}{A_{test}}$$

$A_{control}$ is the absorbance of control

A_{test} is the absorbance of test

- Estimation of superoxide free radical scavenging activity

To the reaction mixture containing 0.1 ml of NBT (1 mg/ml solution in Dmso) and 0.3 ml of the extracts, the compound and standard in DMSO, 1 ml of alkaline DMSO (1 ml DMSO containing, 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4ml and the absorbance was measured at 560nm using UV-visible spectrometer shimadzu, UV-1700, Japan.

The superoxide free radical scavenging activity is calculated as

$$\% \text{ Scavanging} = \frac{A_{test} - A_{control}}{A_{test}} \times 100$$

$A_{control}$ is the absorbance of control

A_{test} is the absorbance of test

- Estimation of reducing power

Various concentration of the extract (*Echinops echinatus)* in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and 1% potassium ferricyanide (2.5 ml)

The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 3000rpm for 10 min.

The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared $FeCl_3$ solution (0.5ml, 0.1%)

The absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Reducing power is given in ascorbic acid equivalent (ASE mi-1) that shows the amount of ascorbic acid expressed in mM those reducing power is the same than that of 1 ml sample (Oyaizu,1986).

6. Statistical analysis

Experimental results were mean ±SEM of three parallel measurements. Linear regression analysis was used to calculate the IC50 value. Student's t-test was used for the comparison between two means for the possible significant interrelation. Data were considered statistically significant only when p value < 0.05.

7. *In vivo* Anti-diabetic activity

- Experimental design

Initially Wistar albino rats of female rat weighing between 190-290g were selected from the animal house.

They were grouped into four groups of five each and were housed in the metabolic cages.

Animal described as fasted had been deprived of food for at least 16 hours but had been allowed free access to water.

Animals fasted overnight were divided into four groups:

Group A: Normal Control group (1% tween 80 in saline)

Group B: Reference standard group Metformin HCl (250 mg/kg p.o.)

Group C: Methanol extract of roots of *Echinops echinatus* (100mg/kg p.o.)

Group E: Methanol extract of roots of *Echinops echinatus* (200 mg/kg p.o.)

Glucose (4 g/kg per o.s) was fed 90 min after pretreatment with vehicle, Echinops echinatus or Metformin HCl.

Initial readings were taken by using the blood sample collected by puncturing retro-orbital venous plexus under ether anaethesia, then blood glucose level was checked after 0, 30 90 and 120min of extract or metformin HCl administration.

The blood glucose levels were estimated by Lowerson ONE TOUCH glucometer with the help of Lifescan (a Johnson & Johnson company). ONE TOUCH plasma calibrated strips.

- Alloxan induce diabetes in rats

Female albino rats of Wistar strain were used. The animals were fasted overnight and weighed (200-280g) then initially alloxan at the dose of 120 mg/kg of body weight was administered by single intraperitoneal injection in rat and 5% glucose solution was given before alloxan induction to prevent mortality after 48 hours of alloxan injection. The glucose level was estimated by Lowerson ONE TOUCH glucometer and the rats with blood glucose level ranging between 150 mg/dl to 450 mg/dl were selected for the experimental study. At this dose the mortality was moderate. This dose of the alloxan was found to produce hyperglycemia hence it was selected for the study respectively for 14 days. The blood glucose levels were estimated on days 0 and 14. The effect of administration of the methanolic extraxt of roots of *Echinops echinatus*. Diabetic rats were estimated on 15^{th} day after the animals were sacrificed by decapitation. Serum lipid profile (Triglyceride, Total Cholesterol, Hdl cholesterol) and serum glutamate oxaloacetae, Transaminase, Serum glutamate pyruvate transaminase on day 15.

- Parameters

Following investigations were carried out with the help of auto-analyzer.

- Serum triglycerides
- Serum Total cholesterol
- Serum HDL
- Serum LDL
- Serum VLDL
- Serum glutamate-oxaloacetate transaminase (SGOT)
- Serum glutamate-pyruvate transaminase (SGPT)
- Serum alkaline phosphatase
- Serum creatinine
- Serum urea
- Serum billirubin
- Serum total protein
- Serum albumin

All the important organs were carefully dissected. Absolute weight of liver, kidney, and pancreas were noted. The organs- Liver, Kidney, and Pancreas were

transferred to 10% formalin for further histo-pathological study.

- Histopathology of pancreas

One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in 10 % neural buffer formalin. After 12 hours liver was embedded in paraffin using conventional methods and cut into 5 cm thick sections and stained using haematoxylin-eosin dye and finally mounted in diphenylxylene. Then the sections were observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Results

1. Physicochemical parameters

See Table 1

2. Total phenolic content

Total Phenolic content of methanolic extract of Echinops echinatus was found to be 0.224 mg/gm. Equivalent to Gallic acid as a standard and reading of UV absorbance was taken on the Shimadzu, Japan, UV-1700, UV-visible spectrometer.

3. Preliminary phytochemical screening

20 gm Echinops echinatus roots powder was taken and extracted by soxhlet apparatus successively with different solvents in ascending (petroleum ether< chloroform< ethyl acetate< methanol < water) order of polarity.

These extracts were tested by different chemical methods to determine the phytochemical constitution. The results of this study confirmed presence of following different phyto- constituents.

4. Antioxidant activity of *Echinops echinatus* Roxb

- DPPH Radical Scavenging Activity of EERE:
- Superoxide free radical scavenging activity of EERE
- Reducing power of EERE

5. Antidiabetic activity of *Echinops echinatus* Roxb.

- Effect of EERE on blood sugar level in normal fasting rats
- Effect of EERE on Serum Triglyceride level.
- Effect on serum cholesterol level in alloxan diabetic rats
- Effect of EERE on Serum HDL level.
- Effect of EERE on Serum LDL level.
- Effect of EERE on Serum VLDL level.
- Effect on Transaminases activity in alloxan diabetic rats SGOT (serum glutamate oxalate transaminase) activity:
- Effect on Transaminases activity in alloxan diabetic rats SGPT (serum glutamate pyruvate transaminase) activity:
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Discussion

The methanolic extract of EERE (100 mg/kg and 200 mg/kg) also shows significant antidiabetic activity against alloxone induced diabetes (120 mg/kg) in rat evidence by significant effect to decrease blood glucose when it was administered in normoglycaemic rats and alloxone induced diabetes rats. Effect seen to reach maximum after 15 days of treatment. Different mechanisms of action to reduce blood glucose level by plant extract which is in normal and Type II diabetic animals by stimulating insulin release from β cells in islet of Langerhans and that alloxone selectively destroys insulin secreting β cells and their effects are irreversible (Fisher.1985) . Alloxone caused body weight reduction , which is reversed by methanolic extract of EERE .and serum cholesterol , serum triglyceride , serum LDL , serum VLDL, serum alkaline phosphate were decreased significantly ($p<0.001$) by Metformin and both extract of *Echinops echinatus* due to 15 days treatment and HDL level were increased by Metformin and both extract of

Echinops echinatus and not significant change in SGOT, SGPT, serum creatinine , serum urea, serum bilirubin , total protein , serum albumin.

Photomicrographs showed normal acini and normal cellular population in the islet of Langerhans in pancreas of vehicle treated rats. Extensive damage to the islet of Langerhans and reduce dimension of islet in alloxone induce diabetic rats. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by both methanolic extract of EERE.

Conclusion

Echinops echinatus Roxb. (Utkanto).

Echinops echinatus showed Moisture content (4%), Foreign matter (0.9%), Total ash (8.50%) and acid insoluble ash (2%), Water soluble ash (17%), Alcohol soluble extractive(12%), hot water soluble extractive (12%), cold water soluble extractive (23%), swelling index (9.2ml), foaming index (<100).

Echinops echinatus root extract was 7.7% w/w in methanolic extract in root powder.

Methanolic extract of *Echinops echinatus* root powder showed the presence of carbohydrates, steroids, terpenoid glycosides, phenolics and tannins are present, whereas alkaloid, proteins, amino acids, saponins, fixed oils, fats and flavonoids were absent.

The total Phenolic content was 0.224 mg/gm expressed as Gallic acid in Methanolic extract of *Echinops echinatus* root powder estimated.

The total antioxidant capacity was 59.34 µg/ml expreesed as ascorbic acid in the Methanolic extract of *Echinops echinatus* root powder determined spectrophotometrically.

Methanolic extract of *Echinops echinatus* root powder showed antioxidant activity of in DPPH model, super oxide model and in reducing power and the IC_{50} value was 73.86 µg/ml, 97.97 µg/ml, 59.10 µg/ml, respectively which may be due to presence of flavonoids, phenols, tannins (phenolic compounds) and Triterpenoids.

Pharmacological evaluation for Anti-diabetic activity

The methanolic extract of EERE (100mg/kg and 200 mg/kg) also shows significant antidiabetic activity against alloxan induced diabetes (120 mg/kg) in rat evidence by significant effect to decrease blood glucose when it was administered in normoglycemic rats and alloxan induced diabetes rats. Effect seen to reach maximum after 15 days of treatment. Different mechanisms of action to reduce blood glucose level by plant extract which is in normal and Type II diabetic animals by stimulating insulin release from β cells in islet of Langerhans and that alloxan selectively destroys insulin secreting β cells and their effects are irreversible. Alloxan caused body weight reduction, which is reversed by methanolic extract of EERE and serum cholesterol, serum triglyceride, serum LDL, serum VLDL, serum alkaline phosphate were decreased significantly ($p<0.001$) by Metformin and both extract of *Echinops echinatus* due to 15 days treatment and HDL level were increased by Metformin and both extract of *Echinops echinatus* and not significant change in SGOT, SGPT, serum creatinine, serum urea, serum bilirubin, total protein, serum albumin.

Photomicrographs showed normal acini and normal cellular population in the islet of Langerhans in presence of vehicle treated rats. Extensive damage to the islet of Langerhans and reduce dimension of islet in alloxan induce diabetic rats. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by both methanolic extract of EERE.

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Physicochemical parameters

Table 1.1 Physico-chemical parameters

WHO PARAMETER	AVERAGE VALUE
Total ash	8.50%
Water soluble	17%
Acid insoluble	2%
Loss on drying	6.20%
Hot water extractive	12%
Cold water extractive	23%
Alcohol extractive	12%
Swelling index	9.2ml
Foaming index	<100

Total phenolic content

Total Phenolic content of methanolic extract of *Echinops echinatus* was found to be 0.224 mg/gm. Equivalent to Gallic acid as a standard and reading of UV absorbance was taken on the Shimadzu, Japan, UV-1700, UV-visible spectrometer.

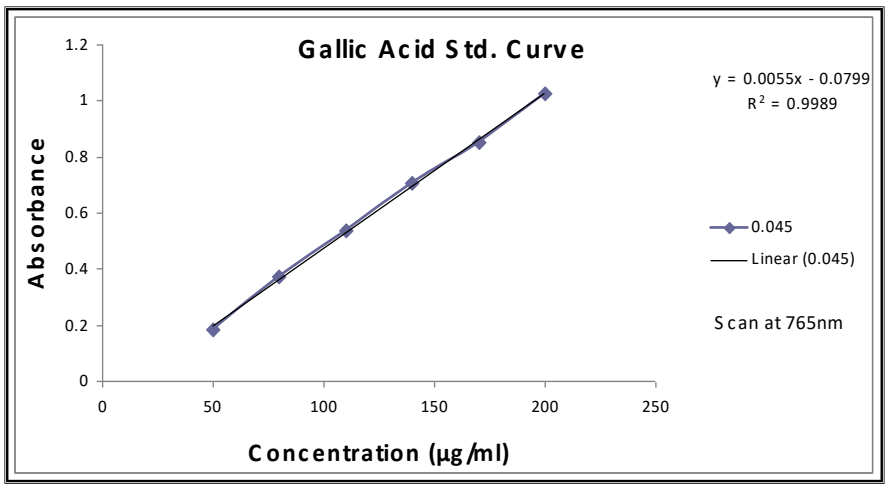

Fig. 2.1 Standard curve of Gallic acid

Preliminary phytochemical screening

20 gm *Echinops echinatus* roots powder was taken and extracted by soxhlet apparatus successively with different solvents in ascending (petroleum ether< chloroform< ethyl acetate< methanol < water) order of polarity.

These extracts were tested by different chemical methods to determine the phytochemical constitution. The results of this study confirmed presence of following different phyto- constituents.

Table 2.1 Preliminary phytoprofile for roots of *Echinops echinatus*

Sr. No.	Solvent	Colour in day light	Consistency	Average value of extractive %w/w
1	Petroleum ether	Yellowish Brown	Sticky	6.1%
2	Benzene	Yellow	Sticky	0.6%
3	Chloroform	Yellow	Dry	1.44%
4	Ethyl acetate	Yellow	Sticky	0.7%
5	Methanol	Sticky brown	Sticky	7.7%
6	Water	Dark brown	Dry	3.3%

Table 2.2 Preliminary phytochemical constitution of roots of *Echinops echinatus*

Classes of compounds	P	B	C	E	M	W
Alkaloid	-	-	-	-	-	-
Carbohydrates	-	+	-	-	+	+
Steroids/Terpenoid	+	+	+	+	+	-
Protiens & Amino acids	-	-	-	-	-	-
Glycosides	+	+	-	-	+	+
Saponins	-	-	-	-	-	-
Fixed oil/Fats	+	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Phenolics	-	-	-	+	+	+
Tannins	-	-	-	-	+	+

P=Petroleum ether; B= Benzene; C = Chloroform; E = Ethyl acetate; M = Methanol; W = Water; '+'= Present; '-'= Absent

6. Antioxidant activity of *Echinops echinatus* Roxb

- DPPH Radical Scavenging Activity of EERE:

Table 4.1: DPPH Radical Scavenging Activity

concentration(µg/ml)	% scavenging ascorbic acid	% scavenging EERE
25	30.46±0.06	32.09±0.08
50	42.41±1.45	47.45±0.78
75	51.07±0.09	59.08±0.95
100	59.78±1.23	68.32±0.34
125	68.76±1.34	79.05±1.23

Data	DPPH Radical Scavenging Activity	
	Ascorbic acid	EERE
Regression equation	y = 0.459x + 22.76	y =0.375x + 22.30
Regression coefficient	0.99	0.995
IC_{50} Value (µg/ml)	59.34	73.86

Mean ± Standard Deviation , p < 0.05

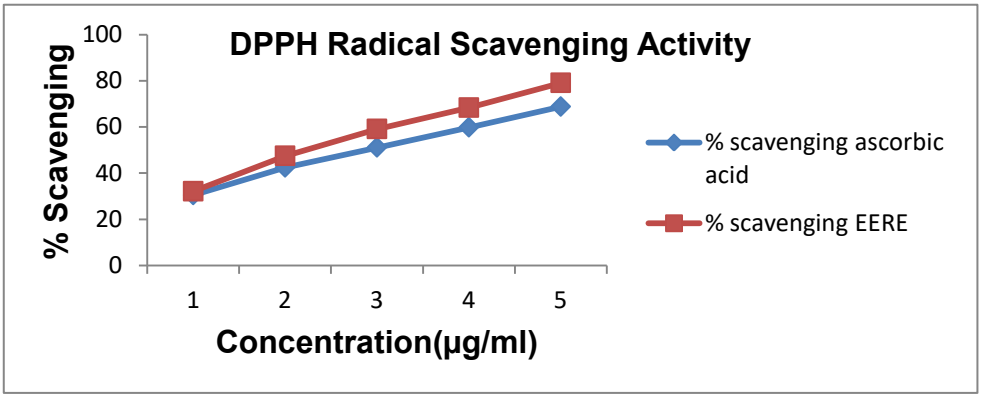

Fig 4.1 DPPH Radical Scavenging Activity

- Superoxide free radical scavenging activity of EERE

Table 4.2 : Superoxide free radical scavenging activity

concentration(µg/ml)	% scavenging of curcumin	% scavenging of EERE
30	35.87±0.67	27.09±0.34
60	46.64±0.52	35.09±2.34
90	57.02±1.34	47.05±0.89
120	67.08±1.08	58±0.65
150	76.08±0.45	69±1.34

Data	Super oxide	
	Curcumin	EERE
Regression equation	y =0.336x + 26.28	y =0.355x + 15.22
Regression coefficient	0.998	0.996
IC_{50} Value(µg/ml)	71.87	97.97

Mean ± Standard Deviation , $p < 0.05$

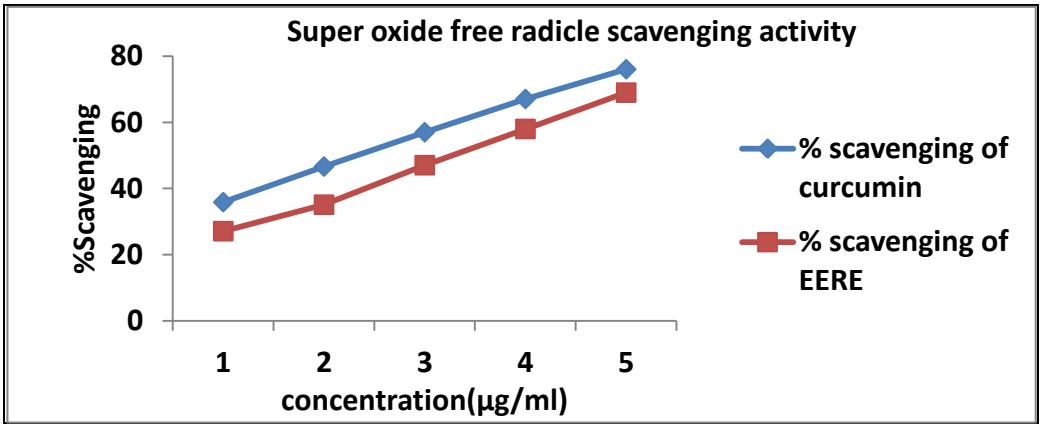

Fig. 4.2 Superoxide free radical scavenging activity

- Reducing power of EERE

Table 4.3: Reducing power of EERE

concentration(µg/ml)	% scavenging ascorbic acid	% scavenging of EERE
25	20±0.87	36.6±1.23
50	31.5±1.34	46±0.98
75	42.4±1.23	57.05±2.56
100	50.07±0.23	69.07±0.76
125	62.08±0.45	73.09±0.69

Data	Reducing power	
	Ascorbic acid	EERE
Regression equation	y =0.410x + 10.39	y =0.384x + 27.54
Regression coefficient	0.996	0.981
IC_{50} Value(µg/ml)	90.6	59.1

Mean ± Standard Deviation , p < 0.05

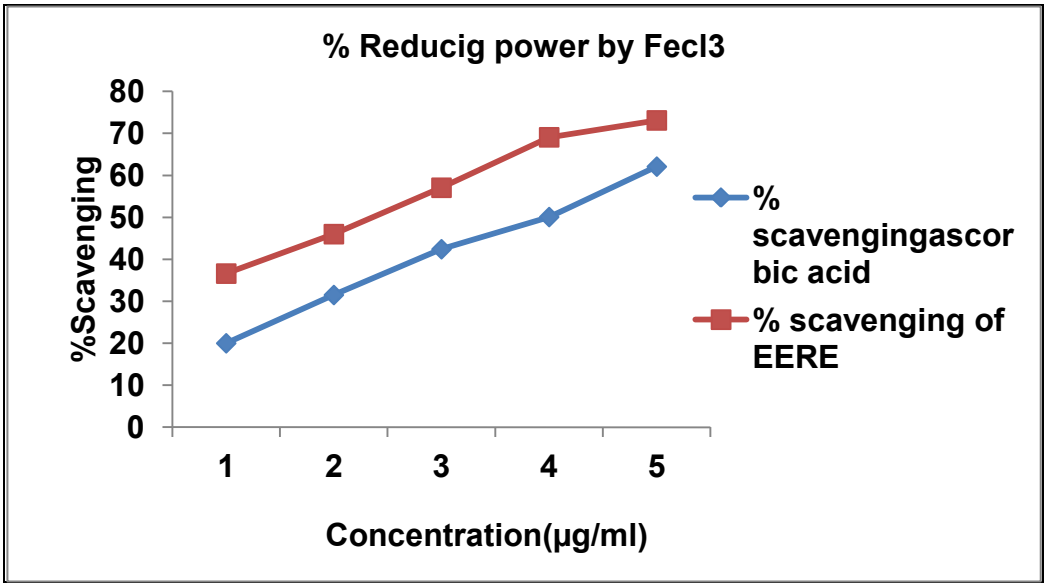

Fig 4.3: % Reducing power of EERE

7. Antidiabetic activity of *Echinops echinatus* Roxb**.**

- Effect of EERE on blood sugar level in normal fasting rats

Table 5.1: Effect of the EERE on blood glucose level in the normal rats.

Group	Dose	Blood sugar level (mg/dl) after glucose load at various time intervals (Mean ± SEM)			
		O min	30min	90min	120min
Control	vehicle	75±1.47	149.25±6.96	120.25±1.38	90.25±1.38
Metformin HCL	250mg/kg	85±1.22*	98±1.75*	95±1.03*	81±1.03*
Test-1	(100mg/kg	79.25±1.31*	129.5±5.24*	85.75±4.50*	82±1.63*
Test-2	200mg/kg)	78.5±2.22*	105±2.8*	81.04±2.8*	77.04±1.09*

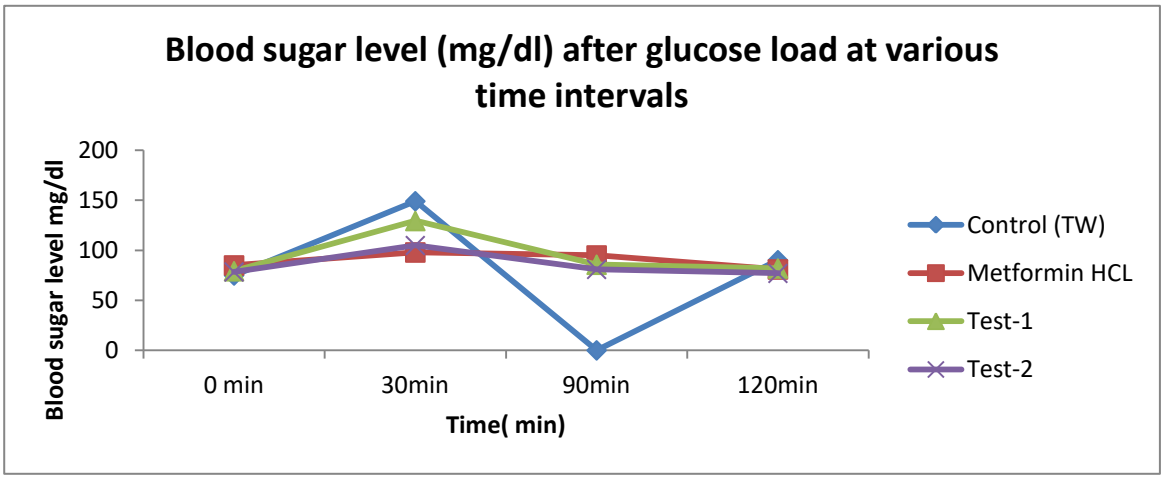

Fig 5.1: Oral Glucose Tolerance Test

Values are expressed as mean ± SEM (n = 5).

*P<0.05 (paired 't' test in comparison to initial values).

The methanolic extract showed a significant reduction in blood glucose levels from 30 min onwards in oral glucose tolerance test.

In normal animals, significant reduction in the blood glucose level was observed as compared to the control.

- Effect of EERE on Serum Triglyceride level.

Table 5.2: Effect of EERE on serum Triglyceride level in alloxan diabetic rats

Group	*Dose mg /kg.*	Serum Triglyceride (mg/dl) Mean ± SEM
Normal	vehicle	90.25±5.793
Diabetec control	-	210±7.88
standard	250mg/kg	95.75±5.58***
Test-1	100 mg/kg	93.50±6.33***
Test-2	200 mg/kg	94.75±4.90***

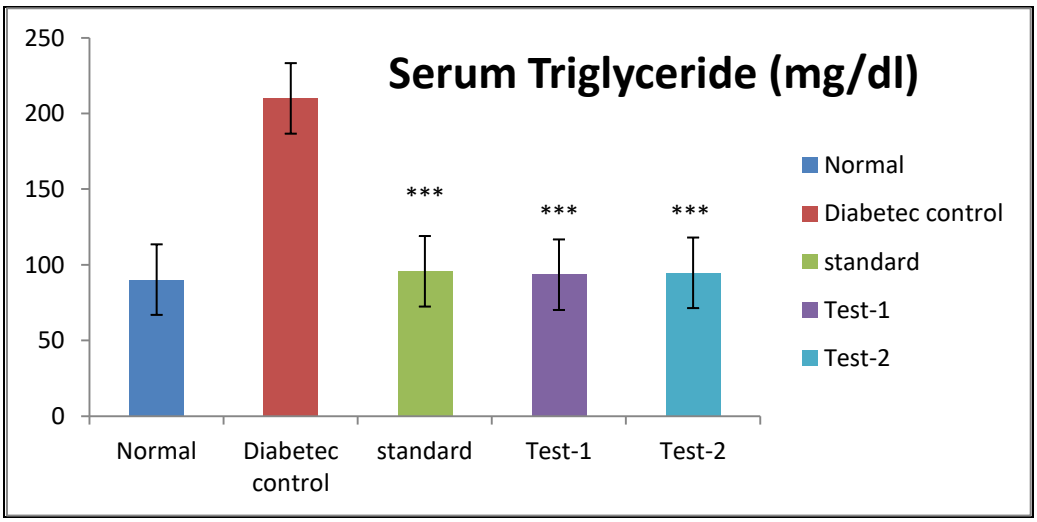

Fig 5.2 Serum Triglyceride (mg/dl)

Values are expressed as mean ± SEM (n = 5).

***p<0.001(dunnet t–test), diabetic control was compared with the vehicle and extract treated groups were compared with diabetic control.

serum triglyceride level was decreased significant by metformin HCL and both extract of *Echinops echinatus* due to 15 days treatment in diabetic rats as compared with diabetic control.

Effect on serum cholesterol level in alloxan diabetic rats

Table 5.3: Effect of EERE on serum cholesterol level in alloxan diabetic rats

Group	*Dose mg /kg.*	Serum Cholesterol (mg/dl) Mean ± SEM
Normal	vehicle	52.75±3.5
Diabetec control	-	144±3.02
standard	250mg/kg	95.75±5.58***
Test-1	100 mg/kg	97.50±4.40***
Test-2	200 mg/kg	94.75±4.905***

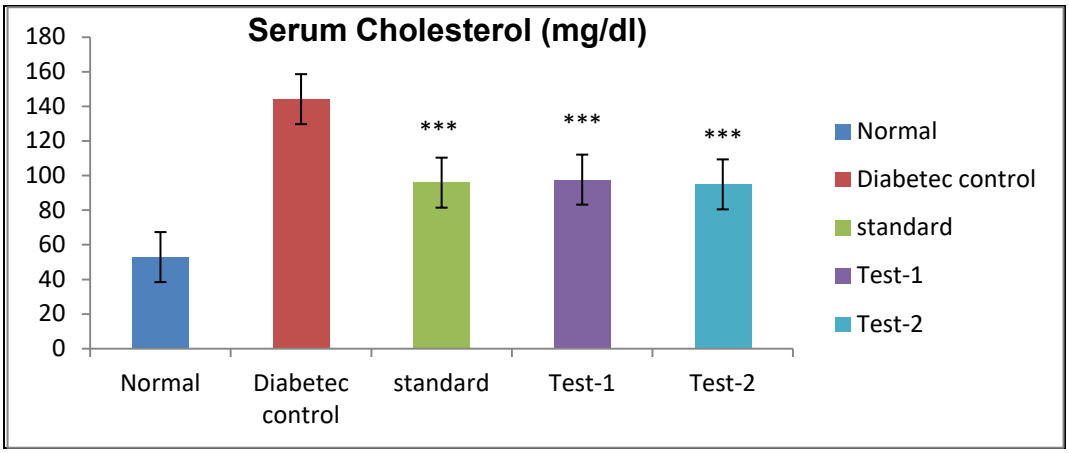

Fig 5.3: Serum Cholesterol (mg/dl)

Values are expressed as mean ± SEM (n = 5).

p<0.001(dunnet t –test),diabetic control was compared with the vehicle and extract treated groups were compared with diabetic control.

serum cholesterol level was decreased significant by metformin HCL and both extract of *Echinops echinatus* due to 15 days treatment in diabetic rats as compared with diabetic control.

- Effect of EERE on Serum HDL level.

Table 5.4: Effect of EERE on serum HDL level in alloxan diabetic rats

Group	*Dose mg /kg.*	HDL(mg/dl) Mean ± SEM
Normal	Vehicle	35.14±1.557
Diabetec control	-	18.73±0.64
standard	250mg/kg	33.25±1.54***
Test-1	100 mg/kg	36.5±1.84***
Test-2	200 mg/kg	34.5±1.93***

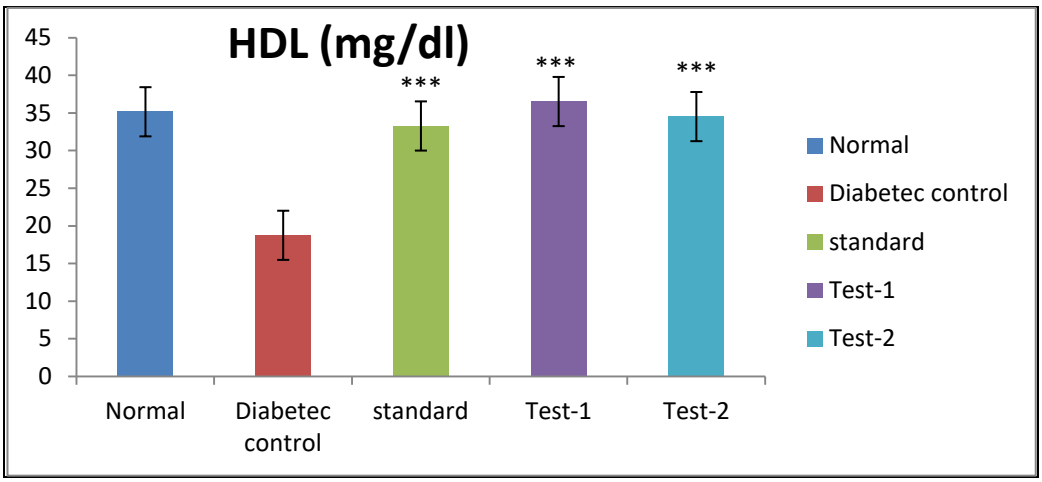

Fig 5.4 Serum HDL (mg/dl)

Values are expressed as mean ± SEM (n = 5).

*** p<0.001(dunnet t –test), diabetic control was compared with the vehicle and extract treated groups were compared with diabetic control.

Serum HDL level was increased significant by metformin HCL and both extract of *Echinops echinatus* due to 15 days treatment in diabetic rats as compared with diabetic control.

Effect of EERE on Serum LDL level.

Table 5.5: Effect of EERE on serum LDL level in alloxan diabetic rats

Group	*Dose mg /kg.*	LDL(mg/dl) Mean ± SEM
Normal	vehicle	54±2.64
Diabetec control	-	176±8.52
standard	250mg/kg	55.25±1.65***
Test-1	100 mg/kg	58.5±1.02***
Test-2	200 mg/kg	62.25±1.65***

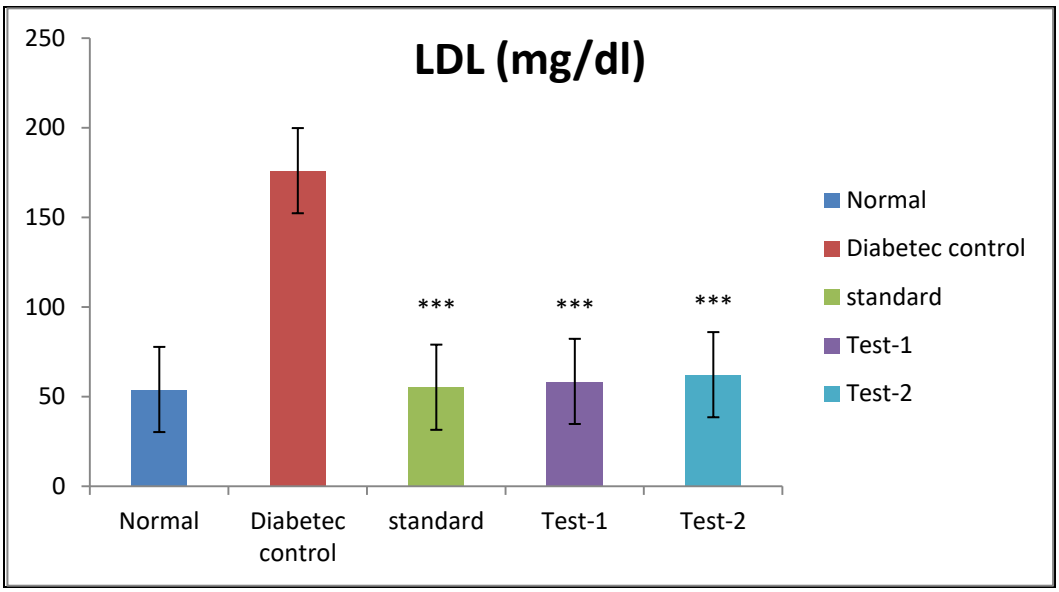

Fig 5.5 Serum LDL (mg/dl)

Values are expressed as mean ± SEM (n = 5).

*** p<0.001(dunnet t –test),diabetic control was compared with the vehicle and extract treated groups were compared with diabetic control.

Serum LDL level was increased significant by metformin HCL and both extract of *Echinops echinatus* due to 15 days treatment in diabetic rats as compared with diabetic control.

- Effect of EERE on Serum VLDL level.

Table 5.6: Effect of EERE on serum VLDL level in alloxan diabetic rats

Group	*Dose mg /kg.*	VLDL(mg/dl) Mean ± SEM
Normal	vehicle	11.98±0.01
Diabetec control	-	24.75±0.85
standard	250mg/kg	13.25±1.10***
Test-1	100 mg/kg	12.75±1.70***
Test-2	200 mg/kg	13±0.91***

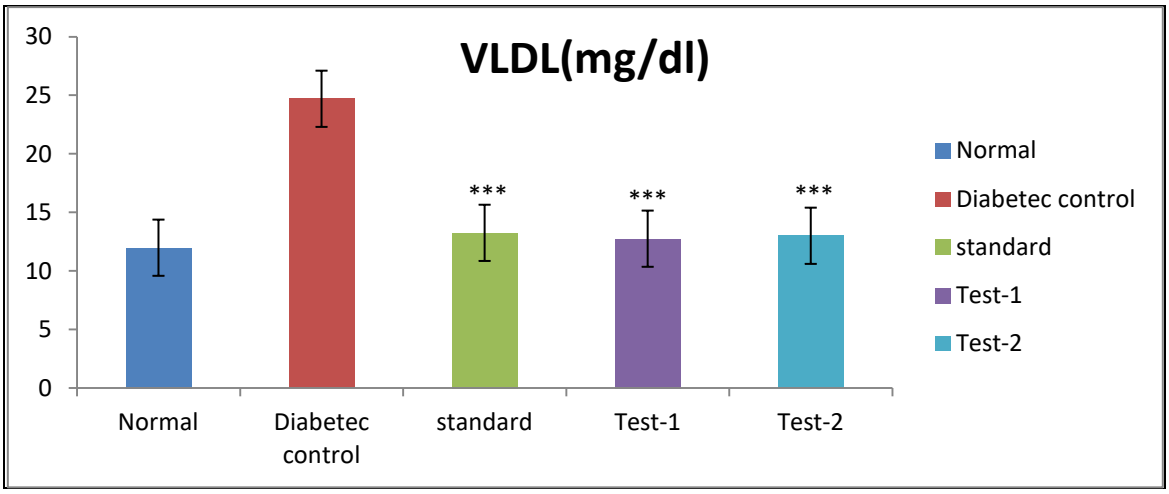

Fig 5.6 Serum VLDL (mg/dl)

Values are expressed as mean ± SEM (n = 5).

*** p<0.001(dunnet t –test),diabetic control was compared with the vehicle and extract treated groups were compared with diabetic control.

Serum VLDL level was decreased significant by metformin HCL and both extract of *Echinops echinatus* due to 15 days treatment in diabetic rats as compared with diabetic control

- Effect on Transaminases activity in alloxan diabetic rats SGOT (serum glutamate oxalate transaminase) activity:

Table 5.7: Effect of EERE on SGOT activity in alloxan diabetic rats

Group	*Dose mg /kg.*	SGOT activity (IU/L) Mean ± SEM
Normal	vehicle	309.25±25.38
Diabetec control	-	288.5±11.19
standard	250mg/kg	337±17.19
Test-1	100 mg/kg	334.75±23.73
Test-2	200 mg/kg	322±20.95

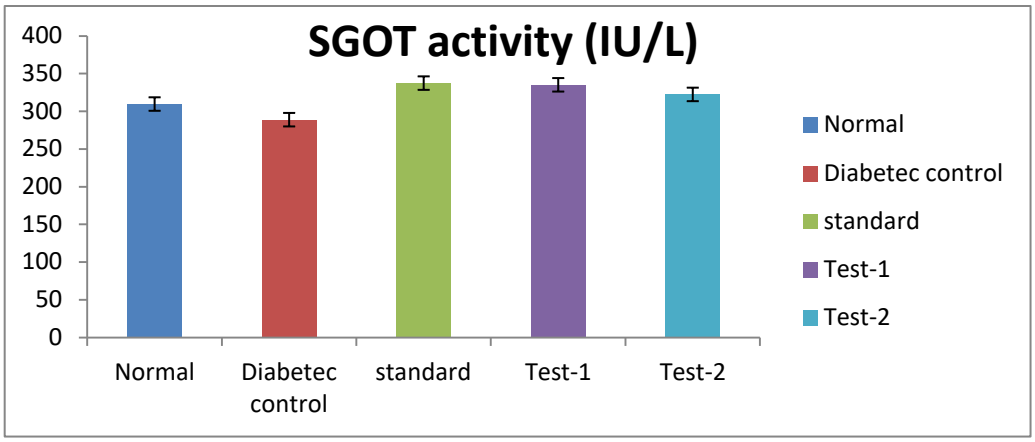

Fig 5.7 SGOT activity (IU/L)
Values are expressed as mean ± SEM (n = 5).

It can be observed from the data presented in Table-5.14 that test drugs administration did not affect SGOT level to significant extent.

- Effect on Transaminases activity in alloxan diabetic rats SGPT (serum glutamate pyruvate transaminase) activity:

Table 5.8: Effect of EERE on SGPT activity in alloxan diabetic rats

Group	*Dose mg /kg.*	SGPT activity (IU/L) Mean ± SEM
Normal	vehicle	54±3.5
Diabetec control	-	63.5±9.17
standard	250mg/kg	59.5±7.37
Test-1	100 mg/kg	68.25±6.5
Test-2	200 mg/kg	61.5±5.69

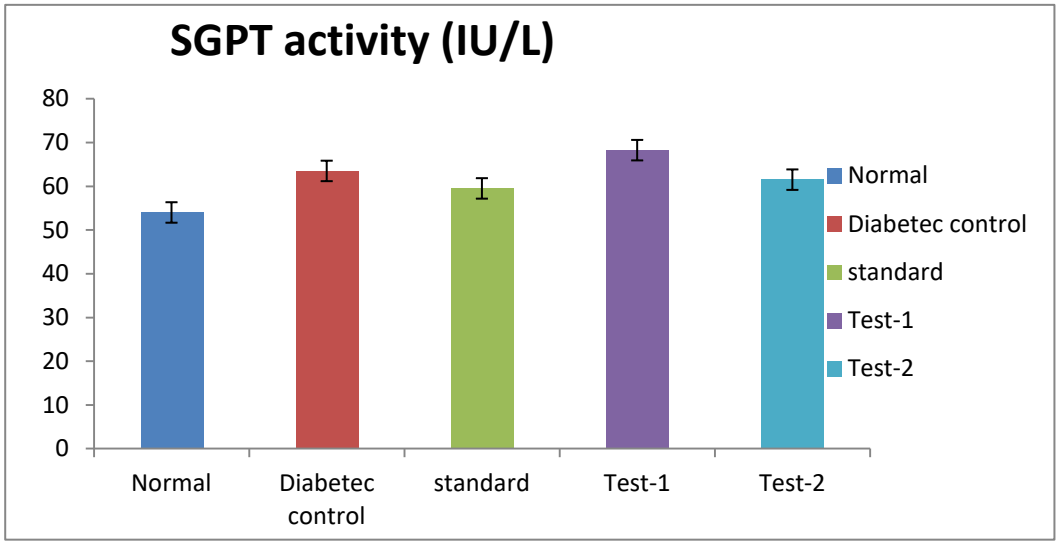

Fig 5.8 SGPT activity (IU/L)
Values are expressed as mean ± SEM (n = 5).

It can be observed from the data presented in Table-5.15 that test drugs administration did not affect SGPT level to significant extent.

Effect on serum alkaline phosphatise in alloxan diabetic rats

Table 5.9: Effect of EERE on serum alkaline phosphatase level in alloxan diabetic rats.

Group	*Dose mg /kg.*	Serum alkaline phosphatase activity (IU/L) Mean ± SEM
Normal	vehicle	160±18.20
Diabetec control	-	407±42.87
standard	250mg/kg	217±15.43***
Test-1	100 mg/kg	170±17.66***
Test-2	200 mg/kg	194.15.54***

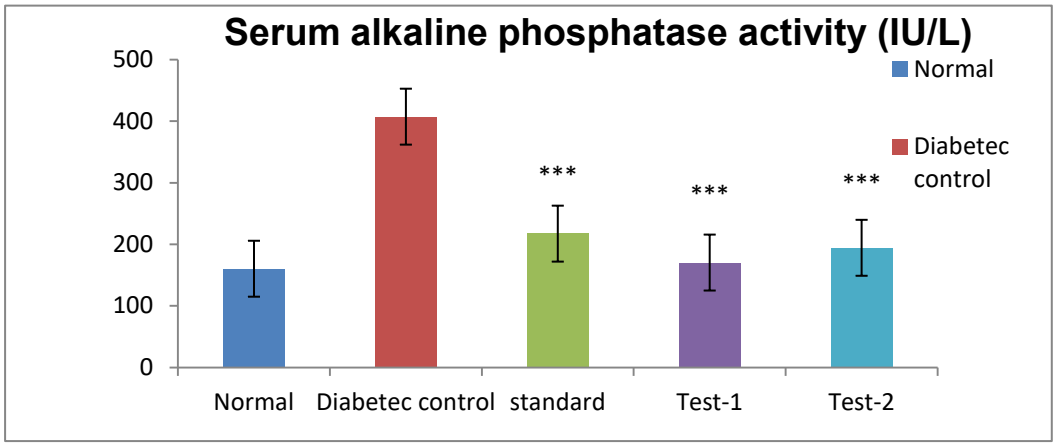

Fig 5.9 Serum alkaline phosphatase activity (IU/L)

Values are expressed as mean ± SEM (n = 5).

*** p<0.001(dunnet t –test),diabetic control was compared with the vehicle and extract treated groups were compared with diabetic control.

Serum alkaline phosphatase level was decreased significant by metformin HCL and both extract of *Echinops echinatus* due to15 days treatment in diabetic rats as compared with diabetic control.

- Effect on serum creatinine in alloxan diabetic rats

Table 5.10: Effect of EERE on serum creatinine level in alloxan diabetic rats

Group	*Dose mg /kg.*	Serum creatinine (mg/dl) Mean ± SEM
Normal	vehicle	1.38±.08
Diabetec control	-	1.36±.18
standard	250mg/kg	1.36±.12
Test-1	100 mg/kg	1.35±1.5
Test-2	200 mg/kg	1.36±2.8

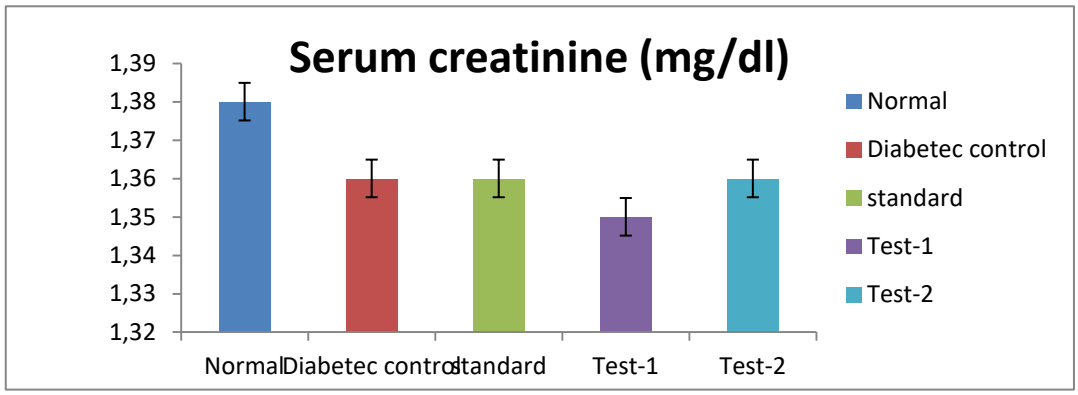

Fig 5.10 Serum creatinine (mg/dl)

Values are expressed as mean ± SEM (n = 5).

It can be observed from the data presented in Table-5.17 that test drugs administration did not affect serum creatinine level to significant extent.

- Effect on serum urea level in alloxan diabetic rats

Table 5.11: Effect of EERE on serum urea level in alloxan diabetic rats

Group	*Dose mg /kg.*	Serum urea (mg/dl) Mean ± SEM
Normal	Vehicle	23.67±3.41
Diabetec control	-	55.21±3.86
standard	250mg/kg	27.44±1.69
Test-1	100 mg/kg	32.28±1.22
Test-2	200 mg/kg	33.25±1.31

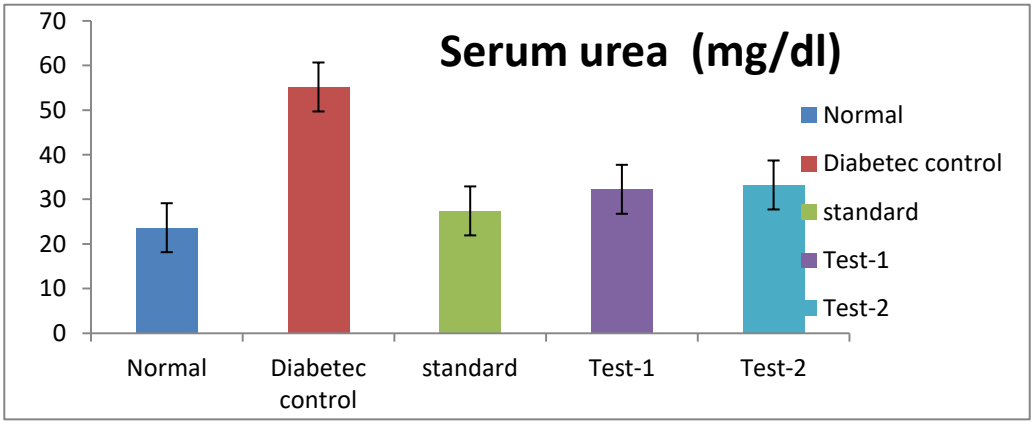

Fig 5.11 Serum urea (mg/dl)

Values are expressed as mean ± SEM (n = 5).

It can be observed from the data presented in Table-5.18 that test drugs administration did not affect serum urea level to significant extent.

Effect on serum bilirubin level in alloxan diabetic rats

Table 5.12: Effect of EERE on Serum Billirubin in alloxan diabetic rats.

Group	*Dose mg /kg.*	Serum bilirubin (mg/dl) Mean ± SEM
Normal	5ml/kg	0.74±0.02
Diabetec control	-	0.91±0.15
standard	250mg/kg	0.71±0.04
Test-1	100 mg/kg	0.67±0.04
Test-2	200 mg/kg	0.89±0.13

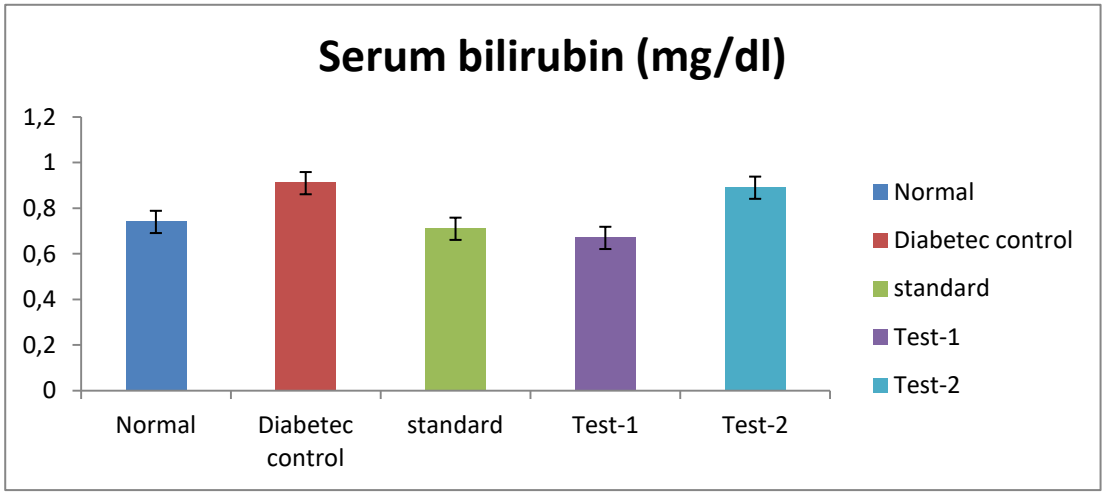

Fig 5.12 Serum bilirubin (mg/dl)
Values are expressed as mean ± SEM (n = 5).

It can be observed from the data presented in Table-5.19 that test drugs administration did not affect serum bilirubin level to significant extent.

Effect on serum total protein in alloxan diabetic rats

Table 5.13: Effect of EERE on serum total protein in alloxan diabetic rats

Group	*Dose mg /kg.*	Serum protein (g/dl) Mean ± SEM
Diabetec control	-	10.1±0.14
standard	250mg/kg	10.75±0.87
Test-1	100 mg/kg	11.04±0.42
Test-2	200 mg/kg	10.91±0.21

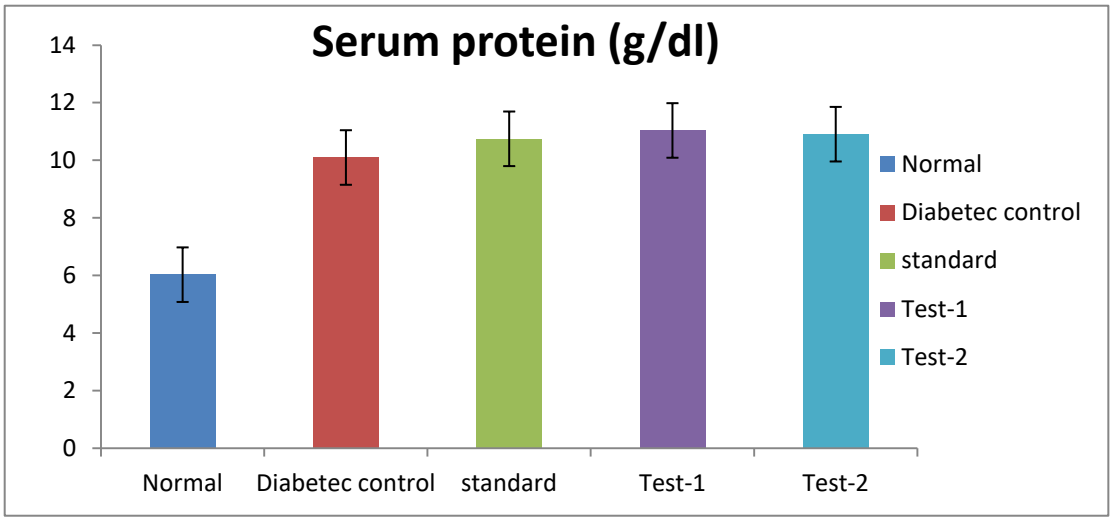

Fig 5.13: Serum protein (g/dl)
Values are expressed as mean ± SEM (n = 5).

It can be observed from the data presented in Table 5.20 that test drugs administration did not affect serum protein level to significant extent.

- Effect on Serum Albumin

Table 5.14: Effect of EERE on serum albumin level in alloxan diabetic rats

Group	*Dose mg /kg.*	Serum albumin (g/dl) Mean ± SEM
Normal	Vehicle	3.57±0.28
Diabetec control	-	4.02±0.54
standard	250mg/kg	4.2±0.31
Test-1	100 mg/kg	4.42±0.71
Test-2	200 mg/kg	5.84±1.07

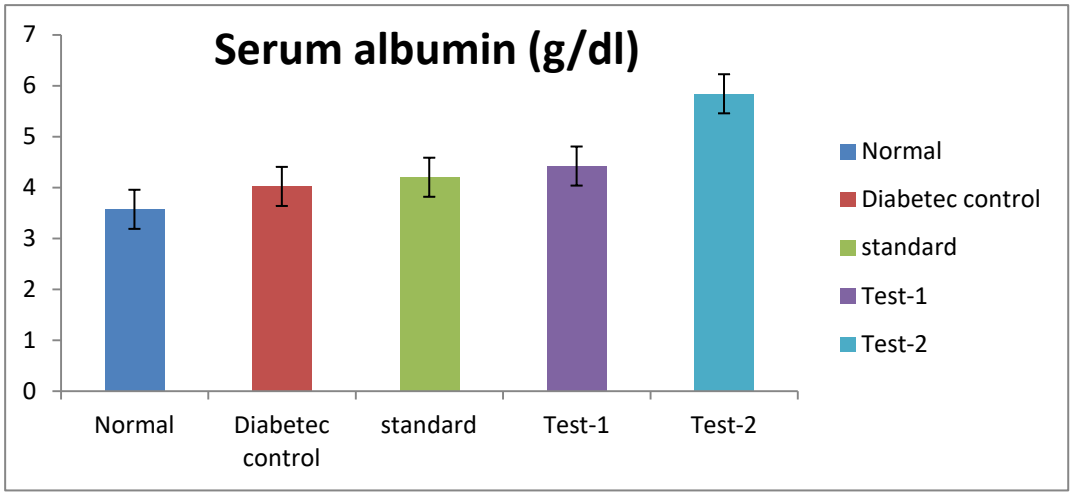

Fig 5.14: Serum albumin (g/dl)

Values are expressed as mean ± SEM (n = 5).

It can be observed from the data presented in Table-5.21 that test drugs administration did not affect serum protein level to significant extent.

Photomicrographs rat pancrease stained by eosino.

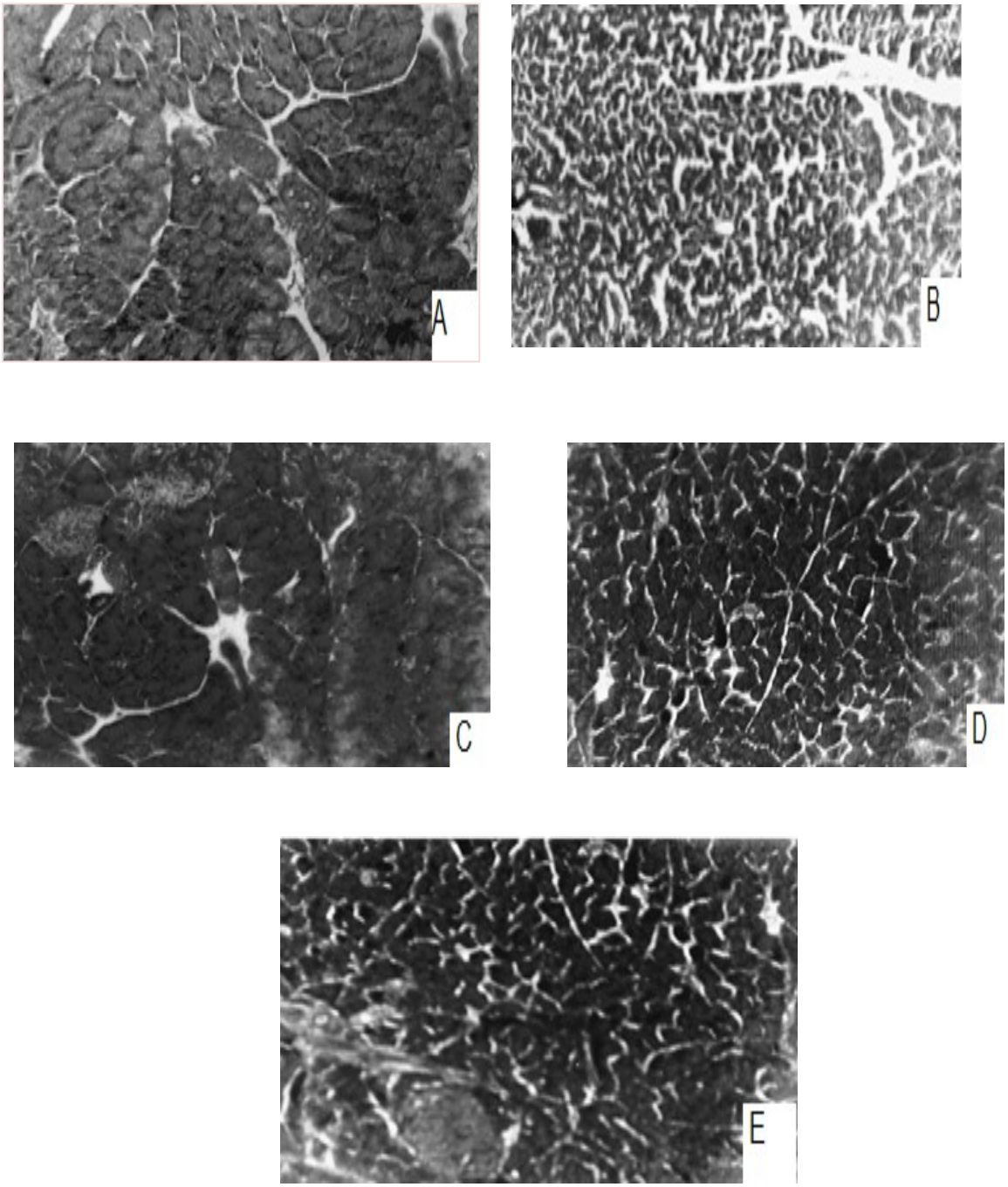

Fig 19 Photomicrographs rat pancrease stained by eosino.

(A) untreated (B) alloxan-induced diabetic rats (C) effects of Metformin HCL,(D methanol extract 100mg/kg (E), methanol extract 200mg/kg, (Microscope magnification (400×).