Evaluation of antidiabetic activity of ethanolic extract of *Barleria noctiflora* (whole plant) in streptozotocin induced hyperglycemia in wistar rats

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Abstract

To evaluate the anti-diabetic effect of a ethanolic extract of aerial parts *Barleria noctiflora*(Acanthaceae) in streptozotocin-diabetic rats. Compared with the control group,the extract showed significant ($p < 0.01$) anti-diabetic efficacy,the effect was observed for 12 hours maximum activity ($p < 0.01$) at 24 h after administration found after extract at 200 and 400 mg /kg body than 100 mg/ kg body wt.The most significant activity was observed with the dose of 200 mg/kg and 400 mg/ kg body weight. Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg orally for 21days treatment were reduced significantly serum glucose level on 14 days ($p<0.05$), 21th days ($p<0.05$ and $p<0.01$) as compared to control groups. I showed that the ethanolic extract of aerial parts of *Barleria noctiflora*(Acanthaceae) showed effective blood-glucose-lowering potential in streptozotocin hyperglycemic rats.Thus *Barleria noctiflora* provided pharmacological support of reduced blood sugar level in diaetic rats.

AIM: To assess antidiabetic activity of aqueous extract of *Barleria noctiflora* (Whole plant) in streptozotocin induced diabetic rats.

Objectives:

1. To prepare Leave extract of *Barleria noctiflora* (Whole plant).
2. Phytochemical investigation of the extracts.
3. To establish pharmacological activities of extracts.
4. Qualitative studies of the extracts.

Methods:

The extraction was carried out with ethanol with using Soxhlet apparatus. The dried,concentrated extracts were assessed for the antidiabetic activity by using *in vivo* studies in rats.

Results:

The alcoholic extract of *Barleria noctiflora* (Whole plant) revealed the presence of variety of chemical constituents like tannins, carbohydrates, flavonoids, saponins, terpenes and phenolic compounds. The ethanolic extract of *Barleria noctiflora* (Whole plant) extract exhibited significant reduction in blood glucose level in STZ induced diabetic rats where as total cholesterol, triglycerides and LDL were reduced and HDL level was increased but statistically not significant compared to standard drug glibenclamide. The significant percentage reduction of glucose level was found to be 43.68% at the dose of 200 mg/kg of ethanolic extract of *Barleria noctiflora* (Whole plant) at 28th day of the treatment.

Conclusion: The ethanolic extract of *Barleria noctiflora* (Whole plant) exhibited significant hypoglycemic property in STZ induced diabetic albino rats.

Key words:*Barleria noctiflora,* antidiabetic activity.

Introduction:

Diabetes mellitus is a chronic metabolic disorder, characterized by hyperglycemia resulting from a variable interactions of here dietary and environmental factors, defects in insulin secretion, insulin action or both.[1]

Today, it is a vulnerable endemic problem all over the globe, affecting carbohydrate, protein, and fat metabolism in addition to damaging liver, kidney, and cells of pancreas.[2]

Currently available oral antidiabetic synthetic drugs in the management of diabetes partially can compensate metabolic derangements, but do not necessarily improve the elementary biochemical lesions.[3]

Moreover, they have accompanied side effects.[4]Furthermore, insulin therapy in insulin dependent diabetes mellitus has several drawbacks such as insulin resistance,[5] develops anorexia nervosa, brain atrophy, and fatty liver[6] after chronic treatment Thus, in modern medicine, no satisfactory effective therapy is still available to control this condition.

Researchers conducted during past few decades on about 45 plants or their products (active, natural principles, and crude extracts) have shown experimental or clinical anti diabetic activity.[8]It is estimated that more than 800 species of plants exhibit hypoglycemic properties, and Wild ginger is one among them.[9]

Wild ginger may have been favorably used for thousands of years; however, modern herbal pharmacology appears to have just begun to appreciate the tremendous therapeutic potential of it.[10-13]

Therefore, because of the side effects associated with the present antidiabetic drugs, there are needed to develop effective, safe and cheap drugs for diabetes management. Such effective, safe and cheap drugs could be obtained by using medicinal plants which have been used by humans to prevent or cure diseases including diabetes since the dawn of civilization[7]. These plant based herbal medicines are thought to be effective, safe and affordable to the common population in the underdeveloped and developing countries of the world. Herbal medicines are effective than the marketed anti diabetic agents.[7]

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Collection and authentication of plant materials :

For this study, the whole plant of *Barleria noctiflora* was collected from ENVIS Centre of medicinal plant Yelahanka Bengaluru, Karnataka.

Preparation of extract

Fresh plant was cleaned and shade dried at room temperature and was powdered mechanically.

The powdered materials was extracted with solvent (ethanol 250 ml) by Soxhlet's extraction method. Finally the ethanol was evaporated using a rotary evaporator, leaving a small yield of extracted plant material (about 2 to 3 ml) in the glass bottom flask. This extract was kept in desiccator till further use and the percentage yield of the plant extract were calculated and noted.

Finally the extract was used for qualitative phytochemical analysis and to evaluate hypoglycaemic activity

Preliminary phytochemical screening of active constituents[61-62]

Preliminary phytochemical screening of *Barleria noctiflora* (Whole plant) extracts were carried out by using standard methods

1. **Test for alkaloids**

The extract was treated with dilute Hydrochloric acid and filtered. Alkaloidal agents allowed to treat with filtrate.

a) **Mayer's–Test**

The little amount of the sample was treated with Mayer's reagent and cream colour indicates the presence of alkaloid.

b) **Dragendroff's–Test**

The little amount of the sample was treated with the Dragendroff's reagent, the presence of reddish-brown precipitate reveals the presence of alkaloid.

c) **Hager's–Test**

The little amount of the sample was Treated with the Hager's reagent and presence of yellow colour precipitate indicates the presence of alkaloid.

d) **Wagner's–Test**

The little amount of the sample was Treated with the Wagner's reagent, the appearance of brown colour precipitate indicates the presence of alkaloid.

2. Test for carbohydrates and glycosides:

The extract was treated with 3ml of alpha–Naphthol in alcohol and to the sides of the test tube concentrated sulphuric acid were added carefully. Formation of violet colour ring at the junction of two liquids shows the presence of carbohydrates.

a) **Fehling's–Test**

The extract was treated with Fehling's solution A and B and heated. Presence of reddish-brown colour precipitate indicates the presence of reducing sugars.

b) **Benedict's–Test**

The extract was treated with Benedict's reagent and heated and presence of reddish orange colour precipitate indicates the presence of reducing sugars.

c) **Barfoed's–Test**

The extract was treated with Barfoed's reagent and heated. Appearance of reddish orange colour precipitate indicates the presence of non-reducing sugars.

3. Test for proteins

a) **Biuret's–test**

When the extracts was treated with copper sulphate solution, followed by the addition of sodium hydroxide solution, appearance of violet colour indicates the presence of proteins

b) **Millon's–Test**

When the extract was treated with Millon's reagent, appearance of pink colour indicates the presence of proteins.

4. Test for steroids

a) **Libermann Burchard Test**

When the extract was treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of acetic anhydride, appearance of green colour indicates the presence of
steroids.

5. Test for sterols

When the extract was treated with 5% potassium hydroxide solution, appearance of pink colour indicates the presence of sterols.

6. Test for phenols

When the extract was treated with neutral ferric chloride solution, appearance of violet colour indicates the presence of phenols. When the extracts were treated with 10% sodium chloride solution, the appearance of cream colour indicates the presence of phenols.

7. Test for tannins

a) When the extract was treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins.

b) When the extract was treated with aqueous bromine solution, appearance of white precipitate indicates the presence of tannins.

8. Test for flavonoids

a) 5ml of the extract solution was hydrolysed with 10 % v/v sulphuric acid and cooled. Later, it was extracted with diethyl ether and divided into three portions in test tubes. 1 ml of diluted sodium carbonate, 1 ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow colour demonstrated the presence of flavonoids.

b) **Shinoda's test**

Dissolved in alcohol, added one piece of magnesium and HCL was added drop wise finally concentrated and heated, magenta colour indicates flavonoids.

9. Test for gums and mucilage

25ml of alcohol with extract was filtered and filtrate swelling nature was examined

10. Test for glycosides

Extract with the glacial acetic acid and ferric chloride solution and concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

11. Test for saponins

a) Foam test

With distilled water foam formed in upper part of tube indicates the saponins.

12. Test for terpenes

Thionyl Chloride and tin , pink colour indicates terpenes.

Chemicals:

Streptozotocin (STZ) was purchased from Quesst International. Glucose, triglycerides, total cholesterol and HDL cholesterol kits were purchased from Span Diagnostics, Gujarat.

Animals:

Male wistar rats (150-200gm) were obtained from animal house of ABIPER. Rats were fed with standard and recommended diet (Amrut Foods Ltd.). After randomization into various groups and before initiation of the experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark/light cycle. Animal kept fasting for 16 hrs All the procedures were performed in accordance to Institutional Animal Ethical Committee.

Determination of acute toxicity: [63]

Principle:

Acute toxicity for ethanolic extract of ***Barleria noctiflora*** (Whole plant) will be done according to the Organization of Economic Co-Operation and Development (OECD) guideline No: 423. To determine acute toxicity of the drug, overnight fasted wistar rats were orally fed with extract in increasing dose levels, with the use of 6 animals of a single sex (normally females) per step. The starting dose level is selected are 5, 50, 300, and 2000 mg/kg After toxicity study , dose of 300 mg/kg was selected as per literature. The test procedure minimizes the number of animals required to estimate the oral acute toxicity[63].

Experimental induction of diabetes mellitus in rats:

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared solution of streptozotocin (50 mg/ kg body weight) dissolved in 0.1 M citrate buffer, pH 4.5. The negative control rats were injected with the same concentration of citrate buffer only (Mohammadi and Naik, 2008). Injected rats with streptazotocin kept for drinking 5%solution of glucose (Gandhi and Sasikumar, 2012). Diabetes mellitus in streptozotocin rats was confirmed by measuring the fasting blood glucose concentration, 72 hours after injection with streptozotocin. The rats with fasting blood glucose above 250 mg/dL were enrolled in the study. The treatment was started on the third day after streptozotocin injection and considered as zero day of treatment.[64]

Preparation of 0.1 molar citrate buffer :

Citric acid (10.5 g) and sodium citrate (14.7 g) were accurately weighted and mixed in 50 ml of distilled water. The volume was made up to 1000 ml with distilled water and the pH was adjusted to 4.5 by sodium hydroxide solution.[65]

EVALUATION OF ANTI- DIABETIC ACTIVITY

Animals were randomly divided into 6 groups of 6 animals in each group and assigned as below. The animals were deprived for food for 16 hours before the experiment, and water is allowed to them, but on the day of experiment water is withdrawn.

Table 1: Group's classification:

TREATMENT	DRUG
Group. I	Vehicle control (I.P route) (06 animals).
Group. II	Diabetic control (Streptozotocin 50mg/Kg I.P route) (06 animals).
Group. III	Diabetic + Glibenclamide (10mg/kg I.P route) (06 animals).
Group. IV	Diabetic +*Barleria noctiflora* (100mg/kg P.O) (06 animals).
Group. V	Diabetic + *Barleria noctiflora* (200mg/kg P.O) (06 animals).
Group. VI	Diabetic + *Barleria noctiflora* (400mg/kg P.O) (06 animals)

Blood glucose estimation:

Fasting blood glucose levels were determined in all experimental rats initially to determine the diabetic status and there after every week during the 28 day study period. Blood was obtained form tail help of sharp razor y snipping and levels of blood glucose were determined by glucometer (Ultra Touch Two, Johnson and Johnson). Tail was sterilized for every time collection of blood. The OGTT was performed on overnight fasted normal rats.

Group I: Glucose (2 g/kg), orally with 0.5 ml of 5% Tween-80 (n =6)
Group II: Glucose (2 g/kg), orally + treated with *B.noctiflora* 100 mg/kg (n =6)
Group III: Glucose (2 g/kg), orally + treated with *B.noctiflora* 200 mg/kg (n =6)
Group IV: Glucose (2 g/kg), orally + treated with *B.noctiflora* 400 mg/kg (n =6)

Serum lipid profile estimation:

At the end of 28 days, blood was collected from inferior vena cava, serum separated for determination of parameters like total cholesterol, HDL- cholesterol and triglycerides using commercially available kits (Span diagnostics). LDL-cholesterol and VLDL cholesterol calculated using the Fried Ewald's formula[66]

VLDL = Triglycerides / 5

LDL = Total cholesterol – (HDL-CH + VLDL-CH)

Liver glycogen estimation

Liver of individual animal was homogenized in 5% w/v trichloroacetic acid and its glycogen content were determined 14 by the method of Carrol .[67]

Glycosylated hemoglobin determination

At the end of 28 days, blood was collected from retro-orbital plexus and subjected for the determination of glycosylated hemoglobin.

Statistical analysis

Mean ± SEM. Used to calculate results.The results were analyzed for statistical significance by one way ANOVA followed by Dun net's Multiple Test for comparison.

Histopathological studies

Processing of isolated pancreas

The animals were sacrificed and their pancreas was isolated. The isolated pancreas is cut into small pieces and preserved in 10% formalin for two days.After 12 hours Pancreas pieces were washed in water. Then dehydration with isopropyl alcohol with following strenght (70%, 80% and 90%) for 12 hours.Using chloroform Clearing was done for 20 minutes. After clearing the organ pieces were subjected to paraffin infiltration in automatic tissue processing unit.

Embedding in paraffin by vacuum

Hard paraffin was melted and the hot paraffin was poured into L-shaped blocks. The pancreas pieces were then dropped into molten paraffin quickly and allowed to cool.

Sectioning

The blocks were cut using microtome blade to get sections of thickness of 5µ. The sections were taken on a microslide on which egg albumin (sticking substance) was applied. The sections were then allowed to remain in an oven at 60^0C for 1 hour. Paraffin melts and egg albumin denatures, thereby fixing tissues to the slide.

Staining

Eosin is an acid stain. Hence it stains all the cell constituents which are basic in nature to pink colour. e.g.: (RNA, Cytoplasm). Haematoxylin is a basic stain which stains all the acidic cell components blue e.g.: DNA in the nucleus.

Procedure

Hydrated the sections by washing in isopropyl alcohol of decreasing strength (100%, 90%, 80% and 70%). Finally washed with water. Stained with haematoxylin for 15 minutes. Rinsed under tap water. Differentiated in 1% acid alcohol by 3 to 10 quick dips checked the differentiation with a microscope. Nuclei were appeared light (or colorless). Washed with tap water.Dipped in lithium carbonate until sections become bright blue (3-5 dips). Washed under running tap water for 10-20 minutes.

Stained with eosin for 15 seconds-2 minutes depending on the age of eosin and depth of the counter stain desired. For even staining results, dip slides several times before allowing them to set in eosin for the desired time. Dehydrated in 95% isopropyl alcohol and absolute isopropyl alcohol until excess eosin was removed, two changes of 2 minutes each were done and checked under microscope. Mounted in DPX (Desterene dibutyl phthalate xylene). The photographs were taken under 500X magnification.

Results:

Preparation of Extract:

Extraction of root of *Barleria noctiflora* was carried out using Ethanol as a solvent. The Percentage yield was 9.58%.

Table 2: The Percentage yield of extract

SL.NO	Extract	Percentage yield
1	Ethanol	9.65%

Preliminary Phytochemistry Screening of the plant extract:

The phytochemical constituents were extracted by using Ethanol as a solvent. The active constituents of Plant extract indicate are alkaloids, carbohydrates, glycosides, steroids, tannins, Phytosterols, flavonoids, proteins and amino acids.

Table 3

SL.NO	***CONSTITUENTS***		***ETHANOLIC EXTRACT***
1	*Alkaloids*	Mayer's Test	+ve
		Dragendroff's Test	+ve
		Hager's Test	+ve
		Wagner's Test	+ve
2	Carbohydrates	Fehling's Test	-ve
		Benedict's Test	+ve
		Barfoed's Test	+ve
3	Proteins	Biuret's test	+ve
		Millon's Test	-ve
4	Steroids	Libermann Burchard Test	-ve
5	Sterols		-ve
6	Phenols		+ve
7	Tannins		+ve
8	Flavonoids		+ve
9	Gums and Mucilage		-ve
10	Glycosides		+ve
11	Saponins		+ve
12	Terpenes		-ve

-ve- indicate the absence of compound

+ve- indicate the presence of compound

Determination of acute toxicity

Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** was studied for acute toxicity at doses of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. As per OECD 423. Guideline Dose of 2000mg/kg showed the toxic symptoms, so according to OECD guideline 423; it is considered as a LD_{50} cutoff value. Doses selected for pharmacological studies by fixed dose methods are:

Ethanolic extract-100mg/kg ($1/20^{th}$ of 2000mg/kg)

Ethanolic extract-200mg/kg ($1/10^{th}$ of 2000mg/kg)

Ethanolic extract-400mg/kg (1/5th of 2000mg/kg)

Oral Glucose tolerance test

In OGTT the serum glucose level (mg/dl) of control fasted rats were significantly increased after the administration of the glucose solution 2g/kg and it started the significance after, 2, 4, 6,12 and 24 hr ($P<0.05$), ($P<0.01$) in control group as compared to normal group.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg and glibenclamide 10mg/kg orally reduced serum glucose level significantly after 2, 4, 6,12 and 24 hr ($p<0.05$, $P<0.01$) when compared to control group.

STREPTOZOCIN INDUCED DIABETIC MODEL:

Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) serum glucose level in STZ induced diabetic rats.

In an STZ induced diabetic rats (Control group) serum glucose level has significantly increased on 14, 21th days ($p<0.001$) in diabetic control rats when compared to normal groups.

Administration of glibenclamide 10 mg/kg by i.p route for 21 days treatment were reduced significantly serum glucose level on 14 days ($p<0.05$) and 21 days ($p<0.01$) as compared to control groups.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg orally for 21days treatment were reduced significantly serum glucose level on 14 days ($p<0.05$), 21th days ($p<0.05$ and $p<0.01$) as compared to control groups.

Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) serum lipid profile level in STZ induced diabetic rats.

In STZ induced diabetic rats serum lipid profile such as total cholesterol, triglycerides, LDL ($p<0.001$) (low density lipids) VLDL (0.01) (very low density lipids) levels were observed significantly increased and HDL level in diabetic control group were seen significantly decreased ($p<0.001$) as compared to normal group.

Administration of glibenclamide 10 mg/kg on serum lipid profile. A decreased in the serum triglycerides, total cholesterol ($p<0.001$), LDL ($p<0.01$), and VLDL (very low density lipids) levels ($p<0.001$), and an increase in the HDL ($p<0.001$) were observed as compared to diabetic control group.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg on serum lipid profile. A decreased in the serum total cholesterol ($p<0.01$), Triglycerides ($p<0.05$ and $p<0.001$), LDL ($p<0.001$), and no changed in VLDL (very low density lipids) levels and an increase in the HDL ($p<0.01$ and $p<0.001$) were observed as compared to diabetic control group.

Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) SGOT, SGPT and ALP in STZ induced diabetic rats.

In STZ induced diabetic rats were observed level of SGOT, as a significantly ($p<0.001$) increased in diabetic rats as compared to normal groups. After treatment with glibenclamide 10mg/kg the SGOT, activities were significantly ($P<0.001$) reduced as compared to diabetic control rats.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100,200 and 400 mg/kg orally were observed level of SGOT significant change in 200 ($p<0.05$) and 400mg/kg group ($p<0.01$) reduction as compared to control group.

SGPT

In STZ induced diabetic rats were observed level of SGPT, as a significantly ($p<0.001$) increased in diabetic rats as compared to normal groups. After treatment with glibenclamide 10mg/kg the SGPT, activities were significantly ($P<0.001$) reduced as compared to diabetic control rats.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg orally were observed level of SGPT significant change in 250 ($p<0.05$) and 500mg/kg group ($p<0.01$) reduction as compared to control group.

ALP

In STZ induced diabetic rats were observed level of ALP, as a significantly ($p<0.001$) increased in diabetic rats as compared to normal groups. After treatment with glibenclamide 10mg/kg the ALP, activities were significantly ($P<0.05$) reduced as compared to diabetic control rats.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100,200 and 400 mg/kg orally were observed level of ALP significant change in 200 ($p<0.05$) and 400mg/kg group ($p<0.01$) reduction as compared to control group.

Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) in –vivo Anti-Oxidant Parameter from Liver Homogenate in STZ-Induced Diabetic Rats.

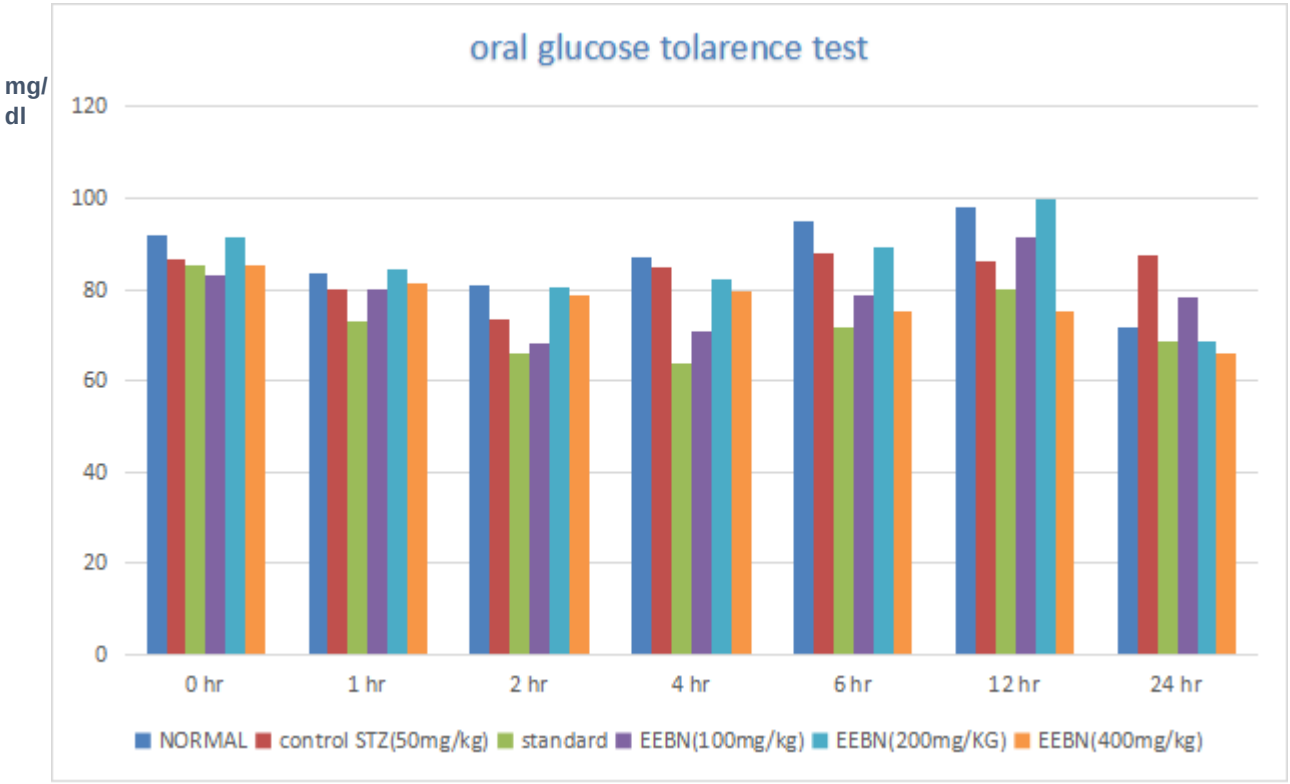
SOD , LPO and CAT levels in experimental animal tissues were summarized . There was significant ($p <0.05$) decrease in SOD and CAT in diabetic-induced groups and significantly ($p<0.001$) increased in LPO level was observed in control group. Treatment with EEBN of 100, 200 and 400 mg/kg body weight and glibenclamide 10 mg/kg body weight increased the activity of SOD, CAT in the liver to near normal and LPO level was decreased as compared to diabeticcontrol group.

Table 4: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats.

Treated groups	0 hr	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr
Normal	92.00±0.73	83.00±2.06	81.11±1.85	86.91±1.07	95.00±1.13	98.18±1.07	71.66±1.17
Control (STZ50mg/kg)	86.50±0.76	80.00±0.57	73.52±1.13b	88.10±0.96c	88.10±0.94b	88.10±0.88c	87.33±4.49b
Standard (Glibenclamide10 mg/kg)	85.4±0.76	73.11±1.45	66.00±1.52*	71.86±1.93*	71.86±1.51*	80.13±1.18*	68.00±2.38**
EEBN (100mg/kg)	83.2±1.23	80.10±1.11	68.33±1.30*	71±0.88*	78.60±1.17*	91.55±1.11ns	78.17±5.63*
EEBN (200mg/kg)	91.3±1.52	84.50±0.88	80.55±1.18*	82.17±0.94*	89.33±0.76*	99.88±0.73ns	68.07±3.64**
EEBN (400mg/kg)	85.54±3.21	81.17±3.36	78.96±4.16*	79.50±3.86*	75.33±3.71*	75.34±1.89*	66.01±7.13**

All the values are mean ± SEM, n=6, ns= not significant, One wayAnalysis of Variance (ANOVA) followed by Dunett'smultiple comparison test, ***p*< 0.05 and **p<0.01, vs. control group and [a]*p*<0.001, vs normal group. EEBN-of Ethanolic extract of *Barleria noctiflora*.

Fig.3: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats.



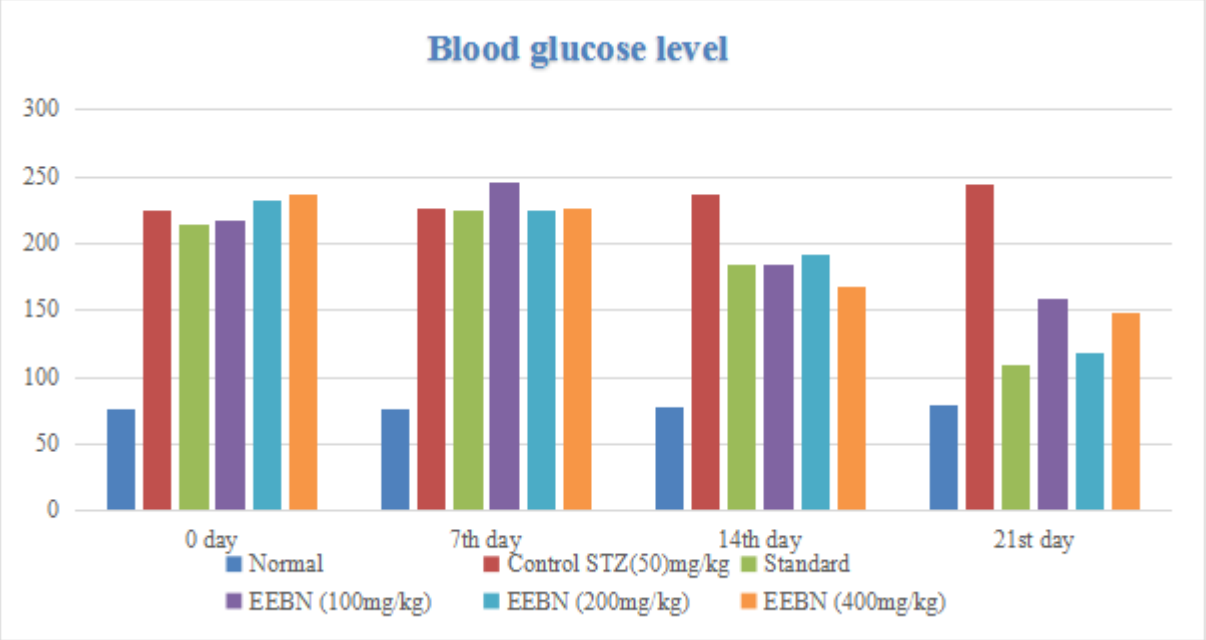
All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test, ***p*< 0.05 and **p<0.01, vs. control group and [a]*p*<0.001, vs normal group. EEBN-of Ethanolic extract of *Barleria noctiflora*.

Table.no.5: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats:

Treated groups	0 Day	7th Day	14th Day	21st Day
Normal	76.50±0.53	76.17±0.68	78.10±0.27	79.77±0.18
Control (STZ50mg/kg)	225.0±0.37	226.1±0.36	237.1±0.21a	244.5±0.25a
Standard (Glibenclamide10mg/kg)	213.9±0.23	224.3±9.17	183.50±10.66**	109.32±0.27***
EEBN(100mg/kg)	216.9±0.22	245.4±9.86	184.56±9.76*	158.3±0.22*
EEBN(200mg/kg)	232.8±0.18	224.8±0.70	191.7±15.16*	117.5±0.14**
EEBN(400mg/kg)	237.10±4.866	225.50±3.342	167.0±3.308***	148.9±11.5***

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, ***p*< 0.05 and **p<0.01, ***p<0.001 vs. control group and [a]*p*<0.001, vs normal group.

Fig.4: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats:



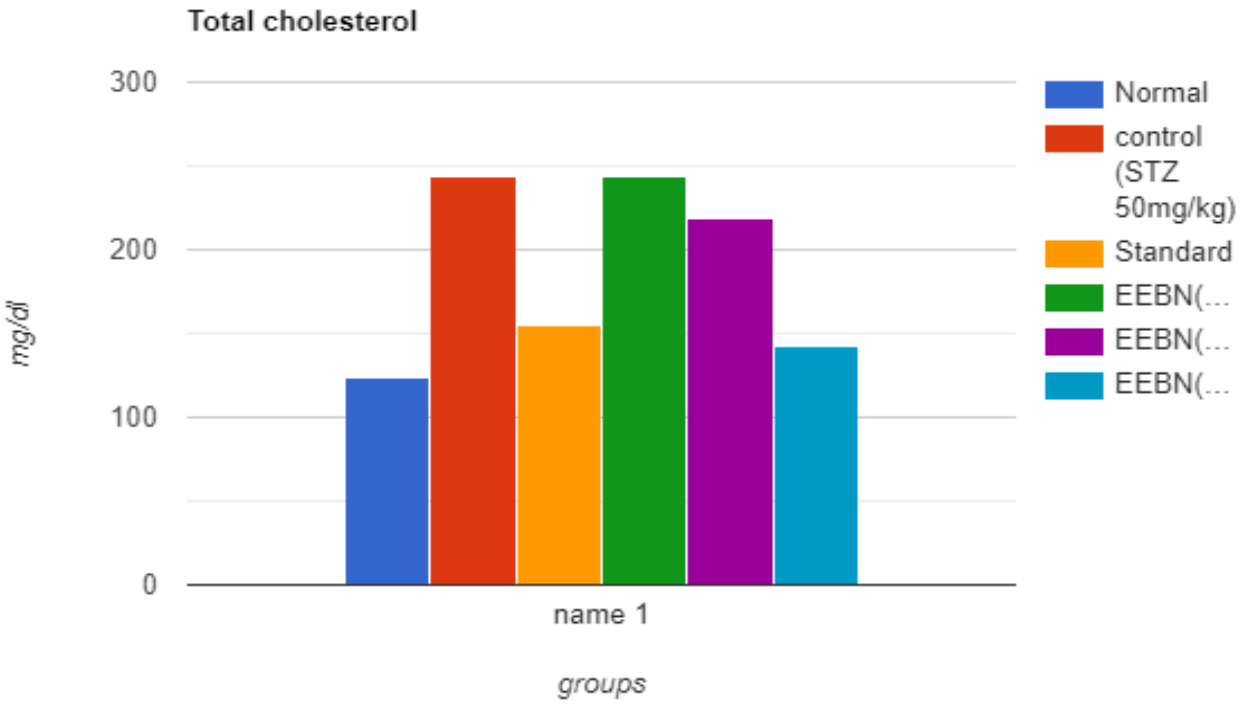
All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test, ***p*< 0.05 **p<0.01 and p<0.001 vs. control group and [a]*p*<0.001, vs normal group.

Table.no.6: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on serum lipid profile in STZ induced diabetic rats:

TREATED GROUPS	TOTAL (mg/dl) CHOLESTEROL	HDL (mg/dl)	LDL (mg/dl)	TRIGLYCERIDES (mg/dl)	VLDL (mg/dl)
Normal	123.7±1.82	52.28±0.74	56.07±2.12	82.04±0.71	16.5±0.14
Control (STZ 50mg/kg)	243.7±4.73a	17.48±0.42a	195.8±7.41a	170.10±2.24a	30.00±5.10b
Standard (Glibenclamide 10mg/kg)	154.5±1.20***	48.93±0.39***	93.01±2.31***	91.88±1.22***	13.00±1.77***
EEBN (100mg/kg)	243.7±4.75ns	18.18±0.48ns	189.01±4.92ns	175.2±2.33ns	35.48±0.46ns
EEBN (200mg/kg)	218.8±8.80**	24.78±1.22**	165.8±8.81*	155.8±7.88*	31.61±1.57ns
EEBN(400mg/kg)	142..9±6.514***	27.39±2.356**	126.9±2.08**	127.8±7.07**	24.07±0.91**

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *p<0.05, **p<0.01 and ***p< 0.001 vs. control group and [a]p<0.001, bp<0.01 vs Sham operated normal.

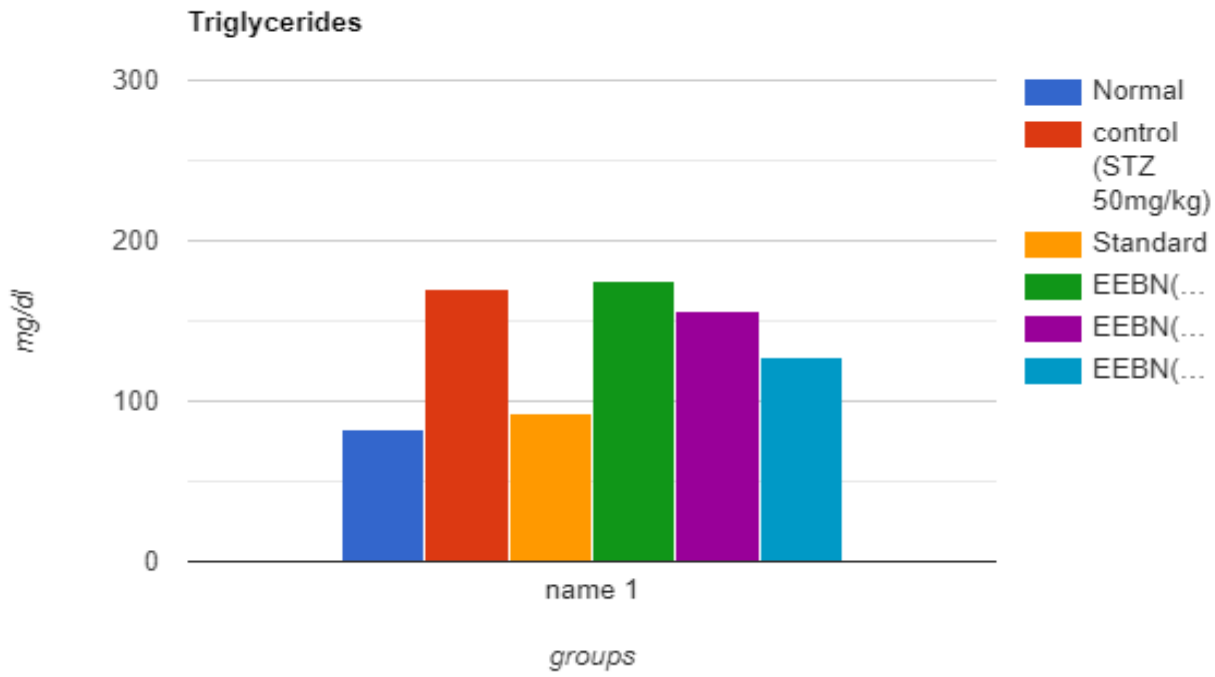
Fig 5: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on cholesterol in STZ induced diabetic rats



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, **p<0.01**, ***p*< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal.

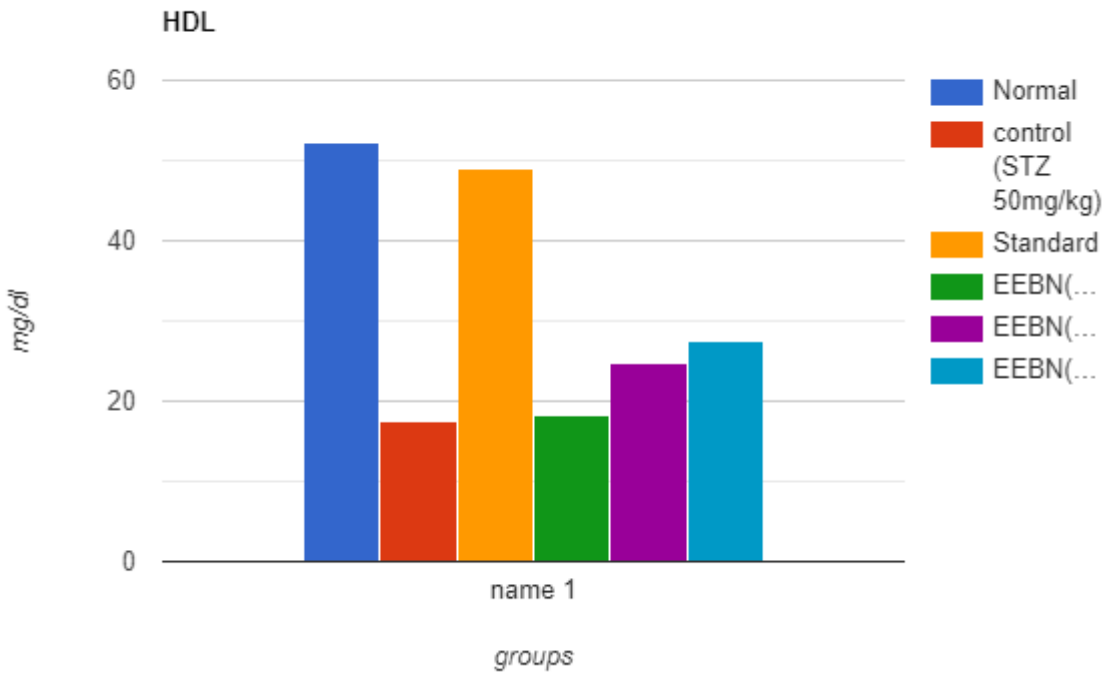
Fig.6: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on Triglycerides in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM ,n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *p<0.05, **p<0.01, ***p< 0.001 vs. control group and [a] p<0.001, vs Sham operated normal.

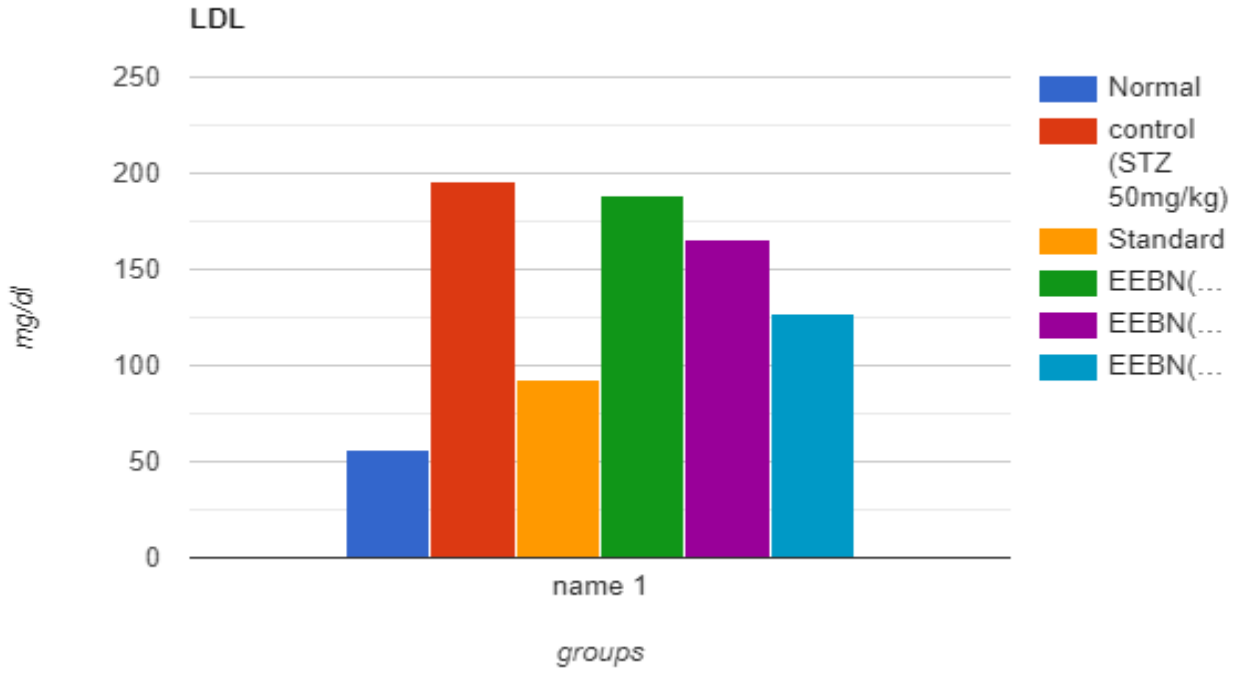
Fig.7: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on HDL in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test,**p<0.01, ***p< 0.001 vs. control group and [a]p<0.001, vs Sham operated normal.

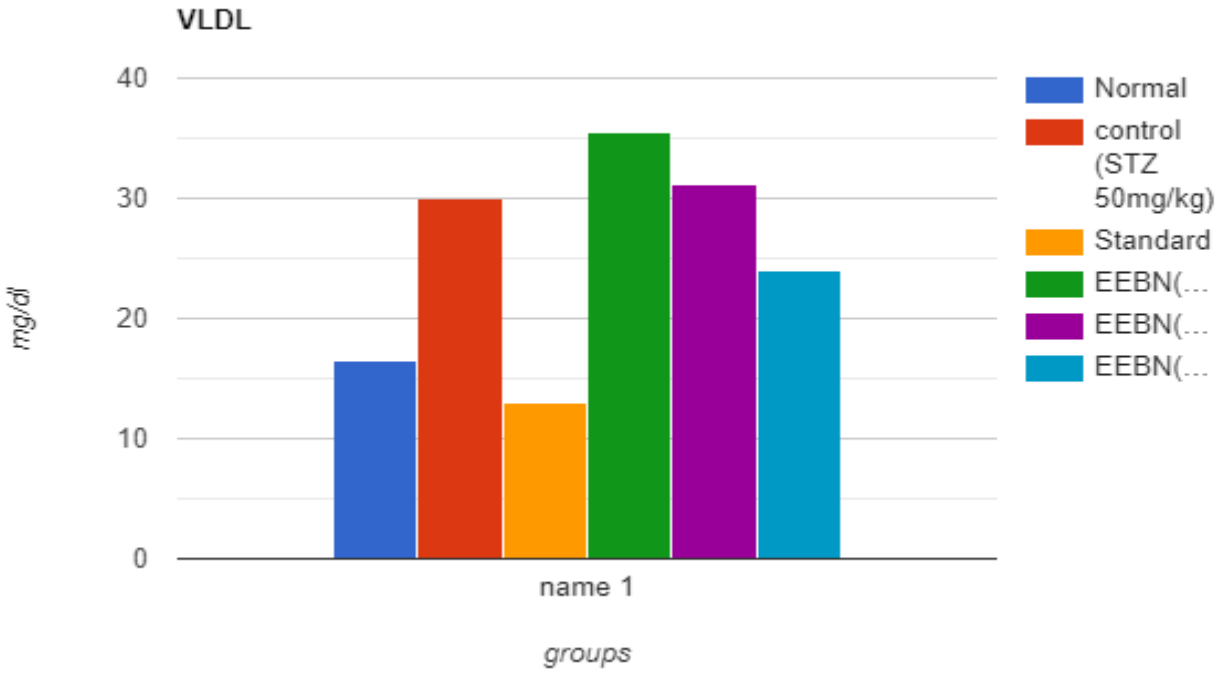
Fig.8: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) LDL in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *****p<0.05, ******p<0.01,*******p< 0.001 vs. control group and ^{a}p<0.001, vs Sham operated normal.

Fig.9: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on VLDL in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

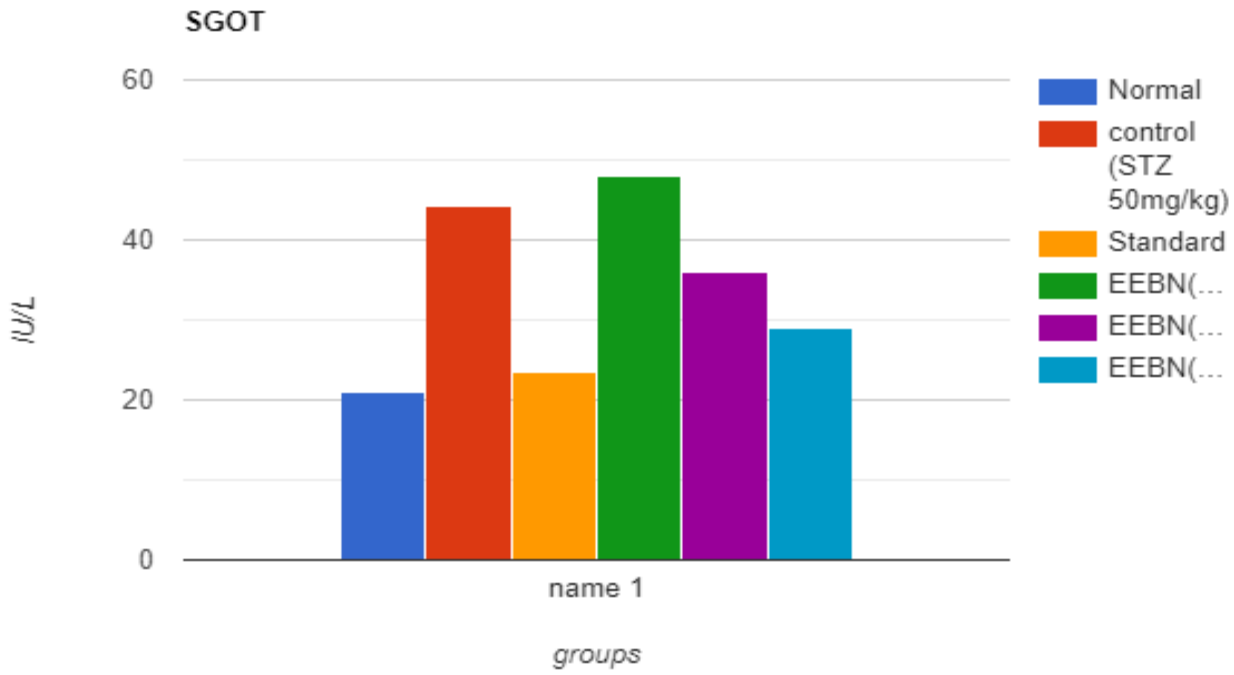
All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test**, ****p<0.01**, ******p*<0.001 vs. control group and ^{b}p<0.01, vs Sham operated normal.

Table.no.7: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on SGOT, SGPT and ALP in STZ induced diabetic rats.

TREATED GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Normal	21.04±0.75	22.41±0.69	90.77±1.27
Control (STZ 50mg/kg)	44.32±0.96a	56.71±0.96a	162.8±1.14a
Standard (Glibenclamide 10mg/kg)	23.58±0.90***	27.22±0.46***	137.40±0.69*
EEBN (100mg/kg)	48.00±1.71ns	43.35±1.14ns	127.81±2.01*
EEBN (200mg/kg)	36.12±1.61**	35.14±2.33**	132.15±2.22*
EEBN (400mg/kg)	29.05±1.253***	31.811±1.45***	133.65±2.91**

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *****p<0.05, **p<0.01**, ******p*< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal.

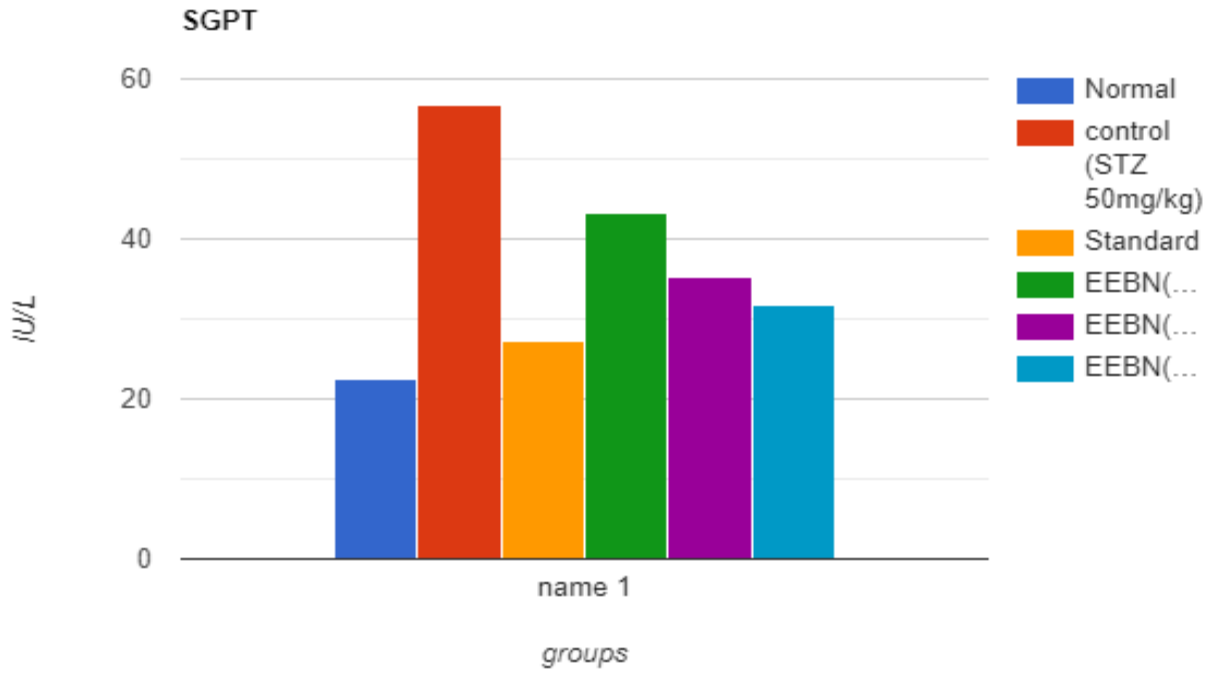
Fig.10: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on SGOT in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM , n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, ***p< 0.001 vs. control group and ^{a}p<0.001, vs Sham operated normal.

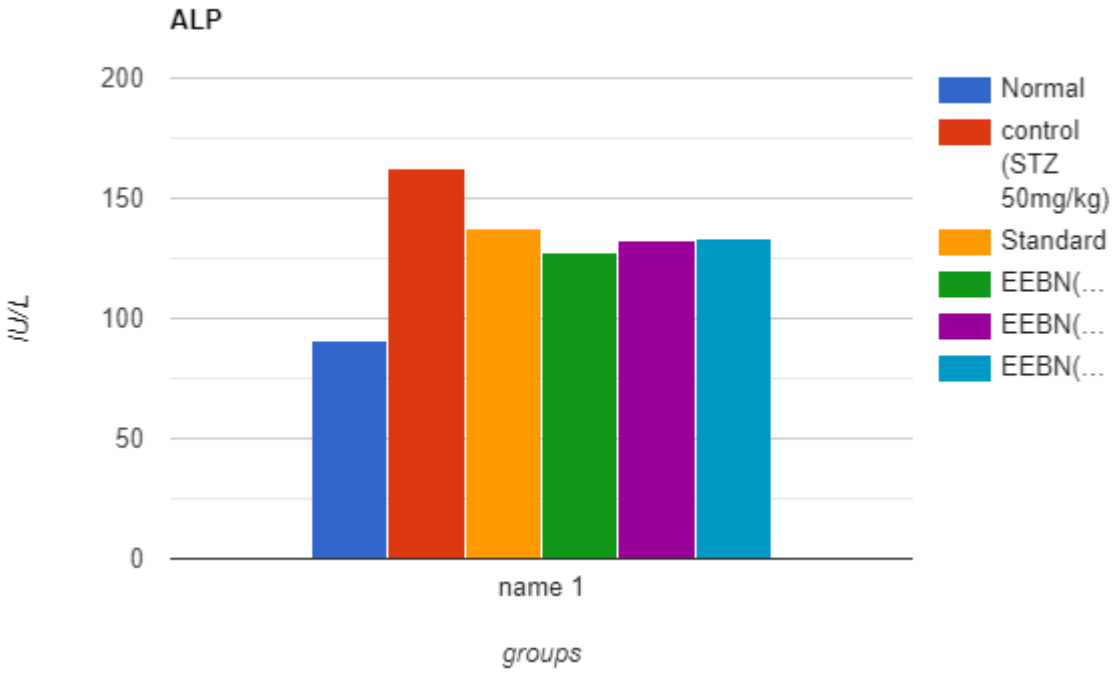
Fig.11: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on SGPT in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, $p<0.01$, ***$p< 0.001$ vs. control group and $^{a}p<0.001$, vs Sham operated normal.

Fig.12: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on ALP in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *p<0.05**, *****p*< 0.01 vs. control group and [a]*p*<0.001, vs Sham operated normal.

Table no.8: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on in –vivo Anti-Oxidant Parameter from Liver Homogenate in STZ induced diabetic rats.

Treatment Group	SOD (Unit/min/gm tissue)	Catalase (µm/mg tissue)	LPO (µm of H2O2/mg tissue)
Normal	45.87±0.66	72.44±0.81	10.11±0.27
Control (STZ 50mg/kg)	27.33±0.84a	31.73±0.84a	25.49±0.59a
Standard (Glibenclamide 10mg/kg)	43.25±0.37***	70.01±0.74***	16.74±0.37***
EEBN (100mg/kg)	37.99±0.71**	57.61±0.91*	15.88±0.65*
EEBN (200mg/kg)	33.784±0.94**	60.88±0.82**	14.99±0.58**
EEBN (400mg/kg)	35.99±1.66**	60.23±2.58***	15.71±1.20**

Preparation of Extract:

Extraction of root of *Barleria noctiflora* was carried out using Ethanol as a solvent. The Percentage yield was 9.58%.

Table 2: The Percentage yield of extract

SL.NO	Extract	Percentage yield
1	Ethanol	9.65%

Preliminary Phytochemistry Screening of the plant extract:

The phytochemical constituents were extracted by using Ethanol as a solvent. The active constituents of Plant extract indicate are alkaloids, carbohydrates, glycosides, steroids, tannins, Phytosterols, flavonoids, proteins and amino acids.

Table 3

SL.NO	***CONSTITUENTS***		***ETHANOLIC EXTRACT***
1	*Alkaloids*	Mayer's Test	+ve
		Dragendroff's Test	+ve
		Hager's Test	+ve
		Wagner's Test	+ve
2	Carbohydrates	Fehling's Test	-ve
		Benedict's Test	+ve
		Barfoed's Test	+ve
3	Proteins	Biuret's test	+ve
		Millon's Test	-ve
4	Steroids	Libermann Burchard Test	-ve
5	Sterols		-ve
6	Phenols		+ve
7	Tannins		+ve
8	Flavonoids		+ve
9	Gums and Mucilage		-ve
10	Glycosides		+ve
11	Saponins		+ve
12	Terpenes		-ve

-ve- indicate the absence of compound

+ve- indicate the presence of compound

Determination of acute toxicity

Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** was studied for acute toxicity at doses of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg. As per OECD 423. Guideline Dose of 2000mg/kg showed the toxic symptoms, so according to OECD guideline 423; it is considered as a LD_{50} cutoff value. Doses selected for pharmacological studies by fixed dose methods are:

Ethanolic extract-100mg/kg ($1/20^{th}$ of 2000mg/kg)

Ethanolic extract-200mg/kg ($1/10^{th}$ of 2000mg/kg)

Ethanolic extract-400mg/kg (1/5th of 2000mg/kg)

Oral Glucose tolerance test

In OGTT the serum glucose level (mg/dl) of control fasted rats were significantly increased after the administration of the glucose solution 2g/kg and it started the significance after, 2, 4, 6,12 and 24 hr ($P<0.05$), ($P<0.01$) in control group as compared to normal group.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg and glibenclamide 10mg/kg orally reduced serum glucose level significantly after 2, 4, 6,12 and 24 hr ($p<0.05$, $P<0.01$) when compared to control group.

STREPTOZOCIN INDUCED DIABETIC MODEL:

Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) serum glucose level in STZ induced diabetic rats.

In an STZ induced diabetic rats (Control group) serum glucose level has significantly increased on 14, 21th days ($p<0.001$) in diabetic control rats when compared to normal groups.

Administration of glibenclamide 10 mg/kg by i.p route for 21 days treatment were reduced significantly serum glucose level on 14 days ($p<0.05$) and 21 days ($p<0.01$) as compared to control groups.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg orally for 21days treatment were reduced significantly serum glucose level on 14 days ($p<0.05$), 21th days ($p<0.05$ and $p<0.01$) as compared to control groups.

Effect of Ethano

lic extract of *Barleria noctiflora* (Whole plant) serum lipid profile level in STZ induced diabetic rats.

In STZ induced diabetic rats serum lipid profile such as total cholesterol, triglycerides, LDL ($p<0.001$) (low density lipids) VLDL (0.01) (very low density lipids) levels were observed significantly increased and HDL level in diabetic control group were seen significantly decreased ($p<0.001$) as compared to normal group.

Administration of glibenclamide 10 mg/kg on serum lipid profile. A decreased in the serum triglycerides, total cholesterol ($p<0.001$), LDL ($p<0.01$), and VLDL (very low density lipids) levels ($p<0.001$), and an increase in the HDL ($p<0.001$) were observed as compared to diabetic control group.

Administration of Ethanolic extract of ***Barleria noctiflora*** **(Whole plant)** 100, 200 and 400 mg/kg on serum lipid profile. A decreased in the serum total cholesterol ($p<0.01$), Triglycerides ($p<0.05$ and $p<0.001$), LDL ($p<0.001$), and no changed in VLDL (very low density lipids) levels and an increase in the HDL ($p<0.01$ and $p<0.001$) were observed as compared to diabetic control group.

Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) SGOT, SGPT and ALP in STZ induced diabetic rats.

In STZ induced diabetic rats were observed level of SGOT, as a significantly ($p<0.001$) increased in diabetic rats as compared to normal groups. After treatment with glibenclamide 10mg/kg the SGOT, activities were significantly ($P<0.001$) reduced as compared to diabetic control rats.

Administration of Ethanolic extract of ***Barleria noctiflora* (Whole plant)** 100,200 and 400 mg/kg orally were observed level of SGOT significant change in 200 ($p<0.05$) and 400mg/kg group ($p<0.01$) reduction as compared to control group.

SGPT

In STZ induced diabetic rats were observed level of SGPT, as a significantly ($p<0.001$) increased in diabetic rats as compared to normal groups. After treatment with glibenclamide 10mg/kg the SGPT, activities were significantly ($P<0.001$) reduced as compared to diabetic control rats.

Administration of Ethanolic extract of ***Barleria noctiflora* (Whole plant)** 100, 200 and 400 mg/kg orally were observed level of SGPT significant change in 250 ($p<0.05$) and 500mg/kg group ($p<0.01$) reduction as compared to control group.

ALP

In STZ induced diabetic rats were observed level of ALP, as a significantly ($p<0.001$) increased in diabetic rats as compared to normal groups. After treatment with glibenclamide 10mg/kg the ALP, activities were significantly ($P<0.05$) reduced as compared to diabetic control rats.

Administration of Ethanolic extract of ***Barleria noctiflora* (Whole plant)** 100,200 and 400 mg/kg orally were observed level of ALP significant change in 200 ($p<0.05$) and 400mg/kg group ($p<0.01$) reduction as compared to control group.

Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) in –vivo Anti-Oxidant Parameter from Liver Homogenate in STZ-Induced Diabetic Rats.

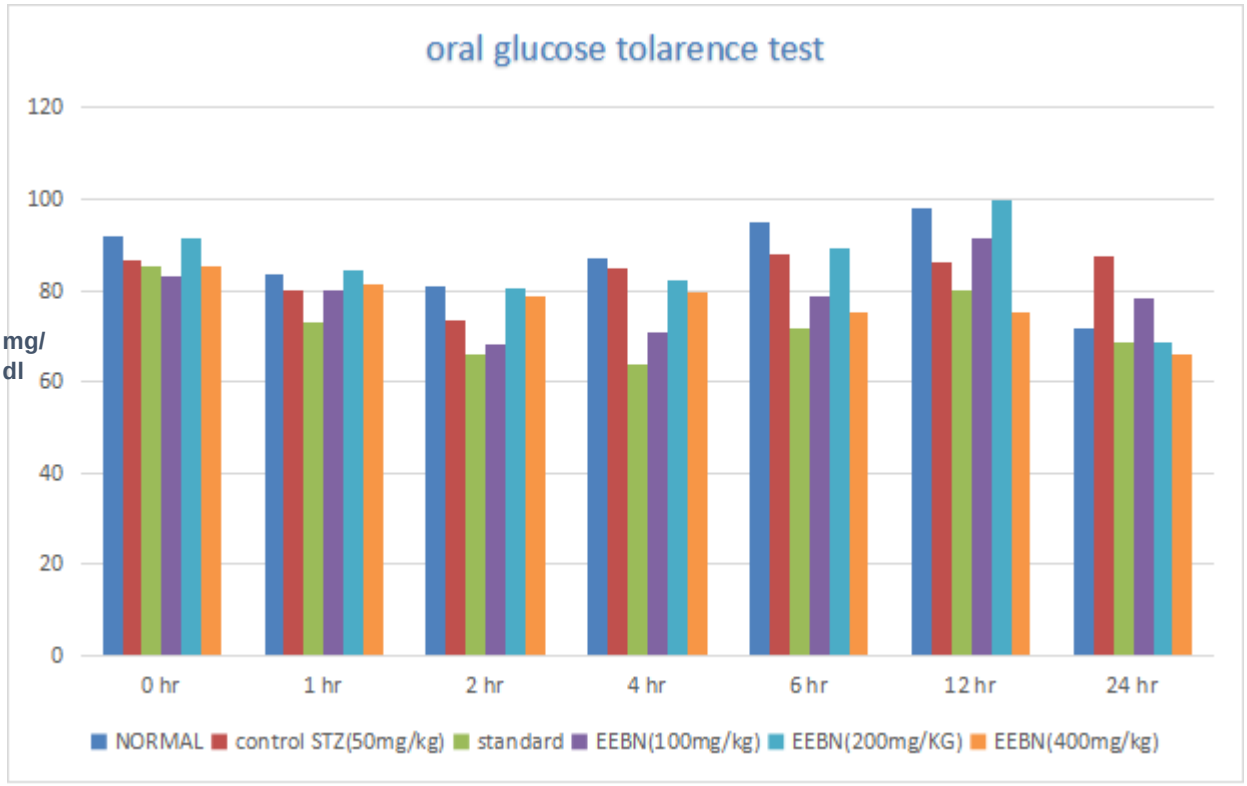
SOD , LPO and CAT levels in experimental animal tissues were summarized . There was significant ($p <0.05$) decrease in SOD and CAT in diabetic-induced groups and significantly ($p<0.001$) increased in LPO level was observed in control group. Treatment with EEBN of 100, 200 and 400 mg/kg body weight and glibenclamide 10 mg/kg body weight increased the activity of SOD, CAT in the liver to near normal and LPO level was decreased as compared to diabeticcontrol group.

Table 4: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats.

Treated groups	0 hr	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr
Normal	92.00±0.73	83.00±2.06	81.11±1.85	86.91±1.07	95.00±1.13	98.18±1.07	71.66±1.17
Control (STZ50mg/kg)	86.50±0.76	80.00±0.57	73.52±1.13b	88.10±0.96c	88.10±0.94b	88.10±0.88c	87.33±4.49b
Standard (Glibenclamide10 mg/kg)	85.4±0.76	73.11±1.45	66.00±1.52*	71.86±1.93*	71.86±1.51*	80.13±1.18*	68.00±2.38**
EEBN (100mg/kg)	83.2±1.23	80.10±1.11	68.33±1.30*	71±0.88*	78.60±1.17*	91.55±1.11ns	78.17±5.63*
EEBN (200mg/kg)	91.3±1.52	84.50±0.88	80.55±1.18*	82.17±0.94*	89.33±0.76*	99.88±0.73ns	68.07±3.64**
EEBN (400mg/kg)	85.54±3.21	81.17±3.36	78.96±4.16*	79.50±3.86*	75.33±3.71*	75.34±1.89*	66.01±7.13**

All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test, ***p*< 0.05 and **p<0.01, vs. control group and [a]*p*<0.001, vs normal group. EEBN-of Ethanolic extract of *Barleria noctiflora*.

Fig.3: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats.



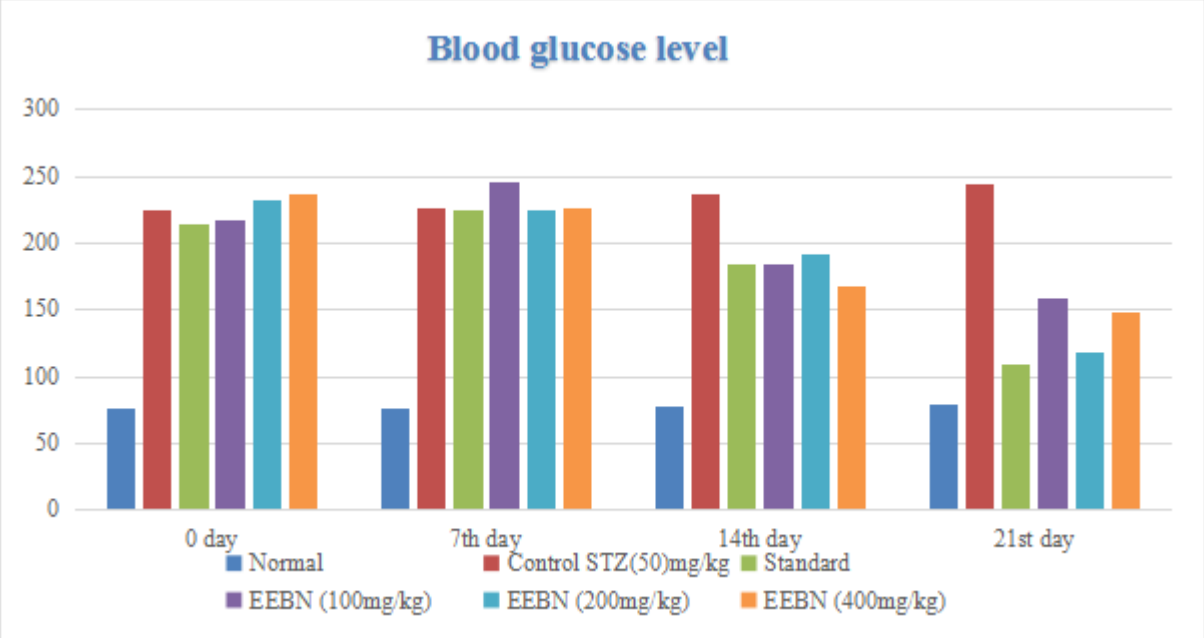
All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test, ***p*< 0.05 and **p<0.01, vs. control group and [a]*p*<0.001, vs normal group. EEBN-of Ethanolic extract of *Barleria noctiflora*.

Table.no.5: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats:

Treated groups	0 Day	7th Day	14th Day	21st Day
Normal	76.50±0.53	76.17±0.68	78.10±0.27	79.77±0.18
Control (STZ50mg/kg)	225.0±0.37	226.1±0.36	237.1±0.21a	244.5±0.25a
Standard (Glibenclamide10mg/kg)	213.9±0.23	224.3±9.17	183.50±10.66**	109.32±0.27***
EEBN(100mg/kg)	216.9±0.22	245.4±9.86	184.56±9.76*	158.3±0.22*
EEBN(200mg/kg)	232.8±0.18	224.8±0.70	191.7±15.16*	117.5±0.14**
EEBN(400mg/kg)	237.10±4.866	225.50±3.342	167.0±3.308***	148.9±11.5***

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, ***p*< 0.05 and **p<0.01, ***p<0.001 vs. control group and [a]*p*<0.001, vs normal group.

Fig.4: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on fasting serum glucose level (OGTT) in STZ induced diabetic rats:



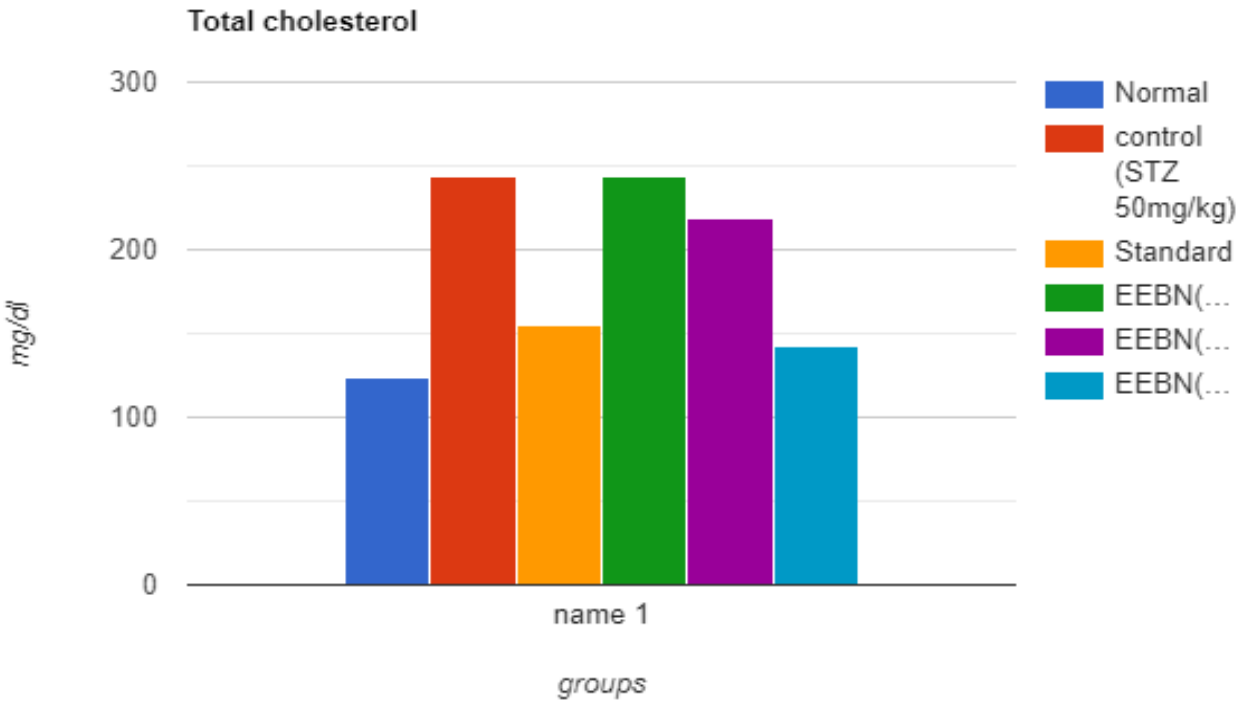
All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test, ***p*< 0.05 **p<0.01 and p<0.001 vs. control group and [a]*p*<0.001, vs normal group.

Table.no.6: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on serum lipid profile in STZ induced diabetic rats:

TREATED GROUPS	TOTAL (mg/dl) CHOLESTEROL	HDL (mg/dl)	LDL (mg/dl)	TRIGLYCERIDES (mg/dl)	VLDL (mg/dl)
Normal	123.7±1.82	52.28±0.74	56.07±2.12	82.04±0.71	16.5±0.14
Control (STZ 50mg/kg)	243.7±4.73a	17.48±0.42a	195.8±7.41a	170.10±2.24a	30.00±5.10b
Standard (Glibenclamide 10mg/kg)	154.5±1.20***	48.93±0.39***	93.01±2.31***	91.88±1.22***	13.00±1.77***
EEBN (100mg/kg)	243.7±4.75ns	18.18±0.48ns	189.01±4.92ns	175.2±2.33ns	35.48±0.46ns
EEBN (200mg/kg)	218.8±8.80**	24.78±1.22**	165.8±8.81*	155.8±7.88*	31.61±1.57ns
EEBN(400mg/kg)	142..9±6.514***	27.39±2.356**	126.9±2.08**	127.8±7.07**	24.07±0.91**

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *p<0.05, **p<0.01 and ***p< 0.001 vs. control group and [a]p<0.001, bp<0.01 vs Sham operated normal.

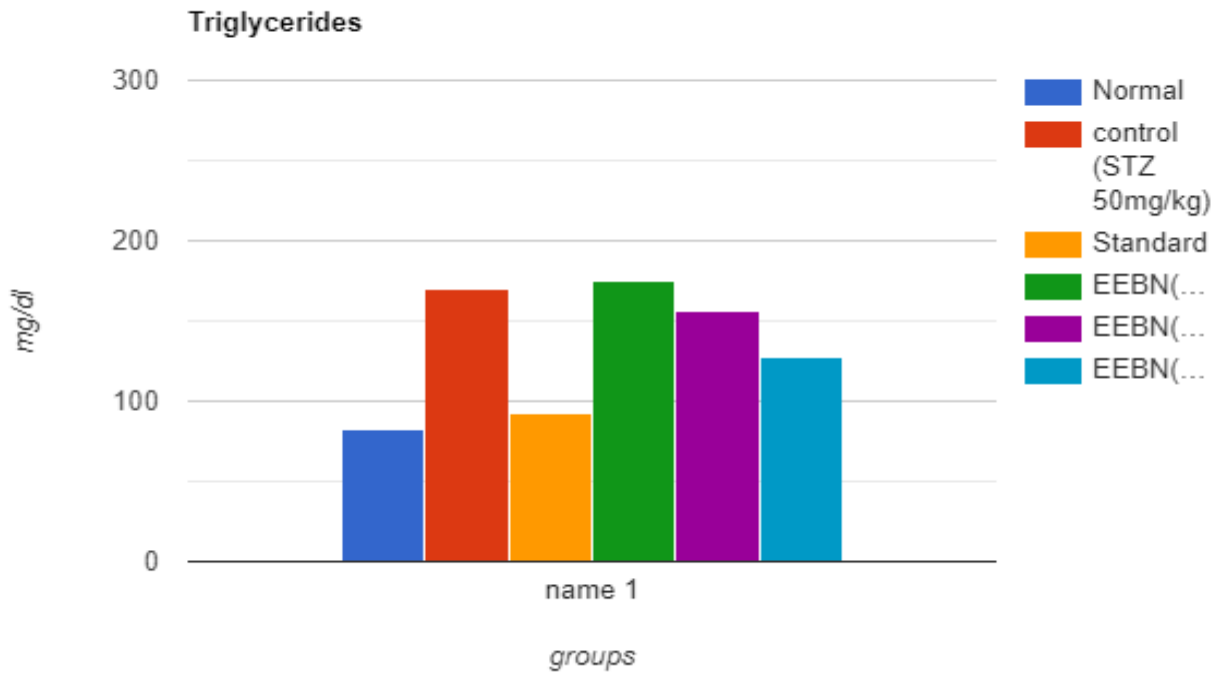
Fig 5: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on cholesterol in STZ induced diabetic rats



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, **p<0.01**, ***p**< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal.

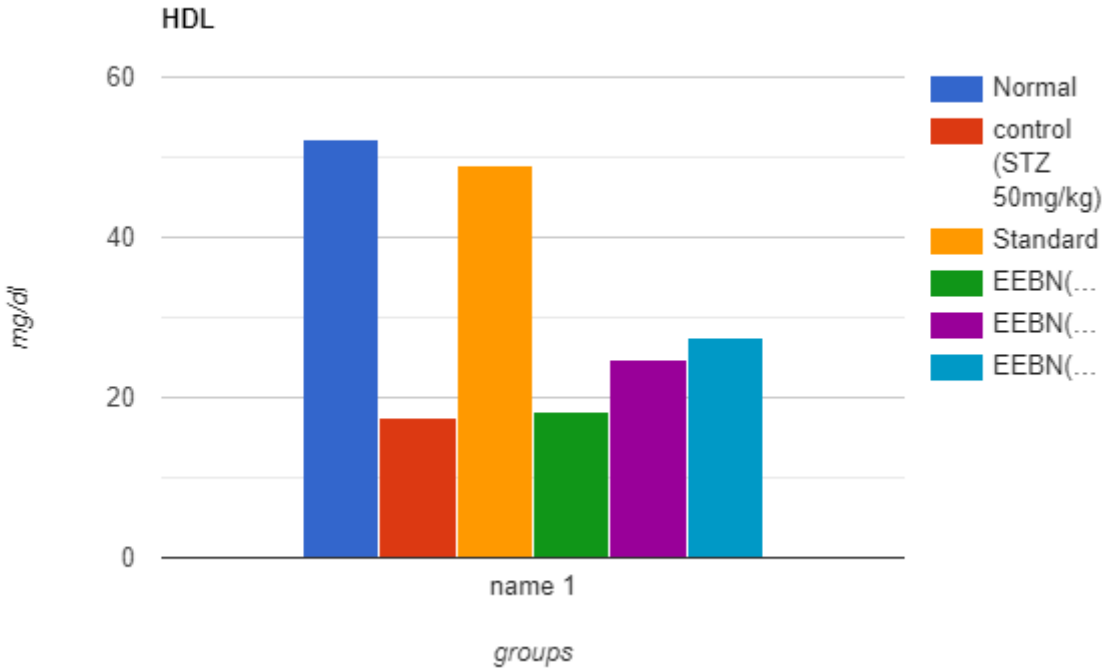
Fig.6: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on Triglycerides in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM ,n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *p<0.05, **p<0.01, ***p< 0.001 vs. control group and [a] p<0.001, vs Sham operated normal.

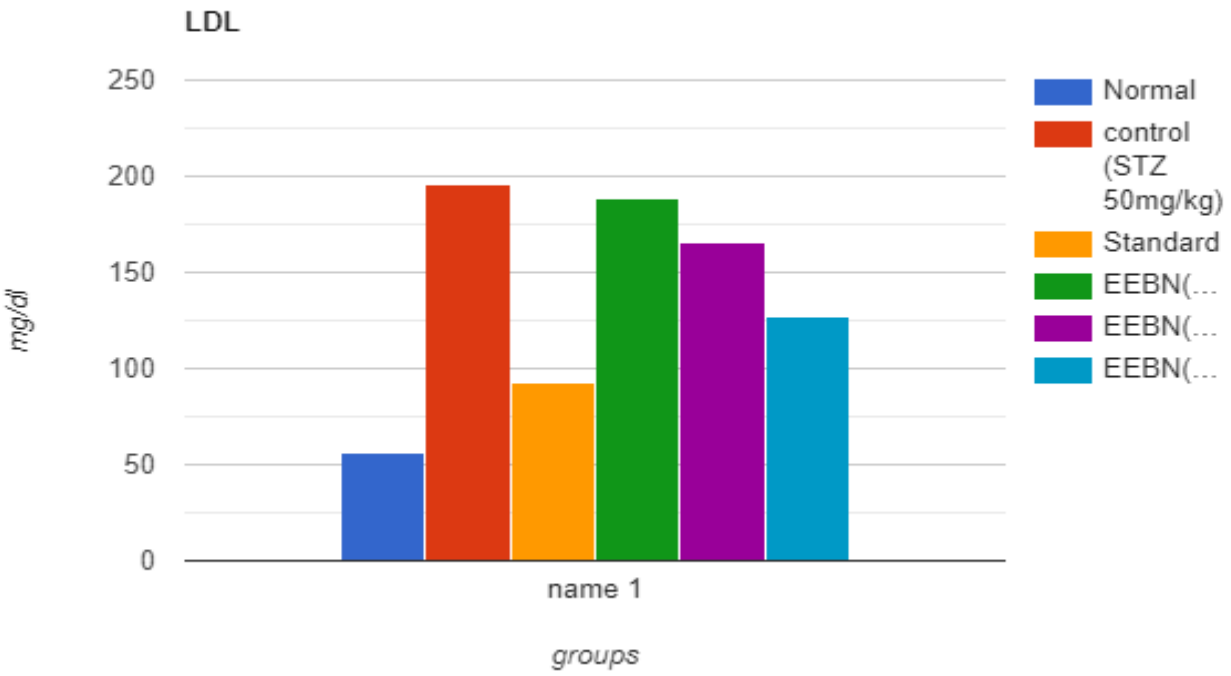
Fig.7: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on HDL in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test,**p<0.01, ***p< 0.001 vs. control group and [a]p<0.001, vs Sham operated normal.

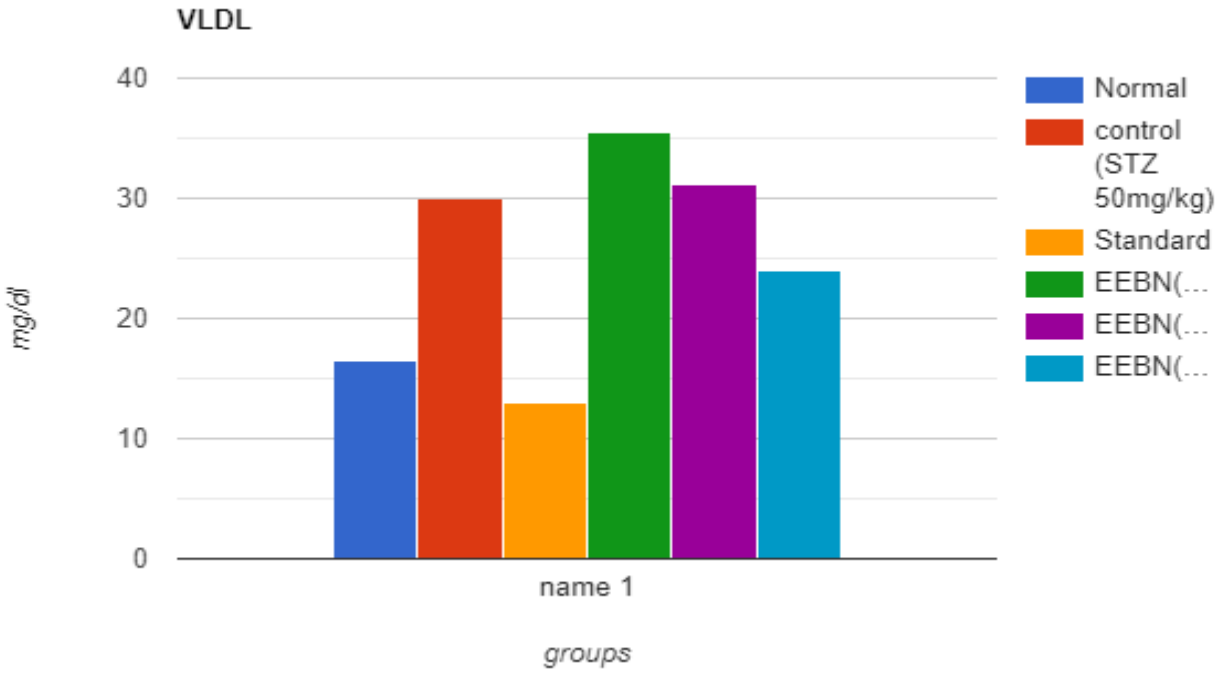
Fig.8: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) LDL in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *****p<0.05, ******p<0.01**,*****p< 0.001 vs. control group and [a]p<0.001, vs Sham operated normal.

Fig.9: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on VLDL in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

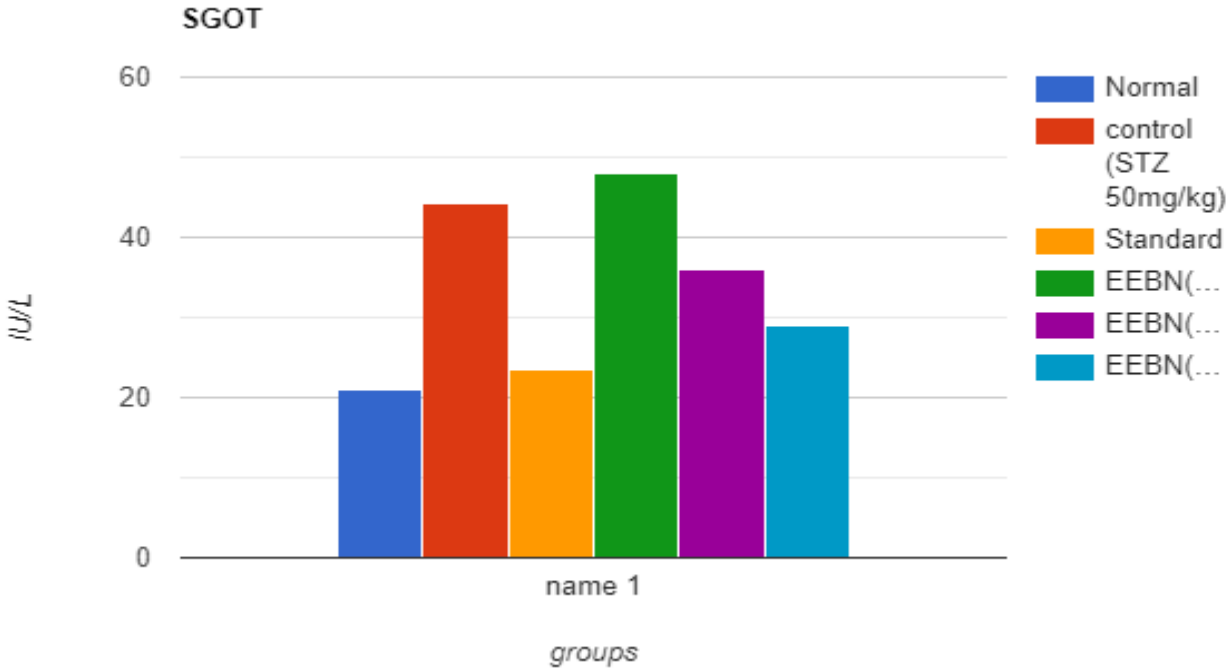
All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test**, ****p*<0.01**, *****p*<0.001 vs. control group and ^{b}p<0.01, vs Sham operated normal.

Table.no.7: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on SGOT, SGPT and ALP in STZ induced diabetic rats.

TREATED GROUPS	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Normal	21.04±0.75	22.41±0.69	90.77±1.27
Control (STZ 50mg/kg)	44.32±0.96a	56.71±0.96a	162.8±1.14a
Standard (Glibenclamide 10mg/kg)	23.58±0.90***	27.22±0.46***	137.40±0.69*
EEBN (100mg/kg)	48.00±1.71ns	43.35±1.14ns	127.81±2.01*
EEBN (200mg/kg)	36.12±1.61**	35.14±2.33**	132.15±2.22*
EEBN (400mg/kg)	29.05±1.253***	31.811±1.45***	133.65±2.91**

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *****p<0.05, **p<0.01**, ******p*< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal.

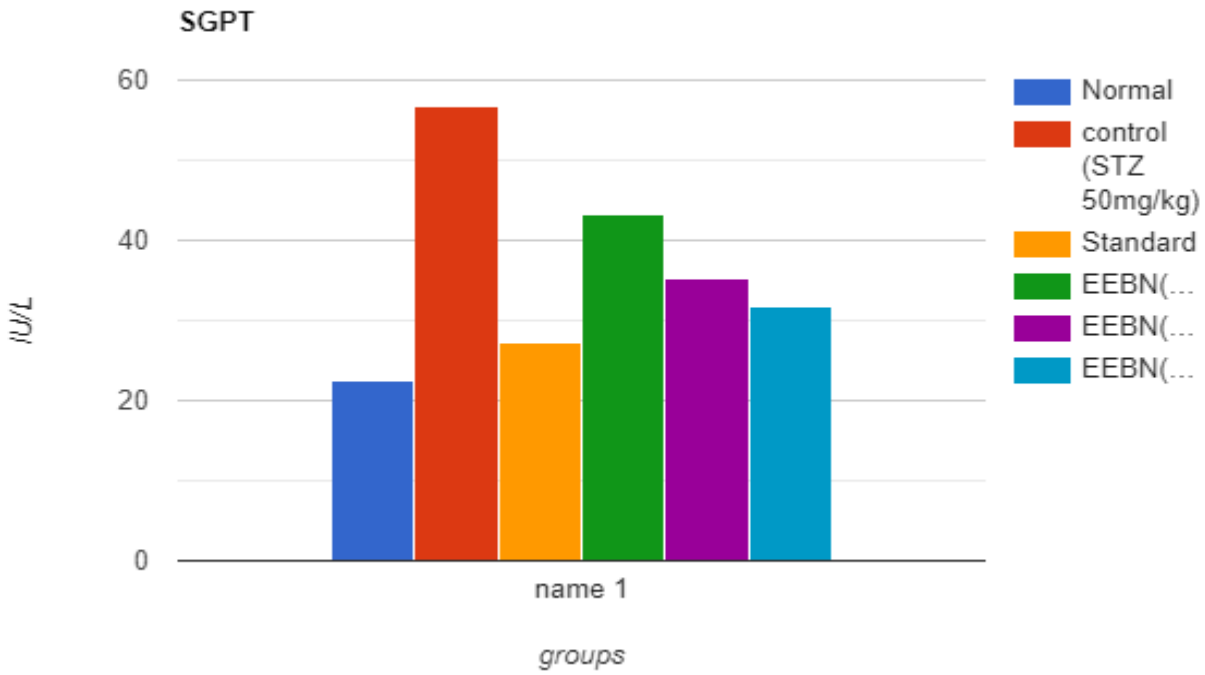
Fig.10: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on SGOT in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM , n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, ***p< 0.001 vs. control group and ^{a}p<0.001, vs Sham operated normal.

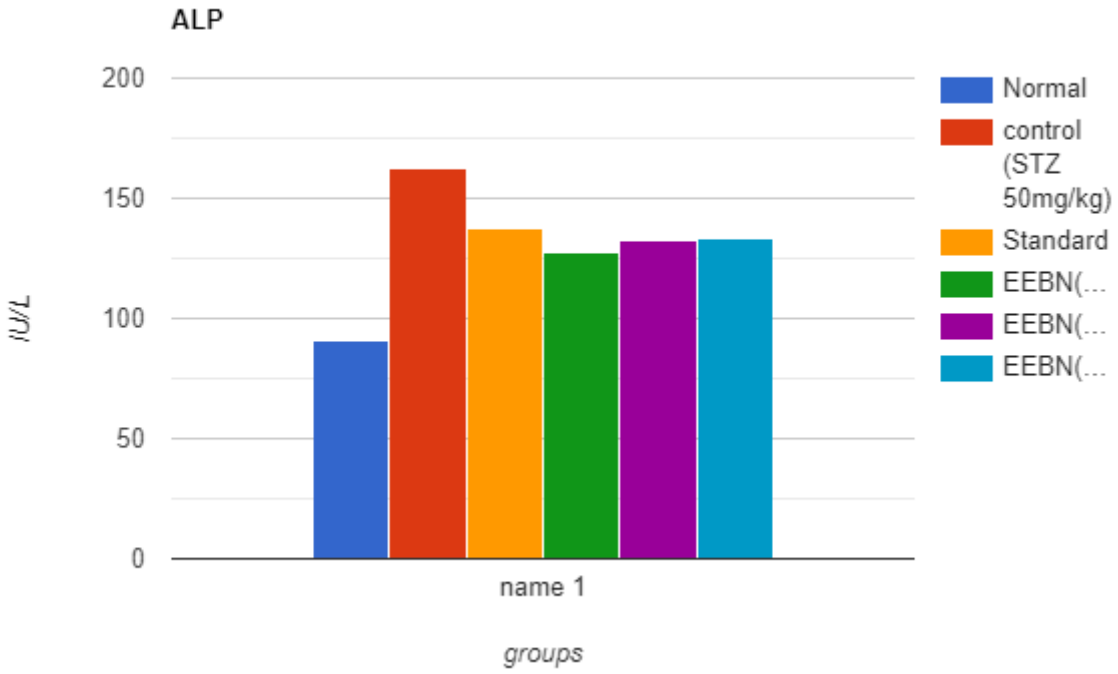
Fig.11: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on SGPT in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, $p<0.01$, ***$p< 0.001$ vs. control group and $^{a}p<0.001$, vs Sham operated normal.

Fig.12: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on ALP in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

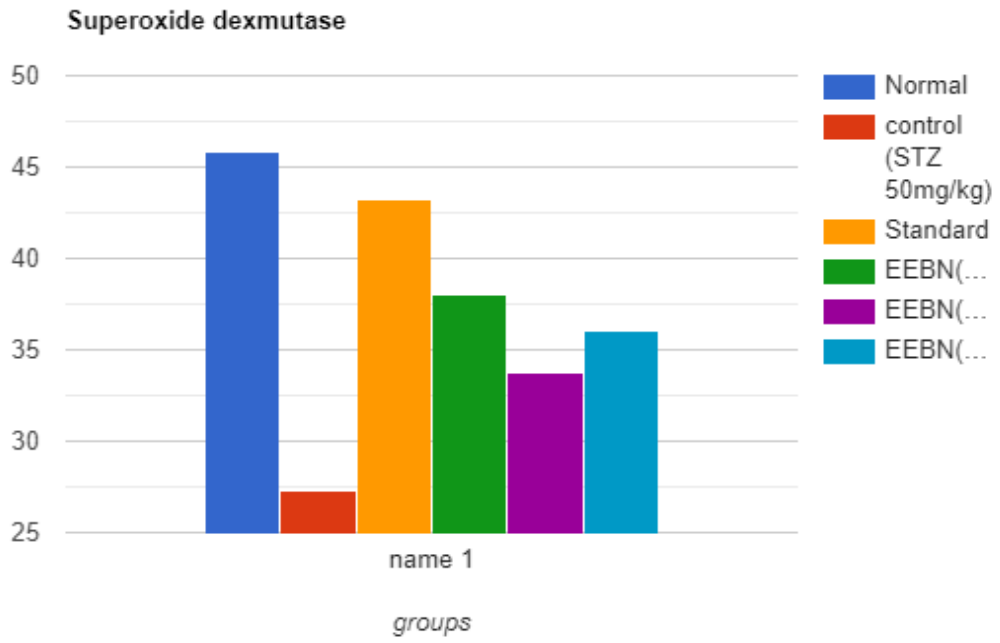
All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, *p<0.05**, *****p*< 0.01 vs. control group and [a]*p*<0.001, vs Sham operated normal.

Table no.8: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on in –vivo Anti-Oxidant Parameter from Liver Homogenate in STZ induced diabetic rats.

Treatment Group	SOD (Unit/min/gm tissue)	Catalase (µm/mg tissue)	LPO (µm of H2O2/mg tissue)
Normal	45.87±0.66	72.44±0.81	10.11±0.27
Control (STZ 50mg/kg)	27.33±0.84a	31.73±0.84a	25.49±0.59a
Standard (Glibenclamide 10mg/kg)	43.25±0.37***	70.01±0.74***	16.74±0.37***
EEBN (100mg/kg)	37.99±0.71**	57.61±0.91*	15.88±0.65*
EEBN (200mg/kg)	33.784±0.94**	60.88±0.82**	14.99±0.58**
EEBN (400mg/kg)	35.99±1.66**	60.23±2.58***	15.71±1.20**

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, p<0.05, **p<0.01**, ***p*< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal

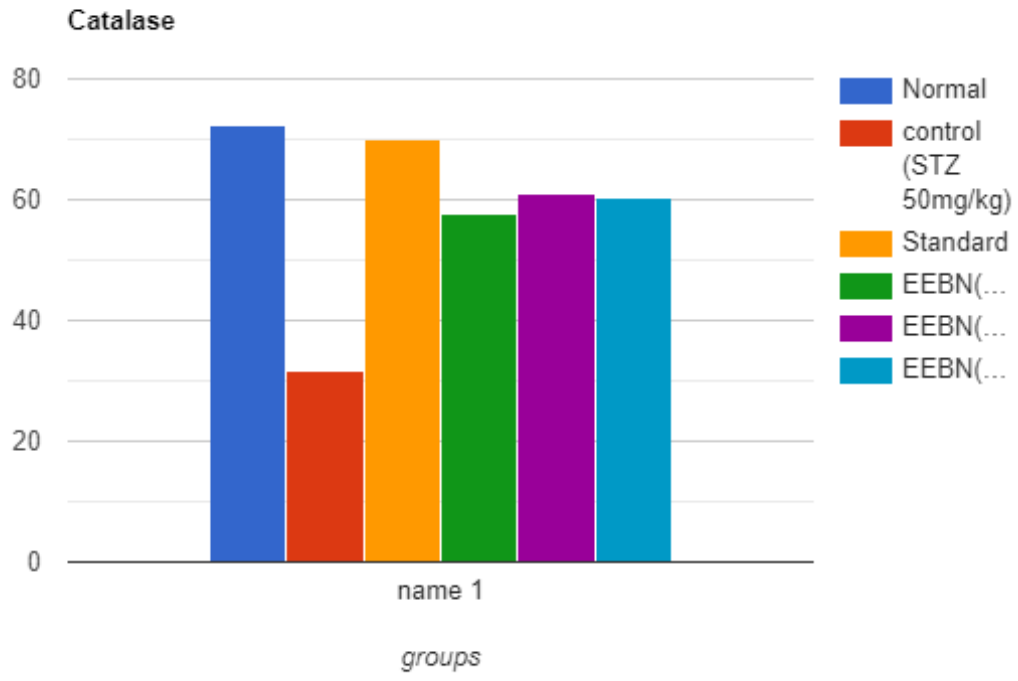
Fig.13: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on in –vivo Anti Oxidant Parameter From Liver Homogenate in STZ induced diabetic rats



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, p<0.05, **p<0.01**, ***p**< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal

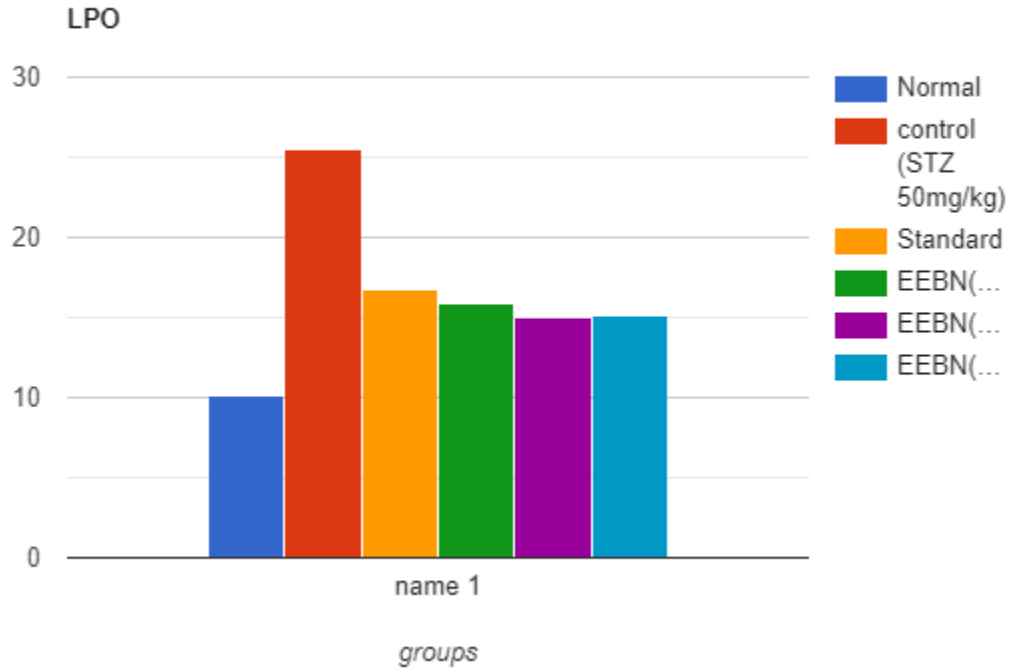
Fig.14: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on in –vivo Anti Oxidant Parameter from Liver Homogenate in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, p<0.05, **p<0.01**, ***p**< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal

Fig.15: Effect of Ethanolic extract of *Barleria noctiflora* (Whole plant) on in –vivo Anti-Oxidant Parameter from Liver Homogenate in STZ induced diabetic rats.



Control (STZ 50mg/kg), Standard (Glibenclamide 10mg/kg), EEBN(100mg/kg), EEBN(200mg/kg), EEBN(400mg/kg).

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, p<0.05, **p<0.01**, ***p**< 0.001 vs. control group and [a]*p*<0.001, vs Sham operated normal

Table 9: Observation in acute toxicity studies at the dose of 2000mg/kg bw p.o dose of *Barleria noctiflora* (Whole plant)

Parameters observed	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6	Day-7	Day-8	Day-9	Day-10	Day-11	Day-12	Day-13	Day-14
Aggressiveness	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alertness	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alopecia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Circling	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Edema	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye closure at touch	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grip strength	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Grooming	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lacrimation	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of writing reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nasal sniffing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Piloerection	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rearing	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Righting reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub tail	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urine stains	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 10: Acute Toxicity studies of ethanolic extract of the plant *Barleria noctiflora*

Parameters observed	1st hour	2nd hour	3rd hour	4th hour
Aggressiveness	+	+	+	+
Alertness	-	-	-	-
Alopecia	-	-	-	-
Circling	-	-	-	-
Diarrhoea	-	-	-	-
Edema	-	-	-	-
Eye closure at touch	+	+	+	+
Grip strength	+	+	+	+
Grooming	+	+	+	+
Lacrimation				
Loss of writing reflex	-	-	-	-
Mortality	-	-	-	-
Nasal sniffing	-	-	-	-
Piloerection	-	-	-	-

Histopathological investigation:

Pancreas of diabetic rats showed reduction in number of ß cell and necrosis along with few surviving β-cells. Severe infiltration of inflammatory cells was also observed. It showed marked degeneration of the Islet of Langerhans and it also showed the fat deposition

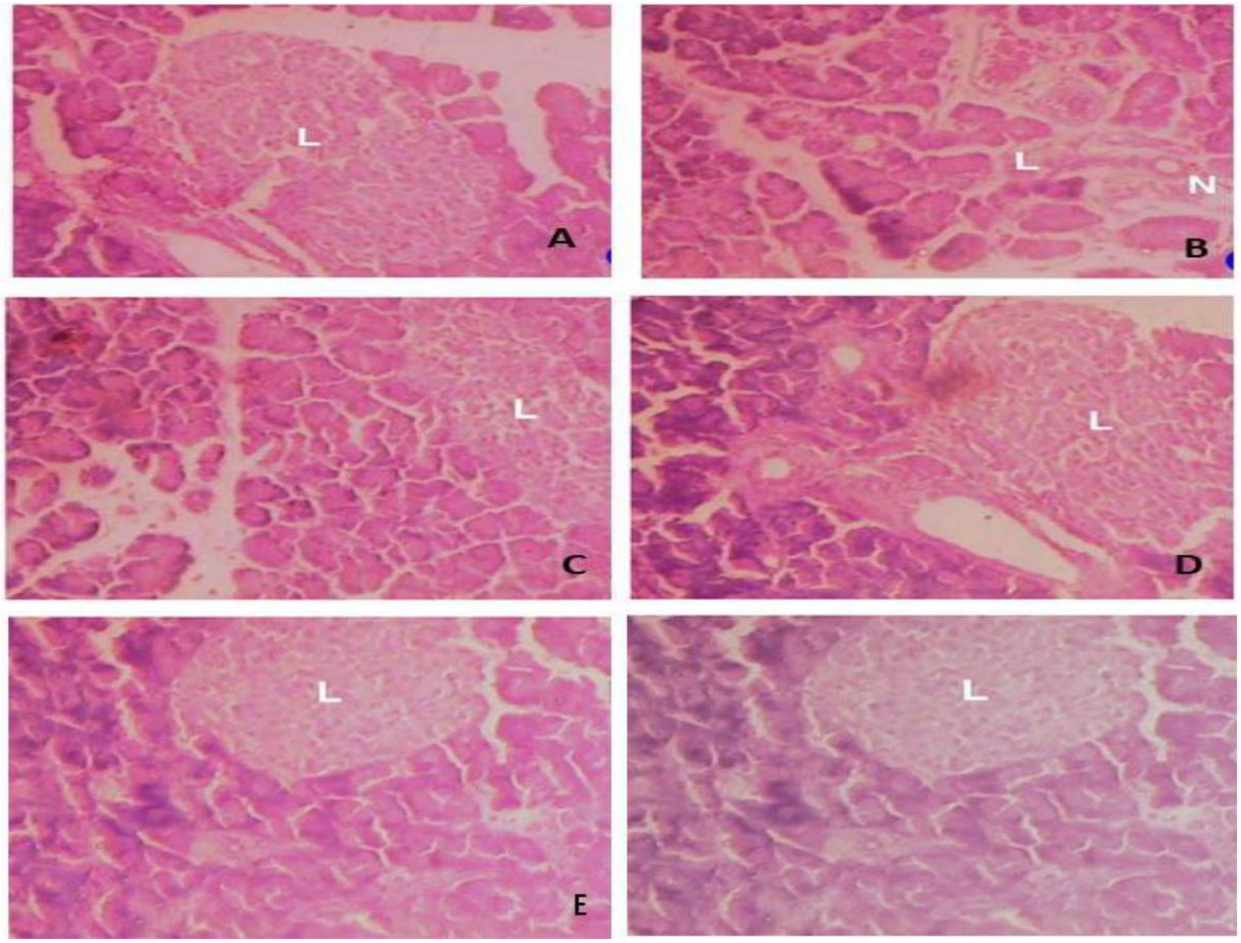
In the reference group, i.e., diabetic rats treated with glibenclamide, pancreas architecture was similar to that observed in control rat and it also showed slight regeneration of the beta cell, less damage to beta cells as compared with the diabetic rat.

Histopathological study of pancreas of diabetic rats treated with the ethanolic extract of *Barleria noctiflora* (Whole plant) at the dose of 100mg/kg, 200mg/kg, 400mg/kg given through orall route and showed significant reduction in the extent of necrosis, inflammation, increased in the number of islet cells of pancreas and less deposition of the fatty material as compared with the diabetic control.

The maximum curative effect against STZ induced diabetic aberrations was achieved with the 400 mg/kg body weight.

Fig.16: Microphotographs of pancreas tissue examined by routine hematoxylin-eosin of STZ

Treated animals:



A. Normal group
B. Control group (STZ50mg/kg)
C. Standard (Glibenclamide 10mg/kg)
D. EEBN (100mg/kg)
E. EEBN (200mg/kg)
F. EEBN (400mg/kg)

DISCUSSION:

The acute toxicity test of *Barleria noctiflora* in mice produced no death or signs of toxicity even at the dose of 2000 mg/kg which shows that the extract was well tolerated and the test doses safe in the animals. Oral administration of Ethanolic extract of *Barleria noctiflora* (Whole plant) showed significant hypoglycemic effects $p<0.01$) against STZ-induced diabetes in rats. The extract significantly lowered the levels of blood glucose.

The ethanolic extract of *Barleria noctiflora* (Whole plant) found to contain wide varieties of chemical constituents such as tannins and phenolic comp., saponins, flavonoids and carbohydrates.

The antidiabetic activity of *Barleria noctiflora* was evaluated in STZ induced diabetic rats by testing its effect on fasting blood glucose level using autoanalyzer (AccuCheckActive®) glucose kit. The fasting blood sugar test is a carbohydrate metabolic test which measures plasma or blood glucose levels after a fast (usually 8–12 h). During fasting the body stimulates the release of the hormone glucagon, which in turn releases glucose into the blood through catabolic processes. Normally, the body produces and processes insulin to counteract the rise in glucose levels but in diabetes, this process does not occur and tested glucose levels normally remain high[68]. Alloxan/STZ are both of the usual substances used for induction of diabetes mellitus and has a destructive effect on the beta (β) cells of the pancreas as previously reported by Jelodar et al[69.]

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted. However, STZ causes diabetes through its ability to destroy the insulin-producing-cells of the pancreas. When there are not enough available beta-cells to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results[70.]

The cytotoxic action of STZ is mediated by reactive oxygen species with simultaneous massive increase in cytosolic calcium concentration leading to rapid destruction of βcells[71].

This results in a decrease in endogenous insulin secretion which paves way for the decreased utilization of glucose by body tissues[72].and consequently elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides[73.]

The present study is the preliminary assessment of the antidiabetic activity of the Ethanolic extract of *Barleria noctiflora* (Whole plant). The extracts showed a dose-dependent fall in FBG in STZ induced diabetic rats. STZ induced diabetes by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation[74]

When Ethanolic extract of *Barleria noctiflora* (Whole plant) were administered to glucose loaded normal rats (OGTT) fasted for 18 hours, reduction in blood glucose levels was observed after 2 hr. The decline reached its maximum at 24 h. In our study, the difference observed between the initial and final fasting blood glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group at the end of the 28days experimental period. Administration of extracts to diabetic rats showed a significant decrease in the fasting blood glucose and an increase in the serum insulin levels. Hence, the possible mechanism by which Ethanolic extract of *Barleria noctiflora* (Whole plant) brings about its hypoglycemic action may be by potentiating the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

Another possible mechanism may be attributed to the rich fiber content of Ethanolic extract of *Barleria noctiflora* (Whole plant). Dietary fibers play a major role in lowering the blood glucose level by slowing the rate of carbohydrate absorption from intestine and are hence beneficial for diabetics, especially type II diabetics.[75]

Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hyper triglyceridemia
Dietary fibers lower the cholesterol and triglyceride levels.[76]Therefore, the significant control of levels of serum lipids in the treated groups may be attributed to the rich fiber content in *Barleria noctiflora* (Whole plant). Induction of diabetes with STZ is associated with a characteristic loss of bodyweight, which is due to increased muscle wasting. and due to loss of tissue proteins.
Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. SGOT, SGPT and ALP are reliable markers of liver function. In STZ induced diabetic rats the liver was necrotized. An increase in the activities of SGOT, SGPT and ALP in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which gives an indication of the hepatotoxic effect of STZ[78]. Treatment of the diabetic rats with Ethanolic extract of *Barleria noctiflora* (Whole plant) reduced the activity of these enzymes in plasma compared to the diabetic untreated group and consequently alleviated liver damage caused by STZ-induced diabetes. Significant reductions in the activities of these enzymes in Ethanolic extract of *Barleria noctiflora* (Whole plant) treated diabetic rats indicated the hepatic protective role in preventing diabetic complications.

CONCLUSION:

In conclusion, the result of the present study indicates that Ethanolic extract of *Barleria noctiflora* (Whole plant) may have active principle(s) that exerts anti-diabetic property. Thus justifies the traditional use of this plant in the treatment of diabetes mellitus. Plant extract of the title plant possesses almost equipotent anti-diabetic activity when compared with reference standard Glibenclamide. However, more efforts are still needed for the isolation, characterization and biological evaluation of the active principle(s) of the *Barleria noctiflora*.
The Whole plant of *Barleria noctiflora* are collected and shade dried. Coarse powder are made from this dried plant and subjected to extraction (soxhlet extraction process). Ethanol is used as solvent. The extracts showed the presence of flavonoids, alkaloids, tannins, saponins and carbohydrates which are responsible for the pharmacological activities.
The acute toxicity study was as per OECD guideline 423 and the dose selected is as follows 100,200 and 400 mg/kg.
The results of the present investigation of Ethanolic extract of *Barleria noctiflora* (Whole plant) showed significant anti-diabetic activity, anti-hyperlipidemic and antioxidant properties against STZ induced diabetic rats.

Summary

In the present investigation Ethanolic extract of *Barleria noctiflora* (Whole plant) has been investigate for the preliminary phytochemical analysis, acute toxicity and antidiabetic activity.

Diabetes is a group of metabolic disorder of carbohydrate, fat, and protein metabolism resulted from destruction of insulin secreting pancreatic beta cells,defects in insulin production, insulin action, or both, characterized by hyperglycemia. In this study the *Barleria noctiflora* (Whole plant) selected for the evaluation of Antidiabetic activity. Preliminary phytochemical analysis revealed that the plant bark possessed phytoconstituents alkaloids, steroids, tannins, Phytosterols, flavonoids, proteins and amino acids.Type II diabetic mellitus was induced in male wistar albino rats body weight of animals 180-220 selected for the study. Evaluation of antidiabetic activity was assessed by using STZ experimental model by administration of STZ 50 mg/kg i.p. for 14 and 21 days.Body weight and serum blood glucose were recorded before administration of Ethanolic extract of *Barleria noctiflora* (Whole plant) and glibenclamide to the animals.Selected animals were then administered the STZ 50mg/kg significantly increased the blood glucose levels in all animals.Administration of Ethanolic extract of *Barleria noctiflora* (Whole plant) and standard glibenclamide were significantly reduced elevated level of serum glucose level.Diabetic control rats had shown the elevated level of serum lipid profile and significantly reduced in Ethanolic extract of *Barleria noctiflora* (Whole plant).Diabetic control rats had exhibited the elevated serum SGOT, SGPT and ALP liver enzyme.Administration of Ethanolic extract of *Barleria noctiflora* (Whole plant) 100, 200 and 400 mg/kg and glibenclamide (50µg/kg) had significantly decreased the elevated level of biochemical markers such as serum SGOT, SGPT and ALP as compared to diabetic control rats.*Invivo* antioxidant enzymes were exhibited significant result in treatment group when compared to diabetic control group.5Animals were sacrificed after 28 days and blood was withdrawn from retro-orbital puncture for various biochemical estimations and pancreas collected for histopathological study.

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