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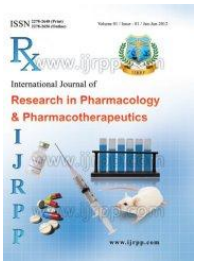

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Antidiabetic activity of ethanolic extract of *Heliotropium indicum* whole plant against alloxan and streptozotocin induced diabetis in rats

Syed Safiullah Ghori*[1], Nafeesunnisa Begum[1], Mohammed Shoibuddin Khateeb[1]

[1]*Pharmacology Research Lab, Anwarul Uloom College of Pharmacy, New Mallepally, Hyderabad 500016, Telangana, India.*

***Corresponding author: Syed Safiullah Ghori**

E-mail id: safiullahghori@gmail.com

ABSTRACT

The present research was carried out to evaluate Antidiabetic activity of *Heliotropium indicum* ethanolic extract in alloxan and streptozotocin induced diabetic rats *Helitropium indicum* whole plant was extracted using ethanol as solvent by Soxhlet apparatus. The extract was subjected to preliminary phytochemical screening. Acute oral toxicity studies were performed to determine test dose. Alloxan and streptozotocin were used for inducing diabetes in albino Wistar rats. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins flavonoids, proteins, and amino acids. Doses up to 2000mg/kg were found to be safe after acute toxicity tests. The results suggested that EHI possess anti diabetic activity against diabetes induced by both alloxan and streptozotocin.

Keywords: *Heliotropium indicum*, ethanolic extract, alloxan and streptozotocin.

INTRODUCTION

Diabetes is a group of metabolic disorders of carbohydrate, fat, and protein metabolism resulting from destruction of insulin secreting pancreatic beta-cells, defects in insulin production, insulin action, or both, characterized by hyperglycemia. The chronic hyperglycemia results in long-term complications of diabetes including peripheral neuropathy causing foot ulcers; autonomic-neuropathy causing stroke, ischaemic heart diseases and peripheral vascular disease, sexual dysfunctions; nephropathy causing renal failure; retinopathy with loss of vision[1]. Lactic acidosis is associated with factors that may relate to diabetes, such as cardiovascular disease, acute myocardial infarction associated with hypoxia and over lactic acid production[2]

The term Diabetes is a multifarious group of disorders characterized by hyperglycemia that has reached epidemic proportions in the current century. Infection is a leading cause of morbidity and mortality among the diabetic population. Diabetes is involved with vascular and renal damage characterized by hypertension, dyslipidemia, micro-albuminuria, macro-albuminuria and glomerular mesangial rise[3].

Diabetes mellitus in its severe forms develops to ketoacidosis leading to stupor, coma and in the absence of effective treatment to death[4].

According to WHO (World health organization) predictions, the prevalence of diabetes is to increase by 35%. Statistical estimations about India reveals that the cases of diabetics which were 15 million in 1995 are expected to increase to 57 million by the year 2025, the highest number of diabetic cases in the world [5].

Heliotropium indicum Linn. commonly known as 'Indian heliotrope' is a common plant in India and some parts of Africa and Bangladesh. H. indicum has been used in different traditional and folklore systems of medicine for curing various diseases. An ethno pharmacological survey revealed that, the traditional healers in Kancheepuram district of Tamil Nadu, India use H. indicum to cure skin diseases, poison bites, stomachache and nervous disorders. The plant is reported to be highly valued in the folklore medicine and is believed to be useful in treating malaria, abdominal pain, fever, dermatitis, venereal diseases, insect bites, menstrual disorder, urticaria, and sore throat[6.] The plant decoction is considered as diuretic and remedy for the treatment of kidney stones[7,8]. The leaf paste is applied externally to cure rheumatism and skin infections[9]. The various tribes of Phulbani district of Odisha use the leaf paste over fresh cuts and wounds and claim for its promising activity. Keeping in view the above ethno medicinal uses an attempt was made to evaluate the antidiabetic potential of *Heliotopium indicum* whole plant ethanolic extract.

MATERIAL AND METHODS

Plant material

The plant *Heliotropium indicum* was collected from an Ayurvedic wholesaler. The plant was identified and authenticated by Asst Prof. Dr.K.Madhava chetty, Head, Department of Botany, S.V. University, Tirupati.

Chemicals

Alloxan-monohydrate (Sigma Aldrich, USA), Metformin (Alembic Pharma) Chloroform (Fisher scientific), Diethy ether (Fisher scientific). All chemicals and reagents were of analytical grade. Diagnostic kits used for estimation of cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT, Total protein and glucose were procured from Robonik Diagnostic Ltd India**.** Autoanalyzer (Robonik), Refrigerator centrifuge (MPW-350R),UV-Spectro-photometer (UV-1601, Shimadzu Corporation, Kyoto, Japan), Mini Lyotrap (LTE Scientific Ltd.), Research centrifuge (Remi industries, Mumbai) and homogenizer (Remi Motors, Mumbai). Dhona balance (M/S Dhona instruments Pvt. Ltd., Kolkata, India).

Experimental Animals

Wistar albino rats of either sex (150–220 g) were obtained from the central animal house of Sigma Institute of Clinical Research Pvt Ltd Hyderabad. The animals were housed at room temperature (22-28 ºC), 12 hr dark and light cycle and given standard laboratory feed and water ad-libitum. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (769/2010/CPCSEA).

METHODS

Preparation of extract

The collected fresh plant material was dried in shade (2 days) and was made to coarse powder with the use of grinder. The powder was weighed separately and transferred to a round bottomed flask and then subjected to continuous heat extraction with soxhlet apparatus using 95% ethanol for 24 hours. Then the extract of ethanol was concentrated. Extract obtained was dried by placing it on a big petriplate on electric water bath (70ºC) and then kept in an oven at 30ºC for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 7.5%.[10]

Qualitative chemical tests

Ethanolic extract of the plant was subjected to chemical tests for the identification of active constituents.

Acute toxicity study

Acute toxicity studies were performed according to OECD-423 guidelines category IV substance (acute toxic class method). (Organization for economic Co-operation and development, 2001).[11]Animals were individually observed for changes in skin, mortality, general behavioral pattern, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma for a time period of 14 days.

Evaluation of Blood Glucose Levels

Blood glucose levels were measured with a portable glucometer on 0, 1, 7 and 14 day. Blood was withdrawn from the rats using tail vein rupture method, and a drop of blood was placed on the glucometer strip loaded in the glucometer for blood glucose determination. During the experiment, blood glucose levels were verified in the interim of each week.

Oral Glucose Tolerance Test

The oral glucose tolerance test was performed in overnight fasted (18 h) normal animals. Rats divided into 5 groups (n = 6) were administered vehicle, extract 1, extract 2 and glibenclamide (10 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the tail vein puncture at 0, 30, 60, 90 and 120 min of extract administration. The fasting blood glucose levels was estimated by glucose oxidase–peroxidase reactive strips

Experimental Models for Evaluation of Anti Hyperglycemic Activity

The anti hyperglycemic activity was performed by two experimental models, allowance and streptozotocin induced hyperglycemia.

Alloxan Induced Hyperglycemia model[12]

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of freshly prepared solution of alloxan (150 mg/kg body weight) in sterile saline after five days of Alloxan administration, blood was collected and plasma glucose levels were determined. The animals were confirmed as diabetic by the elevated plasma glucose levels >200 mg/dl) and were used for the experiment. The animals were assigned randomly into five groups of six animals in each group. The experimental grouping and dosing was as follows

Group1: Normal rats 0.5% tween -80 in distilled water orally

Group 2: Diabetic rats received Alloxan (150 mg/kg)

Group 3: Diabetic rats treated orally with Glibenclamide 10mg/kg

Group 4: Diabetic rats treated orally with ethanolic extract of *Heliotropium indicum* 200mg/kg

Group 5: Diabetic rats treated orally with ethanolic extract of *Heliotropium indicum* 400mg/kg

Streptozotocin Induced Hyperglycemia model[13, 14]

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection (i.p.) of freshly prepared solution of streptozotocin (50 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5)[15]. After seven days of STZ administration, blood was collected and plasma glucose levels were determined. The animals were confirmed as diabetic by the elevated plasma glucose levels (>200 mg/dl) and were used for the experiment. The animals were assigned randomly into five groups of six animals in each group.

Group 1: Normal rats 0.5% tween -80 in distilled water orally.

Group 2: Diabetic rats received STZ (50 mg/kg)

Group 3: Diabetic rats treated orally with Glibenclamide 10 mg/kg

Group 4: Diabetic rats treated orally with Ethanolic extract of *Heliotropium indicum* 200 mg/kg

Group 5: Diabetic rats treated orally with Ethanolic extract of *Heliotropium indicum* 400 mg/kg

Biochemical estimations

On day 14, blood was collected by retro orbital puncture under mild ether anaesthesia from overnight fasted rats and fasting blood sugar was estimated. Serum was separated and analyzed for serum Cholesterol, Triglycerides and HDL, SGOT, SGPT and ALP.

Estimation of Serum total cholesterol (TC) CHOD- PAP[: 16]

This method was used for the estimation of serum cholesterol. In this method the following were pipetted into the reaction vessel using a micro pipette. Test samples (T): 0.02 ml serum, 2.00 ml reaction solution; the standard sample (S): 0.02ml standard and 2.00 ml reaction solution, while for the blank sample (B): 0.02 ml DW and 2.00ml reaction solution. The mixture was mixed well and incubated for 10 minutes at +20 to 25c or 5 minutes at 37c.The absorbance was read at 505/670 nm against the reagent blank

Estimation of serum triglycerides (TG) [17]

GPO-PAP method was used to estimate the serum triglycerides. For this 0.01 ml of serum was taken in a test tube (T) in which 1ml reaction solution was added. In an another test tube (S) 0.01ml standard and 1 ml reaction solution were added. The solution was mixed well and incubated at +20 to 25C for 10 min. The absorbance of standard and test against reagent blank was read at 505 (500-540 nm).

Estimation of HDL-cholesterol[18]

CHOD-PAP method was used to estimate the serum HDL cholesterol level. CHOD-PAP method (Henry, 1974) was used to estimate the serum HDL cholesterol level. For this 2 ml if serum was taken in a test tube and 0.5 ml of precipitation reagent was added. The mixture was shaken thoroughly and left to stand for 10 min at +15 to 25c and then centrifuged for 15 min at 4000rpm. Within 2hr after centrifugation, the clear supernatant was used for the determination of HDL-C. One ml of the supernatant was taken in a test tube (T) and 1 ml of reaction solution was added to it. In a test tube 0.1 ml DW was taken and 1ml reaction solution (B) was added. The mixtures were mixed thoroughly, incubated for 10min at 15-25 c or for 5 min at 37c and measured the absorbance of the sample against reagent blank at 546 nm.

Estimation of liver marker enzymes[19.20, 21]

The estimation of SGOT was done according to Henderson's method, SGPT by Johnson's method and ALP by Teitz method

Histopathological studies

At the end of the study period, animals from both the experimental groups were sacrificed and pancrease was dissected out, washed, 5µm thick section slides were prepared and stained with heamatoxyline-eosin and examined by light microscopy.

Statistical analysis

All the data was expressed as mean± SEM and evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test using Prism Graph pad version 5.0. Values of $P<0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Results of Alloxan induced Hyperglycemia

Effect on serum Total cholesterol level

In alloxan induced diabetic rats (Control group) serum glucose level was significantly increased ($p<0.001$) when compared to normal groups. Administration of EHI 200 and 400 mg/kg, glibenclamide 10 mg/kg orally for 14 days treatment reduced significantly serum glucose level on 7 days ($p<0.01$) and 14 days ($p<0.001$) as compared to control groups

Effect on serum lipid profile level.

In alloxan induced diabetic rats serum lipid profile such as total cholesterol, triglycerides LDL(low-density lipids) VLDL(very low density lipids) levels were observed significantly increased ($p<0.001$) and HDL level in diabetic control group were seen significantly decreased ($p<0.001$) as compared to normal group. Administration of extracts of EHI 200mg/kg 400mg/kg and glibenclamide 10 mg/kg on serum lipid profile. A decrease in the serum triglycerides, ($p<0.001$), total cholesterol ($p<0.001$), LDL ($p<0.001$), and VLDL (very low density lipids) levels ($p<0.001$), and an increase in the HDL (high density lipids) cholesterol levels ($p<0.001$) were observed as compared to diabetic control group.

Effect on serum SGOT, SGPT and ALP

In Alloxan induced diabetic rats the levels of SGOT, SGPT and ALP were significantly ($p<0.001$) increased in diabetic rats as compared to normal groups. After treatment with EHI 200mg/kg 400 mg/kg and glibenclamide 10mg/kg the SGOT, SGPT and ALP level significantly ($P<0.001$) reduced as compared to diabetic control rats.

Fig No 1: Effect of ethanolic extract of *Heliotropium indicum* on OGTT in rats

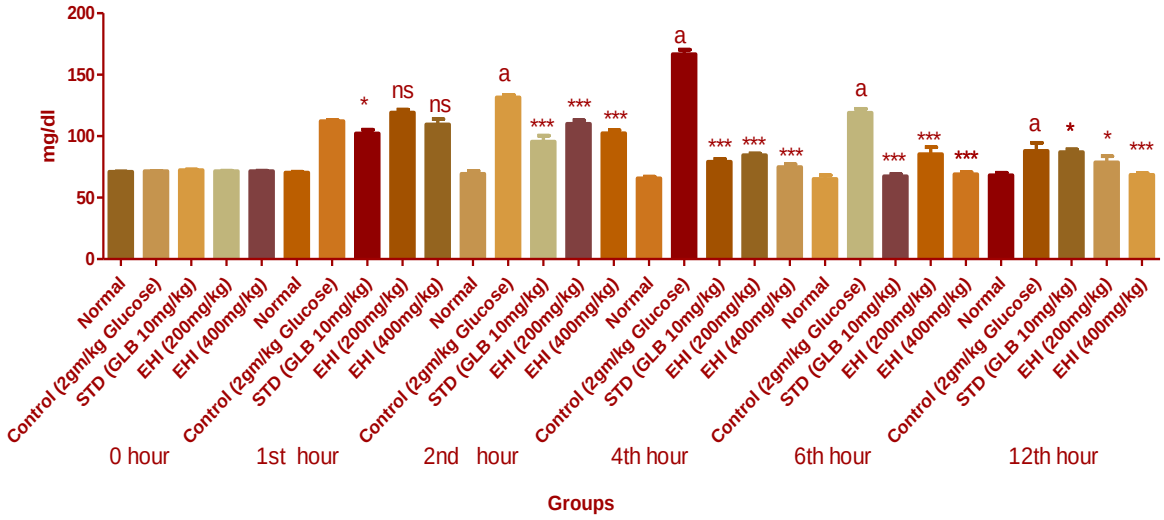


Fig No 2: Effect of ethanolic extract of Heliotropium indicum on serum glucose level in alloxan induced diabetic rats.

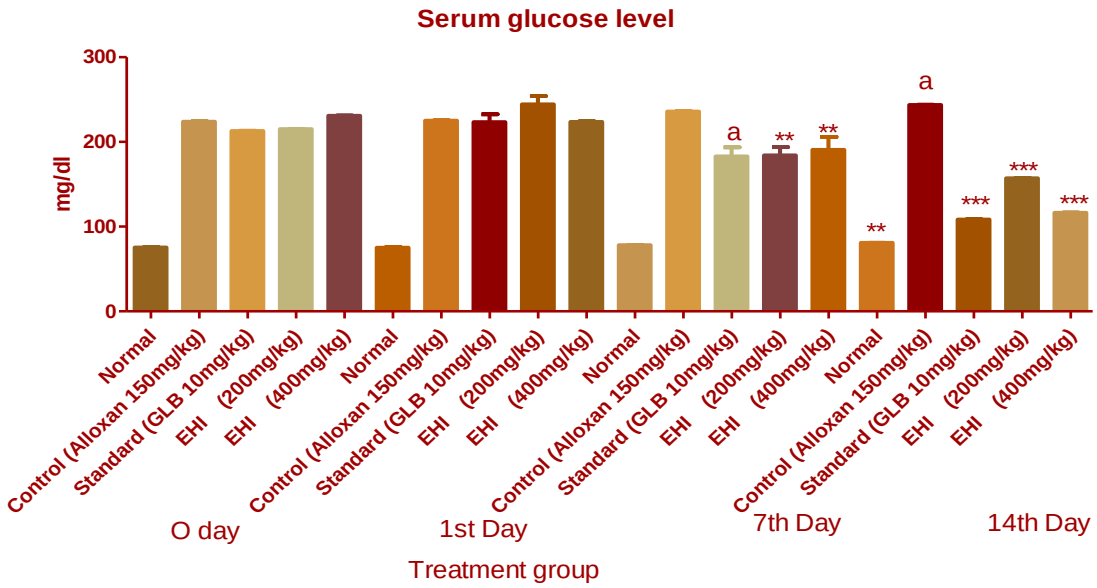
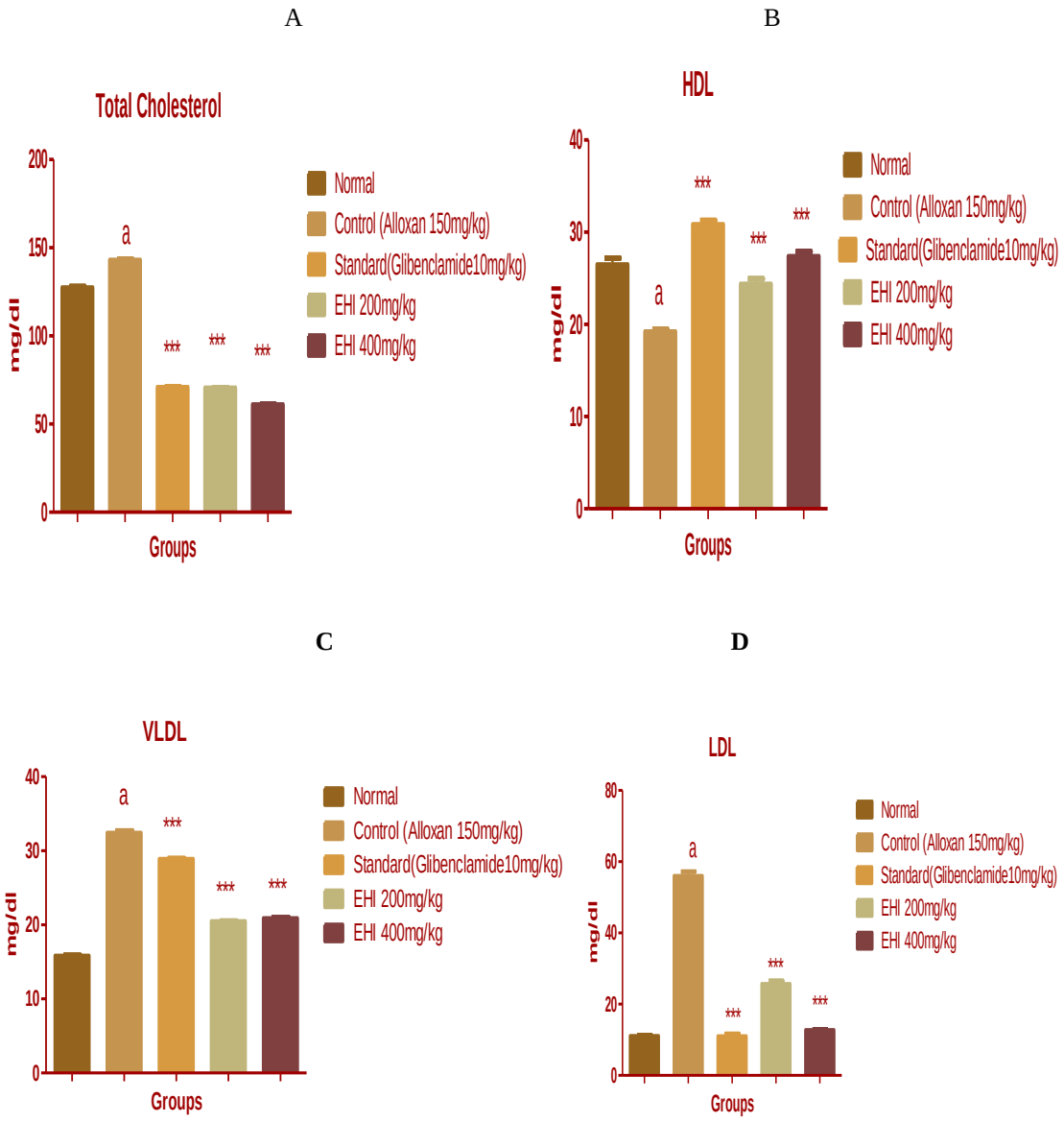


Fig No 3: Effect of ethanolic extract of Heliotropium indicum on serum lipid profile in alloxan induced diabetic rats

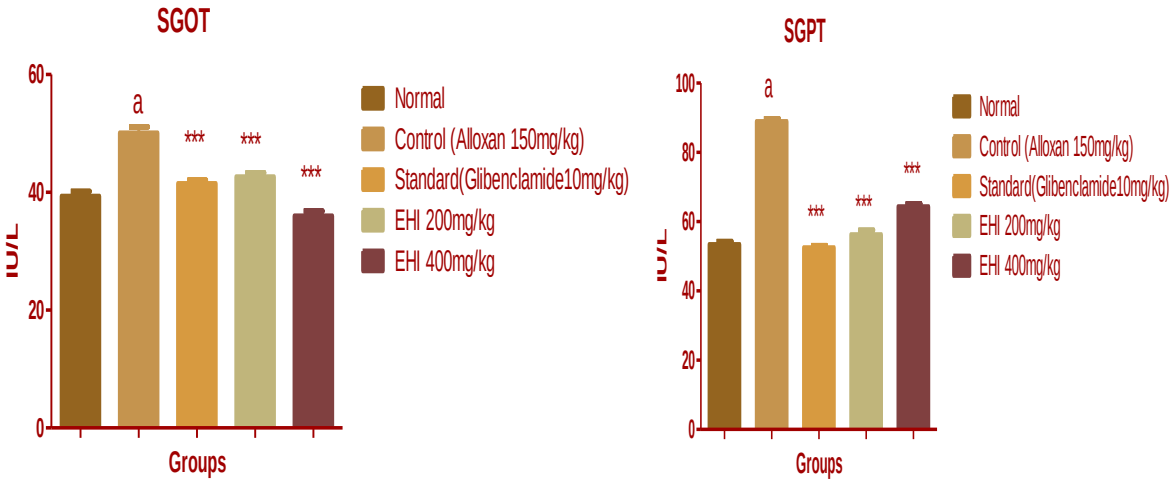


A-D: TOTAL CHOLESTEROL, HDL, VLDL&LDL

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,as control group and [a]p<0.001, as compared normal

Fig No 4: **Effect of ethanolic extract of Heliotropium indicumon liver marker in alloxan induced diabetic rats.**

A **B**



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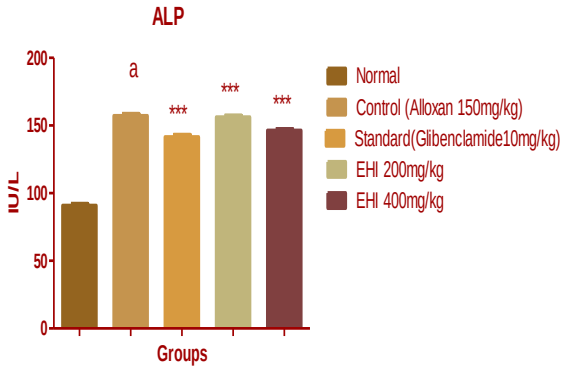


A-C: SGOT, SGPT&ALP

Streptozotocin Model

Effect of Ethanolic Extract of *Heliotropium indicum* on Serum Glucose Levels.

In STZ induced diabetic rats (Control group) serum glucose levels significantly increased on 14, 21 and 28th days ($p<0.001$) in diabetic control rats when compared to normal groups. Administration of glibenclamide 10 mg/kg orally for 28 days treatment, serum glucose level were reduced significantly on 14 day ($p<0.05$) 21 day and 28th day ($p<0.01$) as compared to control groups. Administration of EHI 200 and 400 mg/kg orally for 28 days treatment serum glucose levels were reduced significantly on 14 days ($p<0.05$), 21 days and 28th days ($p<0.05$, $p<0.01$) as compared to control groups.

Effect of ethanolic extract of Heliotropium indicum on serum lipid profile.

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 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 decreased 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 EHI 200 and 400 mg/kg had an effective effect on serum lipid profile. A decrease in the serum total cholesterol ($p<0.01$), Triglycerides ($p<0.05$ and $p<0.001$), LDL ($p<0.001$), and no change 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 *Heliotropium indicum* on SGOT, SGPT and ALP.

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

Administration of EHI 200mg/kg wt of showed a non-significant change in SGOT and SGPT but ALP level was significantly reduced as compared to control group. Administration of EHI 400mg/kg produced a significant ($p<0.001$) reduction in the level of SGOT, SGPT and ALP as compared to control group.

Fig No.5 Effect of ethanolic extract of Heliotropium indicum on serum glucose level in STZ induced diabetic rats.

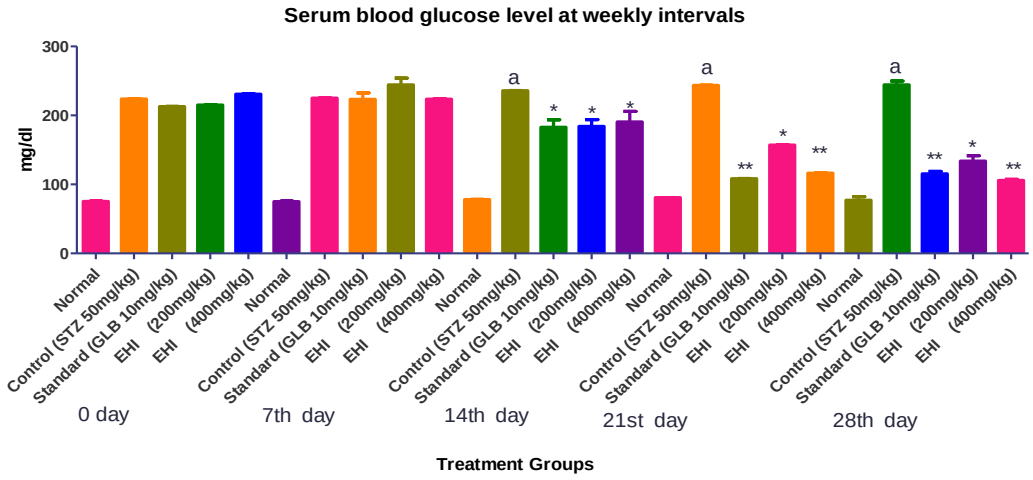
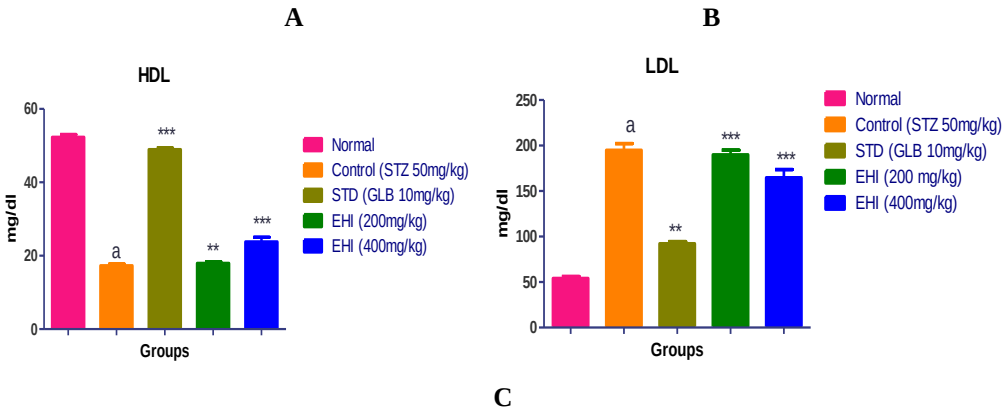


Fig No.6 Effect of ethanolic extract of Heliotropium indicum on serum lipid profile in STZ induced diabetic rats.



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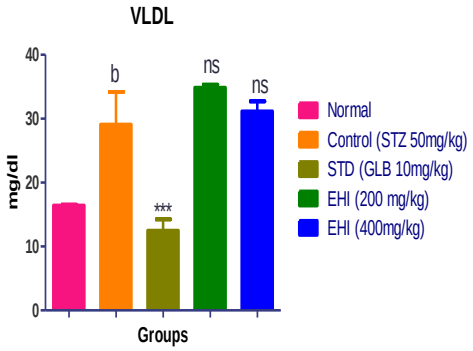
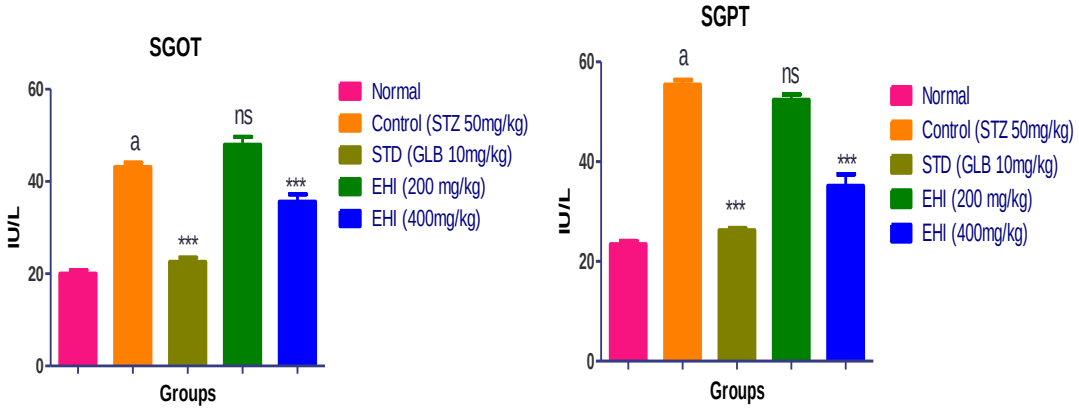


A-C: HDL, VLDL&LDL

Fig No 7: Effect of ethanolic extract of Heliotropium indicum on liver marker in streptozotocin induced diabetic rats.

A **B**



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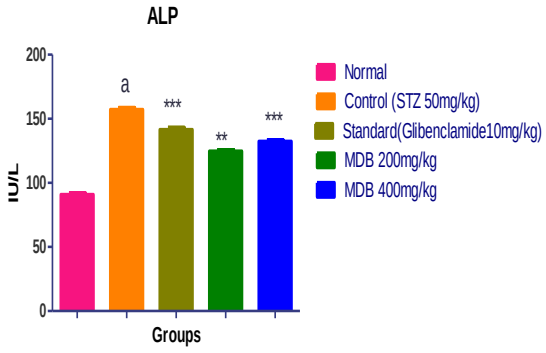


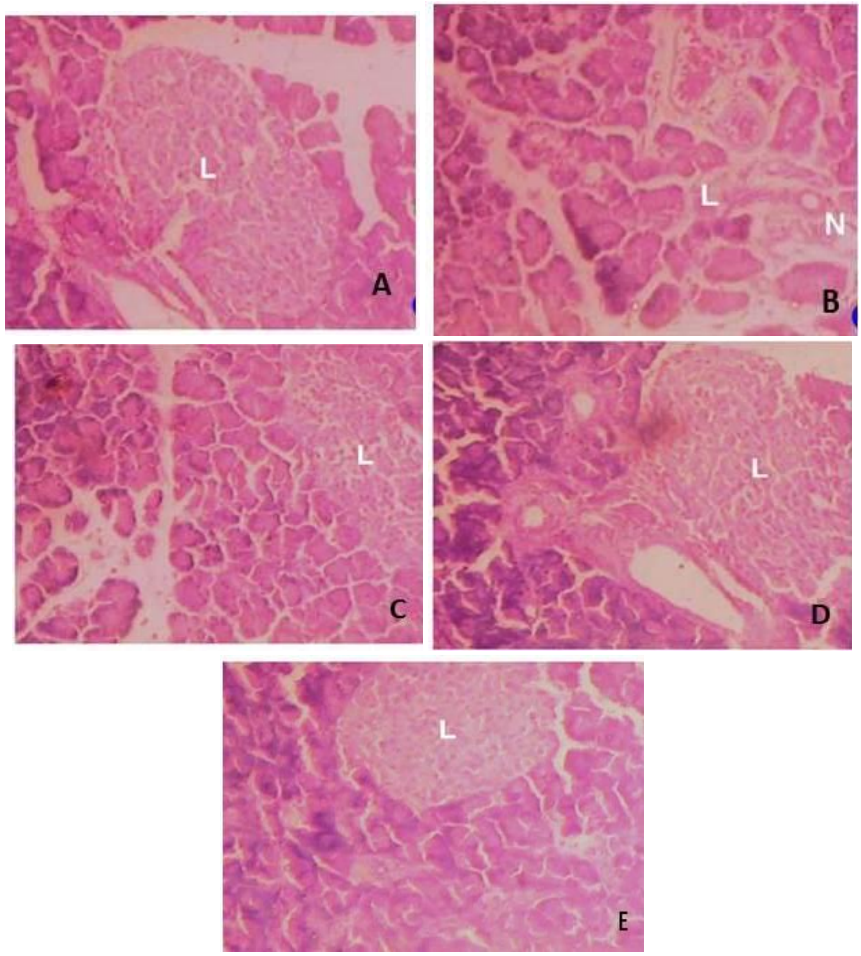
A-C: SGOT, SGPT&ALP

Histopathology of Pancreas

The pancreas of control rats showed normal appearance of islet cells. The alloxan treated rats showed vascularization, necrotic changes, reduced and damaged islet cells. Oral administration of ethanolic extract of Heliotropium indicum at a dose of 200 and 400 mg/kg body weight to alloxan treated

rats showed markedly reduced extent of necrosis, vascularization and reduced islet cells. In the reference group, i.e., alloxan with Glibenclamide pancreatic architecture was similar to that observed in the control rats. The maximum curative effect against alloxan induced diabetic aberrations was achieved with EHI 400 mg/kg body weight.

Fig.8. Microphotographs of pancreas examined by routine hematoxylin-eosin of alloxan treated animals



A: Normal group, B: Control group (alloxan 150mg/kg), C: Glibenclamide 10mg/kg
D: EHI 200mg/kg, E: EHI 400mg/kg.

Discussion

The acute toxicity test of *Heliotropium indicum* in mice showed 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 were safe in the animals. The antidiabetic activity of Heliotropium indicum was evaluated in alloxan/ STZ induced diabetic rats by testing its effect on fasting blood glucose level using autoanalyzer 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 glucose levels normally remain high[22].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[23]

Pancreas is the primary organ involved in sensing the organisms dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted[3].However, alloxan/STZ causes diabetes through its ability to destroy the insulin-producing-cells of the pancreas[24] leading to stage where not enough available beta-cells remain to supply sufficient insulin to meet the needs of the body, insulin-dependent diabetes results[25]

The cytotoxic action of alloxan/STZ is mediated by reactive oxygen species with simultaneously cause a massive increase in cytosolic calcium concentration leading to rapid destruction of β-cells[26]. This results in a decrease in endogenous insulin secretion which paves way for the decreased utilization of glucose by body tissues[27] and consequently elevation of blood glucose level, decreased protein content, increased levels of cholesterol and triglycerides[28]. The present study is the preliminary assessment of the antidiabetic activity of the ethanolic extracts of *Heliotropium indicum*. The extracts showed a dose-dependent fall in fasting blood glucose in alloxan induced diabetic rats. Alloxan/STZ induces 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[27]When EHI extracts were administered to glucose loaded normal rats (OGTT) fasted for 18 hours, reduction in blood glucose levels was observed after 60 min. The decline reached its maximum at 2 h. In the 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 14 days 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 EHI brings about its hypoglycaemic 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 EHI. 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.[28]

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[29]. Therefore, the significant control of serum lipid levels in the treated groups may be attributed to the rich fiber content in *Heliotropium indicum*. Induction of diabetes with alloxan/STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue proteins[30]

CONCLUSION

In conclusion, the result of the present study indicates that ethanolic extract of *Heliotropium indicum* whole plant may have active principle(s) that exerts anti-diabetic property. Thus this justifies the traditional use of this plant in the treatment of diabetes mellitus. The extract possesses almost equipotent antidiabetic 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 *Heliotropium indicum*.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

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