Research Article

Amelioration of Hyperglycemia by Hydroalcoholic Extract of Leaves of *Ecbolium viride* against Experimental Type II Model in Rats

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ABSTRACT

Objective: To evaluate the antidiabetic activity of hydroalcoholic extract of leaves of *Ecbolium viride* against streptozotocin induced hyperglycemia in wistar albino rats.

Method: Blood glucose levels of streptozotocin-induced hyperglycemia in wistar rats were monitored after the administration of *Ecbolium viride* extract (100 and 400 mg/kg) to diabetic rats for 21 days. Fasting plasma glucose levels, serum insulin levels, serum lipid profiles and changes in body weight were evaluated in normal rats while liver glycogen levels and pancreatic TBARS levels were evaluated additionally in diabetic rats.

Results: The diabetic groups treated with the hydro alcoholic leaf extract were compared with standard metformin. The findings of the study support the antidiabetic claims of *Ecbolium viride*.

Conclusions: The results suggest that the leaf extract of *Ecbolium viride* possesses antidiabetic activity, which might be a potential source for isolation of new orally active agent in the treatment of diabetes and its associated complications.

Keywords: *Ecbolium viride*, Antidiabetic activity, Streptozotocin, Hydro-alcoholic extract.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases globally, affecting the quality of human life of all ages across the world. [1, 2] The disease has become a global public health problem affecting the socio-economic status of the individual. [3] It is an age long, serious heterogeneous disorder characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from vascular diseases.[4] Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycemia. [5] The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. It is frequently associated with the development of microvascular and macrovascular diseases such as neuropathy, nephropathy, cardiovascular and cerebrovascular diseases. [6] Several oral hypoglycemic drugs are available for managing diabetes since it is incurable but they suffer from generally inadequate efficacy and number of serious adverse effects. [7,8] Hence, the shift to the use of plant source, a new hopeful approach that has long been authenticated by World Health Organization in its general assembly [9]. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications. *Ecbolium viride* is commonly known as Green Shrimp plant belonging to the family Acanthaceae and distributed throughout in Western Ghats, North East India, Africa, SriLanka, Nepal, Malaya. Alston (Acanthaceae) is a perennial woody under shrub. It is commonly known as Green Shrimp plant. They can be easily identified by their intense green leaves and greenish blue flowers. Traditionally different parts of the plant like roots, leaves, stem and whole plant which are used in folklore medicine for several medicinal purposes like cancer, jaundice and rheumatism. [10,11] Hence the present study was carried out to evaluate the antidiabetic activity of Hydroalcoholic Extract of Leaves of *Ecbolium viride* against streptozotocin induced hyperglycemia in albino wistar rats.

MATERIALS AND METHODS

Collection and Authentication of plant material

Fresh leaves of *Ecbolium viride* were collected from, Tamil Nadu, India. Botanical identification of the plant was done by Dr. S. Mutheeswaran, Scientist, Xavier Research Foundation, Palayamkottai, Tamil Nadu, India with voucher number XCH40429. The leaves were dried under shade, sliced into small pieces, pulverized using a mechanical grinder, passed through 40-mesh sieve, and stored in an airtight container for further use.

Preparation of Plant Extract

About 100 gms of powdered leaves were extracted with the solvents (70% ethanol) in Soxhlet Apparatus and then it was filtered by muslin cloth and evaporated to dryness by using Rotary Evaporator to obtain a desiccated extract, which was stored at 4 °C until further use. The percentage yield of the extract was 8.9 %. [12]

Phytochemical screening

The extracts of the plant material were screened for the presence of alkaloids, carbohydrates, phenols, gums and mucilage, flavonoids, steroids, proteins, tannins and saponins using standard qualitative methods as described by Harborne (1973)

Animals

Wistar Albino rats of either sex weighing between 150-200 grams wistar albino rats (180-200 g) of either sexes obtained from Animal House, Aditya Bangalore Institute of Pharmacy Education and Research, Yelahanka, Bengaluru, were used for the study. The animals were housed in cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had free access to water. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee on 4/12/2021 and it was approved by the same before beginning the experiment (Protocol Proposal no. 67/161/CPCSEA).

Acute toxicity study of the extract

Acute toxicity test was done on plant extracts after the animals had been fasted overnight while only taking water. The weight of each mouse was recorded before administering the extract. Randomly the animals were divided into a control and treatment groups, each group consisting of six mice. The control group received only the vehicle (1% Tween 80) and each treatment group received orally the 70% ethanol extracts of *Ecbolium viride* in a dose of 1000, 2000 and 5000 mg/kg. Animals were kept under close observation for explicit toxicities and/or behavioral changes like restlessness, tremor, diarrhea, sluggishness, loss of weight, and paralysis at regular intervals for the first four hours after administering the extract , and then they were observed daily for two weeks for any change in general behavior and/or other physical activities. Food was available after four h of administration of the extracts. [14]

Induction of experimental diabetes

Thirty male albino rats of weighing (180-200 g) were used for the study. First, the rats were fasted overnight after which they were given a single intra-peritoneal injection (*i.p*) of 65 mg/kg b.w. of streptozotocin (STZ) dissolved in 0.1 mL fresh cold citrate buffer pH 4.5.Confirmation of diabetes was done 72 h after STZ induction, using a One Touch Glucometer. Blood samples were obtained from the tail puncture of the rats. Animals with fasting blood glucose ≥ 200 mg/dL, after 10 days of STZ induction were considered diabetic and included in the study as diabetic animals.

Experimental design

Rats were randomly allocated into 5 groups (n=6). Group 1 which served as negative control received normal saline (10 mL/kg), group 2 is Diabetic control, group 3 served as standard and group 4 & 5 received (100 and 400 mg/kg orally) of the leaf extract.

Determination of blood glucose levels Measurement and weight

Blood glucose level was estimated using biochemistry analyzer (GOD POD method) on 0, 1,7, 14 and 21st day of experiment. At the end of study (on 21day), blood samples were collected by retro orbital puncture from anesthetized (slight exposure to ether) rats.

Hypolipidemic activity and Biochemical analysis

After treatment, blood samples were collected from the retro -orbital puncture of all rats of all groups at 1, 2, 3, 6, 10, 16, and 24th hour and analyzed for content of blood glucose using Glucometer (Bio Land, Germany). Then all the rats were sacrificed, and approximately 1-2 mL of blood was collected directly from the heart with the help of disposable syringes. The blood samples were transferred to centrifuge tubes and allowed to centrifuge at 4000 rpm for 10 min; serum was collected used to determine total cholesterol (TC) and serum triglycerides (TG). Serum total cholesterol and triglycerides were estimated at 505 and 546 nm, respectively, using cholesterol oxidase/ p-amino antipyrine (CHO / PAP) method and glycerol 3- phosphate oxidase (GPO) method, respectively according to manufacturer's protocol.

Statistical Analysis

All the values are expressed as mean ± standard error of the mean (S.E.M) for groups of six animals, each data was analyzed by One way Analysis of Variance (ANOVA) and compared by using Tukey's Kramer multiple comparison test. $P<0.05$ was considered as significant.

RESULTS

Phytochemical screening

The main chemical constituents found in these plants are alkaloids, carbohydrates, glycosides, phenols, phytosterols, thiols, gums, mucilage, flavonoids, terpenes, steroids, proteins, tannins, resins.[15]

Acute oral toxicity study

Acute toxicity study revealed that the leaf extract of *Ecbolium viride* caused no mortality in both doses (2 g/kg) within the first 24 h as well as for the following 14 days. Physical and behavioral observations of the experimental rats also indicated no visible signs of overt toxicity like lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea and convulsion. This suggests that LD_{50} of the extract is greater than 2 g/kg.

Anti Diabetic Activity:

The effects of different doses of hydro alcoholic extracts of *Ecbolium viride* on blood glucose (mmol/L), serum total cholesterol & triglycerides (mmol/L) were investigated within control and streptozotocin induced diabetic rats. Metformin HCl (150 mg/mL) was used as a standard anti-diabetic agent.

Effects of hydro-alcoholic extracts of *Ecbolium viride* on Blood Glucose: A decrease in blood glucose level was observed in animals treated with *Ecbolium viride* at 0, 1, 2, 3, 6, 10, 16, and 24th hr

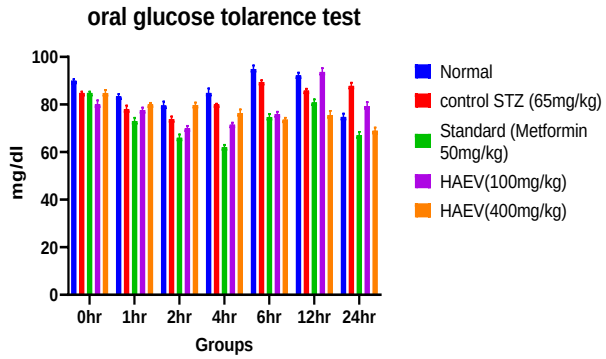

Figure 1: Effect of Hydro-alcoholic extract of *Ecbolium viride* on fasting serum glucose level (OGTT) in STZ induced diabetic rats. (In hours)

All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test

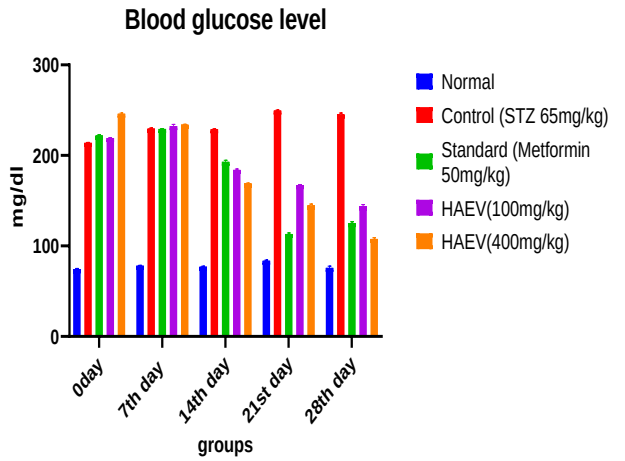

Figure 2: Effect of hydro-alcoholic extract of *Ecbolium viride* on fasting serum glucose level (OGTT) in STZ induced diabetic rats. (In weeks)

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test.

After the glucose administration, the peak of blood glucose level was increased rapidly from the fasting glucose value and then subsequently decreased. Both doses of plant extracts exhibited noticeable blood glucose lowering effect at 4 hour. High dose of extract 400 mg/kg body weight showed more blood glucose lowering capacity, whereas, that of metformin -treated rats showed a comparable result to that of normal control rats. The significant reduction of the peak levels of blood sugar within 4 hour manifests the antidiabetogenic potential of *Ecbolium viride* extract in rat models. This result showed an appreciable improvement of the glucose tolerance test which could be attributed to the insulin mimetic activity of the plant's extract by restoring the delayed insulin response.

The results from the study clearly indicated that the standard and plant extract treated animals showed a significant reduction in glucose levels when compare to diabetic control. *Ecbolium viride* (400 mg/ kg) and metformin (50 mg/kg) treated animals reversed the hyperglycemia induced by STZ to normal levels after two weeks of administration onwards.

Effect of Hydro-alcoholic extract of *Ecbolium viride* on serum lipid level

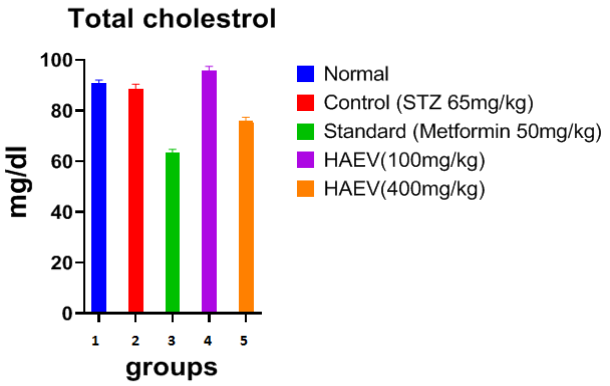

Figure 3: Effect of Hydro-alcoholic extract of *Ecbolium viride*on cholesterol in STZ induced diabetic rats

All the values are mean ± SEM, n=6, ns=Not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test,

As shown in fig 3, elevated total cholesterol level was observed in the diabetic control compared with that of normal controls. Administration of the extract to STZ-diabetic rats protected the alterations of total cholesterol.

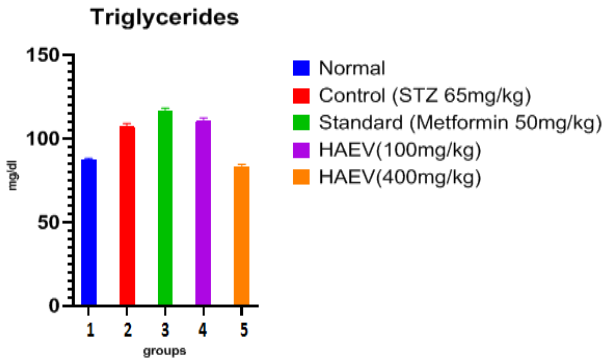

Figure 4: Effect of Hydro-alcoholic extract of *Ecbolium viride* on Triglycerides in STZ induced diabetic rats

As shown in fig 4, High doses of *Ecbolium viride* exhibited reduction in TG level were observed in the diabetic control compared with that of normal control.

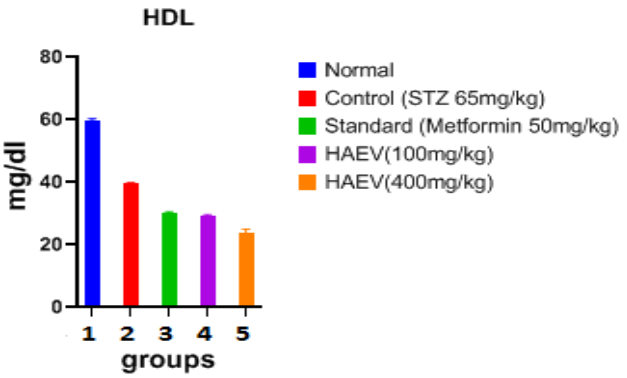

Figure 5: Effect of Hydro-alcoholic extract of *Ecbolium viride* on HDL in STZ induced diabetic rats

Treatment of the diabetic rats with hydro-alcoholic extract of *Ecbolium viride* reduced HDL level compared to the diabetic group

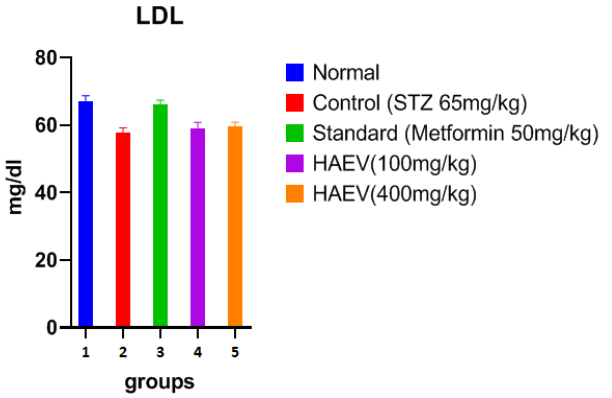

Figure 6: Effect of Hydro-alcoholic extract of *Ecbolium viride* on LDL in STZ induced diabetic rats

Treatment of the diabetic rats with hydroalcholic extract of *Ecbolium viride* reduced LDL level compared to the diabetic group

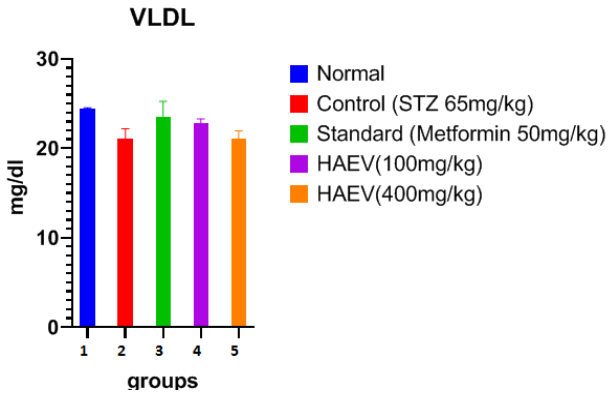

Figure 7: Effect of Hydro-alcoholic extract of *Ecbolium viride* on VLDL in STZ induced diabetic rats

All the values are mean ± SEM, n=6, ns= not significant, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test**.**

Treatment of the diabetic rats with hydro-alcoholic extract of *Ecbolium viride* reduced VLDL level compared to the diabetic group

Effect of Hydro-alcoholic extract of *Ecbolium viride* on Liver function test

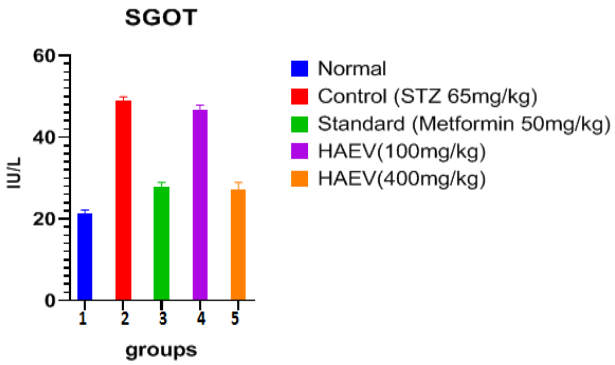

Figure 8: Effect of Hydro-alcoholic extract of *Ecbolium viride* on SGOT in STZ induced diabetic rats

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test.

The liver biomarkers such as SGOT found to be normal in Hydroalcholic extract of *Ecbolium viride* and metformin - treated rats, but the diabetic control showed elevated SGOT levels.

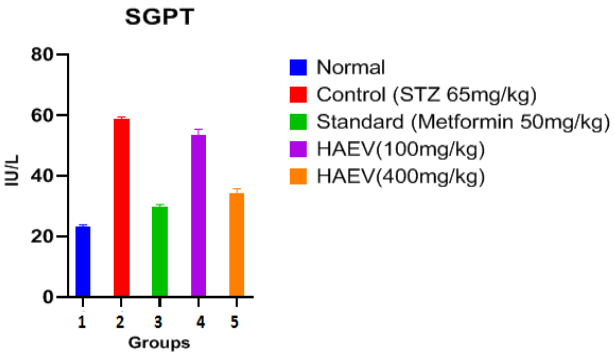

Figure 9: Effect of Hydro-alcoholic extract of *Ecbolium viride* on SGPT in STZ induced diabetic rats

All the values are mean ± SEM, n=6, ns=Not significant, one way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test. All the values are mean ± SEM, n=6.

The liver biomarkers such as SGPT found to be normal in Hydroalcholic extract of *Ecbolium viride* and metformin - treated rats, but the diabetic control showed elevated SGPT levels.

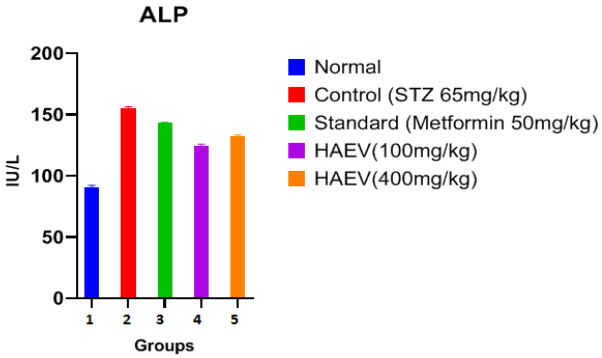

Figure 10: Effect of Hydro-alcoholic extract of *Ecbolium viride* on ALP in STZ induced diabetic rats

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test.

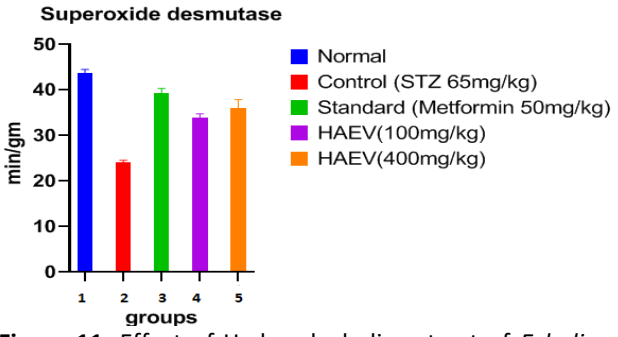

Figure 11: Effect of Hydro-alcoholic extract of *Ecbolium viride* on *in–vivo* Anti-Oxidant Parameter from Liver Homogenate in STZ induced diabetic

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test.

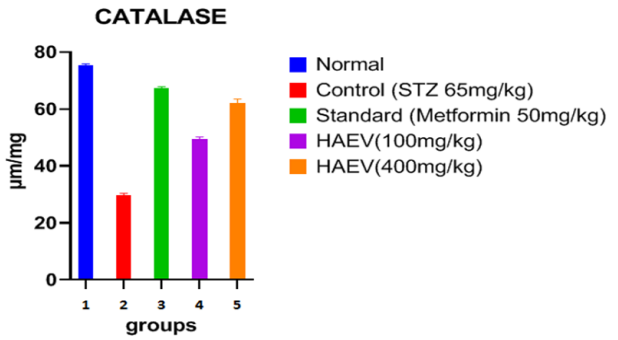

Figure 12: Effect of Hydro-alcoholic extract of *Ecbolium viride* on in –vivo Anti-Oxidant Parameter from Liver Homogenate in STZ induced diabetic rats

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test.

The liver biomarkers such as ALP found to be normal in Hydroalcholic extract of *Ecbolium viride* and metformin - treated rats, but the diabetic control showed elevated ALP levels.

The result showed that the diabetic control had a significantly lower superoxide dismutase (SOD) while the treated groups and normal control group had relatively higher SOD level.

The result showed that the diabetic control had a significantly low level of Catalase while the treated groups and normal control group had relatively higher Catalase level.

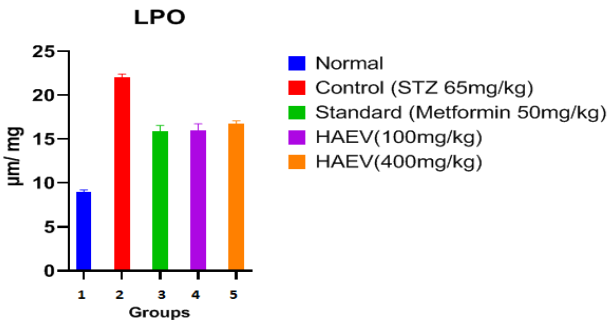

Figure 13: Effect of Hydro-alcoholic extract of *Ecbolium viride* on in–vivo Anti-Oxidant Parameter from Liver Homogenate in STZ induced diabetic rats

All the values are mean ± SEM, n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test.

The result showed that the diabetic control had a significantly high level of LPO while the treated groups and normal control group had relatively low level of LPO level.

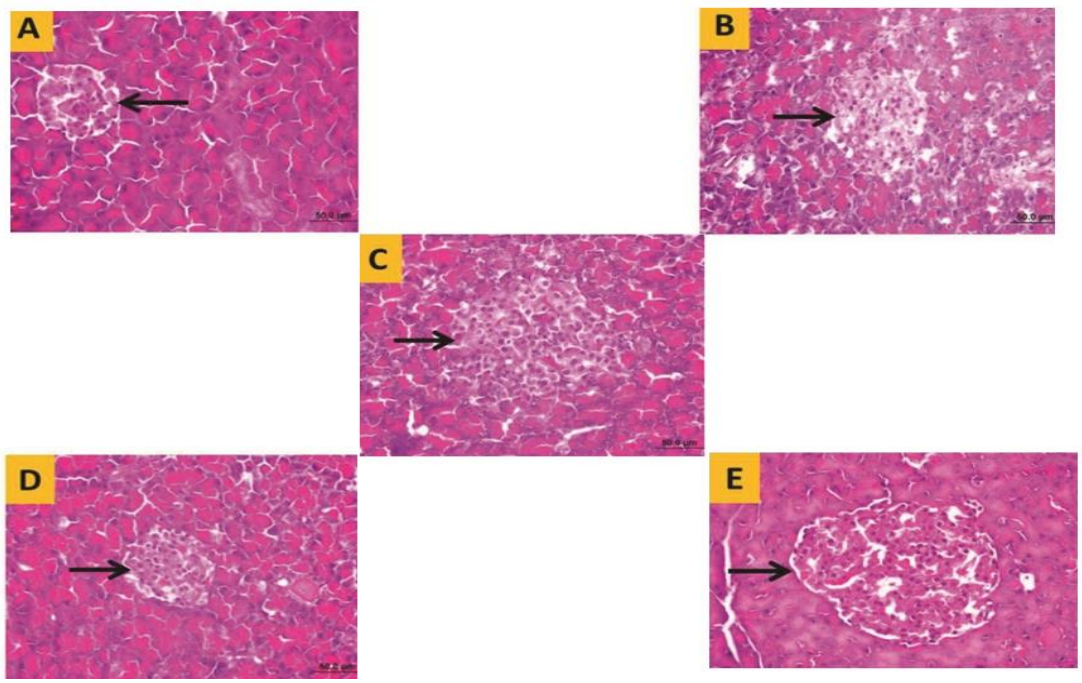

Figure 14: Microphotographs of pancreas tissue examined by routine hematoxylin-eosin of STZ treated animals A: Normal group, B: Control group (STZ 65mg/kg), C: Metformin (50mg/kg), D: HAEV (100mg/kg), E: HAEV (400mg/kg)

Histopathology of Pancreas

The histopathological changes were observed in control and experimental group rats. The control rat's pancreas showed that the normal appearance of islet cells. The STZ treated rats showed vacuolization, necrotic changes and reduced islet cells of pancreas damage was observed. Oral administration of Hydro-alcoholic extract of *Ecbolium viride* at the dose of 100 and 400 mg/kg body weight to STZ treated rats showed markedly reduced the extent of necrosis, vacuolization and reduced islet cells of pancreas. In the reference group, i.e., STZ with metformin, pancreas architecture was similar to that observed in the control rats. The maximum curative effect against STZ induced diabetic aberrations was achieved with the 400 mg/kg body weight.

DISCUSSION

The acute toxicity test of *Ecbolium viride* 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 hydroalcholic extract of *Ecbolium viride* showed significant hypoglycemic effects against STZ-induced diabetes in rats. The antidiabetic activity of *Ecbolium viride* was evaluated in STZ induced diabetic rats by testing its effect on fasting blood glucose level using autoanalyzer (Accu Check Active®) glucose kit. 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. STZ is usual substances used for induction of diabetes mellitus and has a destructive effect on the beta (β) cells of the pancreas

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. When hydro-alcoholic extract of *Ecbolium viride* extracts 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 hydro-alcoholic extract of *Ecbolium viride* 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 hydro-alcoholic extract of *Ecbolium viride*. 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.

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. Therefore, the significant control of levels of serum lipids in the treated groups may be attributed to the rich fiber content in hydroalcoholic extract of *Ecbolium viride*. Induction of diabetes with STZ is associated with a characteristic loss of body weight, 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. Treatment of the diabetic rats with hydro-alcoholic extract of *Ecbolium viride* 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 hydro-alcoholic extract of *Ecbolium viride* treated diabetic rats indicated the hepatic protective role in preventing diabetic complications.

Antioxidants or phytochemical agents protect against ROS generation and in some circumstances promoting free radical/ROS in others. Excessive antioxidant action can adversely affect some physiological processes. It is known that some of these phytochemicals in plants have balanced biochemistry i.e. having a redox mixture of being in oxidized form and partly in a reduced form depending on concentration. Hyperglycemia is also responsible for cause of inactivation of anti-oxidative enzymes.[15] This however, needs further investigation. It has been reported that plant phytochemicals not only depend on individual levels but also on the ratios of various components and their redox states. It might be possible that due to having dual property of anti-diabetic and antioxidant activities this natural medicine can better manage the diabetes and diabetes associated complications which usually accompanied by increased oxidative stress.

CONCLUSION

In conclusion, the result of the present study indicates that hydro-alcoholic extract of *Ecbolium viride* 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 metformin. However, more efforts are still needed for the isolation, characterization and biological evaluation of the active principle(s) of *Ecbolium viride.*

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