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ASSESSMENT OF PANCREATIC BETA CELL PROTECTION AND ROLE OF ETHANOLIC EXTRACT OF *Vitex trifolia* L IN ANTIDIABETIC ACTIVITY USING STREPTOZOCIN MODEL

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ABSTRACT

Pancreatic beta cells play a major role in the management of Type II Diabetes mellitus. In the present study we have been evaluated the anti-diabetic and pancreatic protective effects of Ethanolic extract of *Vitex trifolia.L* in Streptozocin (STZ) induced diabetes model. Ethanolic extracts (*VTE*) were orally administered at the dose of 100, 300 and 500 mg/kg and metformin was used as a standard anti-diabetic drug at 10 mg/kg dose orally. Blood glucose, Liver and kidney parameters, lipid profiles, pancreatic oxidative stress parameters were evaluated. Oral glucose tolerance test (OGTT) was performed. Treatment with extracts resulted in a significant decrease in diabetes incidence with a marked reduction in the blood glucose levels dose dependent and time dependent. Glucose tolerance by animals was significantly improved after treatment with plant extract. Histopathological studies also indicated pancreatic beta-cell protective effects of *VTE* with increased beta-cell number and size. Our results demonstrate that *VTE* can be a possible remedy to treat type 2 diabetes and associated complications.

Keywords: *Vitex trifolia* L.; streptozotocin; blood glucose; pancreatic beta-cell protective effect.

INTRODUCTION

Type II Diabetes is a diverse metabolic disorder becomes a major global health issue, characterized by high glucose levels associated with the disturbance of carbohydrate, fat, and protein metabolism [1]. According to the International Diabetes Federation there will be 10.1% increase of cases by the year 2035 that means 80% could be type 2 diabetic cases [2]. Predominant approaches for management of diabetes is combination of modifications of lifestyle with yoga, meditation and some exercise and the use of oral hypoglycaemic or insulin therapy, if needed.

The typical perspective treatment comprises effects like gastric irritation, weight gain, hematologic adverse effects, and liver or kidney dysfunction and risk of cardiovascular disease [3]. The final objective of all the types of treatment is to produce safe antidiabetic effect without adverse effect. Herbal drugs met these standards since from vedas time with protected pharmacological actions; it leads the development, modification and derivatization for developing new drugs.

Vitex trifolia.L plant from Vitex genus, which is widely used in traditional medicine, nearly 270 species were identified worldwide, belongs to the family Lamiaceae [4]. It is an aromatic coastal deciduous shrub which can grow 1 to 4 m long. It contains poly phenolic Compounds [5], flavonoids [6], proteins [7], tannins [8], mono, di, tri terpenes [9], phytosterols, and saponins [10]. Based on the ethnobotanical reports and traditional claims *Vitex trifolia.L*. is a potential plant for the treatment of many ailments like anti-inflammatory, wound healing, malaria, diabetes mellitus, influenza and veneral disorders [11]. As the scientific evidence for antidiabetic activity of this plant has not explored, the present aim of this work is to evaluate the antidiabetic effect of 90% ethanolic extract *Vitex trifolia.L* of streptozotocin-induced diabetic mice.

MATERIALS AND METHODS

Plant Material

The Plant leaves of *Vitex trifolia L.* were collected from Davanagere locality in Karnataka in the month of March and authenticated by botanist. The leaves were cleaned, shade dried, and coarsely powdered using mechanical device.

Extraction

Nearly 500 g of coarse powder of leaves of the *Vitex trifolia .L* were extracted with 90% ethanol using Soxhlet extractor for 24 hours and dried in desiccators [12]. Collected and stored ethanolic extract of *Vitex trifolia .L.*

Experimental Animals

Male albino rats of Wister strain weighing between 200-250g were selected for experimental protocol. Acclimation was done in animal house with maintained standard laboratory conditions, fed with commercial pellet (Hindustan Lever Ltd. Bangalore) and had free access to water.

Acute Oral Toxicity Study

The approval for the activity was taken by submitting protocol to the institutional animal ethics committee; the acute toxicity study was performed as per the OECD (Organisation for Economic Co-operation and Development) guidelines 423. Based on sensitivity six healthy female Swiss albino mice were selected weighed around 20-25gms, fed with laboratory graded diet *ad libitiu* [13]. Animals were kept in clean polypropylene cages, for the bedding sawdust was used and animals were maintained in well ventilated state with 12 hours light, allowed to adapt the laboratory conditions for minimum 10 days prior to the experiment [14]. Animals were kept on overnight fasting allowed only with water, before 3-4 hours of experiment providing water also stopped [15]. Each animal was weighed and marked to determine the appropriate quantities of dosing. The animals dosed once with 2000 mg/kg of the extract and monitored for 14 days to observe general clinical symptoms and mortality. No animal was died till the end of study reveals the 2000 mg/kg dose to be safe. Thus, 100,300,500 mg/kg were chosen for subsequent experimentation.

Induction of Diabetes

To induce diabetes, streptozotocin (STZ) was dissolved in 0.1M sodium citrate buffer (at pH of 4.5) to obtain dose of 55mg/kg [16]. The drug was administered to the rats through oral route, which were under fasting condition for 12 hours prior to the experiment. The induction of diabetes affirmed after 3 days of the STZ administration, animals were tested for its fasting blood glucose levels more than 250 mg/dl were considered as diabetic/hyperglycaemic for the proceeding of experiment [17].

Experimental Design

For the STZ impelled diabetic study the albino rats were assembled in 6 groups, each group comprised of 6 animals.

- Group I: Normal control
- Group II: STZ induced diabetes rats' group.

- Group III: STZ induced diabetes rats treated with *VTE* Low dose (100mg / kg).
- Group IV: STZ induced diabetes rats treated with *VTE* Mid dose (300mg / kg).
- Group V: STZ induced diabetes rats treated with *VTE* High dose (500mg / kg).
- Group VI: STZ induced diabetes rats treated with Standard Metformin (10mg / kg).

The rats were subjected for the treatment of 28 days, the animals which were fasted overnight and the blood sample was collected through retro-orbital method under light anaesthesia on the 7th, 14th and 21st day respectively [18], each time blood samples were sent to analyse the variant parameters of blood, such as blood glucose [19], SGOT [20], SGPT [21], ALP [22], Creatinine [23]. The changes in the animal body weight and water, food intake were observed and recorded throughout the treatment period.

After completion of 28 days of study animals were sacrificed using high doses of anaesthesia and pancreas was collected and measured. In the pancreatic tissue the oxidative stress marker like malondialdehyde (MDA) levels, antioxidant marker glutathione (GSH) levels [24] and estimation of tissue nitrosative stress levels were measured [25]. The tissue samples of all animals from each group were subjected for histological studies.

Histopathological Evaluation of Pancreas: After sacrificing the animals, part of the pancreatic tissue was removed and fixed in 10% formalin saline. Formalin-fixed tissues were processed according to the standard histological tissue processing [26].

Statistical Analysis: All the experimental values were expressed as mean ± standard error of the mean (SEM). The statistical significance among the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test.

RESULTS AND DISCUSSION

Acute Oral Toxicity Test

In the current work, leaf ethanolic extract of *Vitex trifolia.L.* Did not produce any behavioural changes like restlessness, motor activity, breathing and diarrhoea in the first 24 h and up to 14 days cage side observation at 2000 mg/kg dose and no mortality was observed.

Blood Glucose Levels

During the experimental period, upon STZ administration, a significant increase in the blood glucose levels was observed in all groups except normal control. Remarkably the anti-hyperglycaemic condition was observed dose-dependently in VTE extract-treated animals. The high dose of VTE exhibited nearly similar to standard drug Metformin depicted in Fig. 1.

The efficacy of *VTE* on serum SGOT, SGPT in diabetic rats were depicted in Figs. 2 & 3 and the data was represented as (mean±SEM) (n=6). *** $P<0.001$ vs normal control group and $P<0.001$ vs STZ group.

The measured biochemical parameters were significantly altered in STZ-induced diabetic rats compared to normal control rats. In diabetic rats, administration of both doses of high doses of 500mg/kg and standard drug reduced SGOT, SGPT compared to diabetic control rats. The high doses of *VTE* (500 mg/kg) treatment showed significantly ($P<0.001$) higher reduction of SGOT, SGPT levels compared to low doses (100 mg/kg) dose.

ALP levels were significantly altered in STZ-induced diabetic rats compared to normal control rats. The *VTE* 500 mg/kg treatment showed significantly higher reduction of ALP levels compared to *VTE* 100 mg/kg dose. The same is depicted in Fig. 4.

Creatinine levels were shown in Fig. 5, significantly altered in STZ-induced diabetic rats compared to normal control rats. The *VTE* 500 mg/kg treatment showed significantly higher reduction of Creatinine levels compared to *VTE* 100 mg/kg dose.

Effect on OGTT

OGTT was done to assess the status of glucose intolerance and glucose disposal behaviour of animals. It is noted from the blood glucose profile that upon glucose challenge the levels were greatly increased in STZ animals compared to

control animals. The glucose values were increased rapidly and reach close to 600 mg/dl within 15 min post glucose challenge. Glucose levels have not come down to normal levels even after 90 min in STZ animals, in contrast, the glucose levels have come down to normal levels in control animals, indicates the glucose intolerance in STZ animals compared to normal control animals. Interestingly, treatments with extract showed significant effects on glucose profiles with dose-dependent improvement in glucose disposal behavior. A high dose of extract has normalized the glucose intolerance close to normal control animals. A similar kind of improved glucose tolerance was observed in metformin-treated groups.

Pancreatic Beta Cell Protection

The oxidative degradation of lipids were determined by evaluating the concentration of thiobarbituric acid reactive substances (TBARS) which expressed regarding malondialdehyde (MDA) content. There was a 2.48 fold increase in the MDA levels in diabetic control groups compared to normal control animals. Further, treatment with plant extract produced 1.18, 1.86, 2.11 fold reduction, respectively in 100, 300 and 500 mg/kg groups compared to diabetic control pancreatic tissues. Moreover, metformin treatment also produced a 2.1 fold reduction in the MDA levels.

The endogenous antioxidant GSH was found to be 2.12 folds significantly decreased in STZ control pancreatic tissues compared to control animals. Treatment with extracts resulted in 1.15, 1.24 and 1.95 fold increase in the GSH levels. The high dose of extract produced a similar kind of increase like standard drug metformin

The NO levels which is a marker of reactive nitrogen species status, suggested that there is an increase in nitrosative stress in diabetic control pancreas compared to non-diabetic control animals. There was a 2.51 fold increase in the NO levels in pancreatic tissues of STZ treated groups compared to normal pancreas. Further, treatment with extracts dose-dependently produced a reduction in pancreatic nitrosative stress, with a high dose group producing 2.14 fold reduction in the NO levels compared to diabetic control pancreas.

In diabetic control groups, the histology of pancreas showed the proliferation of ductular epithelial cells and increased ductular fibrosis. Treatment with plant extract produced dose-dependent improvement of damaged histological features. Normal histological features were observed on standard anti-diabetic drug metformin treated animals. The number of pancreatic beta cells and the average size of beta-cell and pancreatic cell areas were decreased in STZ groups compared to non-diabetic control pancreas. Treatment with extracts produced progressive and dose-dependent improvement in the pancreatic beta-cell number and beta-cell sizes.

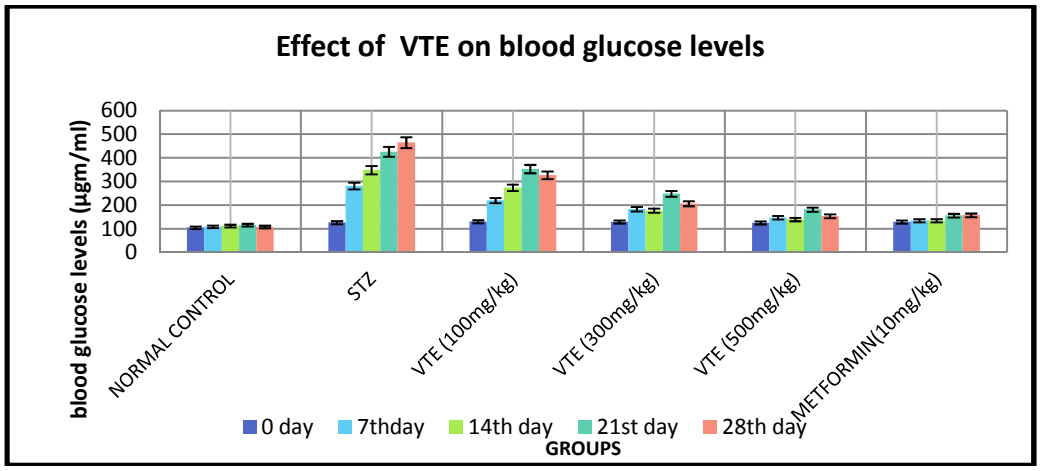

Fig. 1. Effect of VTE on blood glucose levels evaluated in STZ induced model

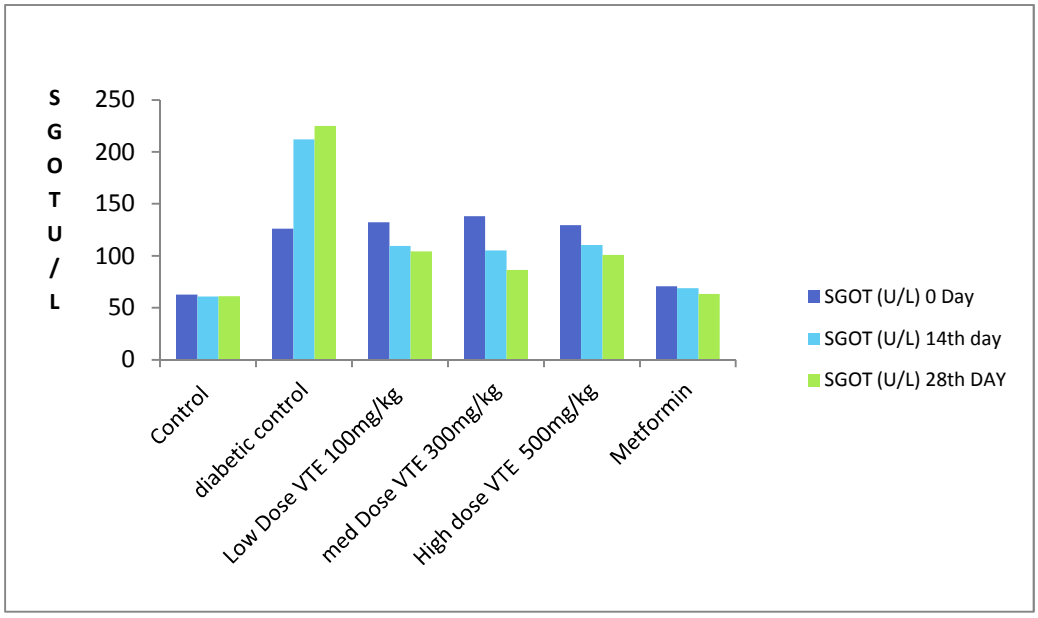

Fig. 2. Effect of VTE on SGOT levels evaluated in STZ induced model

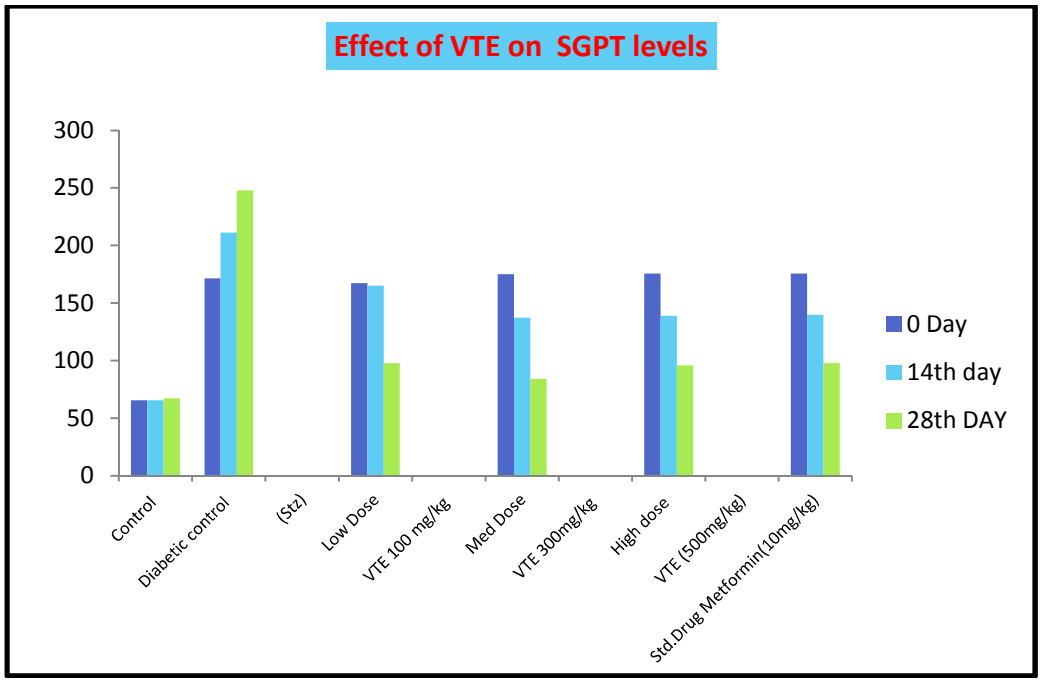

Fig. 3. Effect of VTE on SGPT levels evaluated in STZ induced model

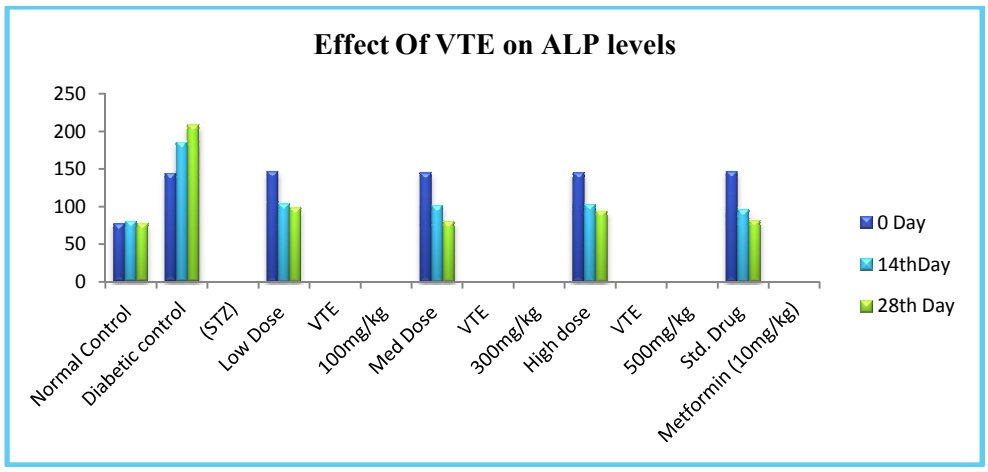

Fig. 4. Effect of VTE on ALP levels evaluated in STZ induced model

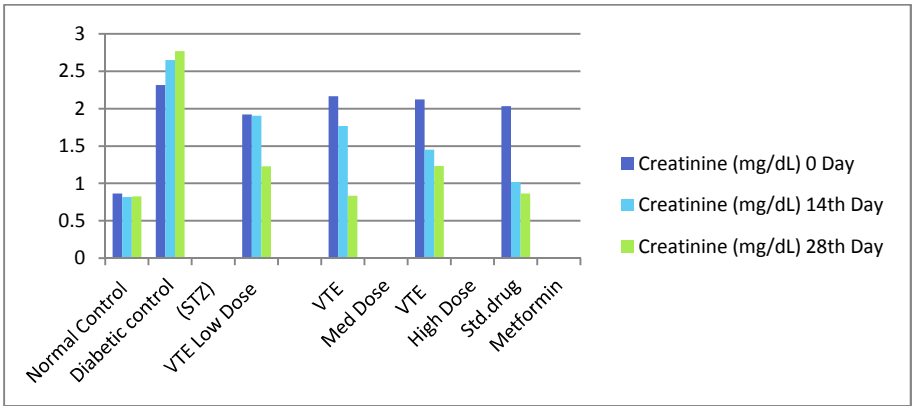

Fig. 5. Effect of VTE on Creatinine levels evaluated in STZ induced model

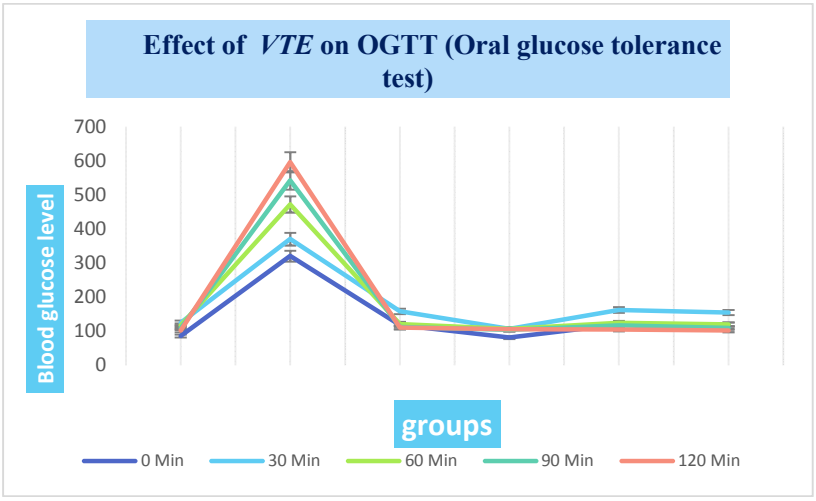

Fig. 6. Effect of VTE on OGTT levels evaluated in STZ induced model

Table 1. Effect of Oral administration of *VTE* on antioxidant parameters in STZ diabetic models

GP	MDA(µM/mg)	GSH(µM/mg)	NO(µM/mg)	TG(mg/dl)	CHL(mg/dl)
I	9.60±3.49	296.18±4.91	16.37±4.22	88.6±4.52	96.21±2.17
II	24.30±8.60	125.89±1.21	41.4±5.94	196.63±4.58	246.91±4.41
III	18.96±4.17	158.28±4.66	31.97±8.39	171.11±7.08	182.39±4.63***
IV	14.62±6.86*	206.71±4.34	25.02±5.22**	122.36±2.55***	130.19±3.90***
V	11.22±5.28***	263.76±1.59*	19.29±8.87***	109.46±4.25***	113.77±1.27***
VI	10.75±4.41***	297.25±4.05**	20.10±5.98***	105.56±3.55***	114.89±4.59***

Data was represented as mean±SEM (n=6).Statistically significant *P<0.05, **P <0.01 and ***P<0.001 vs normal Control group

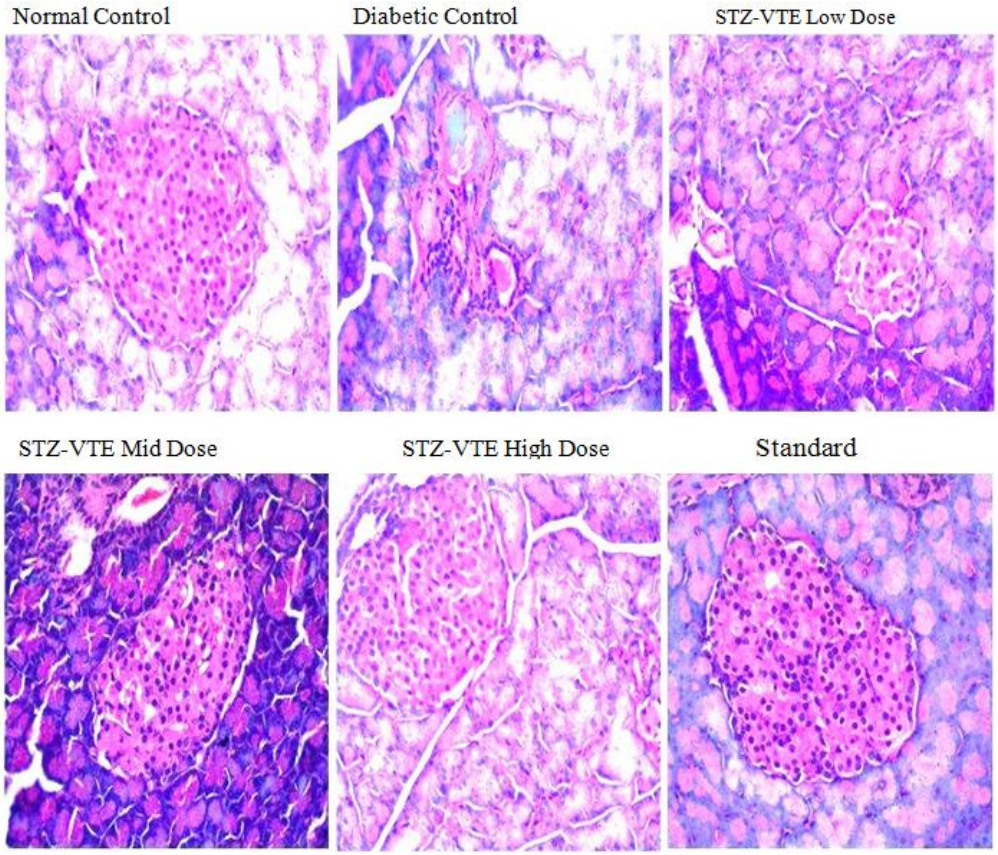

Fig. 7. Effect of VTE on Histological Features of Pancreas (The Hematoxylin and Eosin (H&E) staining on pancreatic tissues)

Discussion

Type II Diabetes is a complex metabolic disorder which is due to the relative deficiency of insulin and sometimes with insulin resistance [27]. The current study was intended to explore the protection of beta cells in antidiabetic activity of *Vitex trifolia*.L in diabetic rats. Here single intraperitoneal (IP) administration of 55mg/Kg streptozotocin made substantial increase in blood glucose levels is due to damage of insulin secreting pancreatic β cells which lead to less glucose uptake by peripheral tissue [28]. In this model we observed that severe damage of pancreatic beta cells within 1 week of post administration of STZ (55mg/kg), Current work

explored that high dose (500mg/kg) of ethanolic extract of *Vitex trifolia.L* leaves lowered blood glucose levels which is nearly similar to the standard drug Metformin (10mg/kg). The antidiabetic effect may be due to the presence of flavonoids, triterpenes, steroids, saponins and phenolic compounds which can stimulate insulin secretion through their antioxidant activities [29]. A liver enzyme plays a major role in gluconeogenesis (formation of glucose by using by-products). The prevalence of elevated liver enzymes, including SGOT, SGOP, and ALP usually help to detect chronic liver disease by monitoring their concentration [30]. The high levels of Creatinine was observed in stz induced diabetic rats, high dose (500 mg/kg) of ethanolic extract of *Vitex trifolia*.L leaves reduced significantly these enzymes. The OGTT test evaluated the improved glucose tolerance. Malondialdehyde (MDA) is polyunsaturated fatty acid formed due to peroxidation in the cells and increase in free radicals causes overproduction of MDA [31]. It is the marker of oxidative stress and there is a vast increase in diabetic control animals, numerous works also reported increased oxidative stress and in metabolic syndrome conditions and STZ is known to produce an abundant increase in oxidative stress conditions in pancreatic tissues. The MDA levels induced oxidative stress are greatly increased in STZ control pancreatic tissues compared to non-diabetic control pancreas. GSH, low molecular weight non-protein this, modulates physiological levels of ROS and is involved in the cell's oxidative stress response [32]. It was observed that there was a reduction in GSH levels in the liver of diabetic rats compared to control rats. The administration of different doses of plant extract and standard drug significantly reversed these changes to near-normal level. Whereas, treatment with high dose of VTE (500 mg/kg) showed an intense improvement with significant elevation of endogenous antioxidant GSH. Nitrosative stress is another parameter involves in pancreatic damage [33], increased NO levels in diabetic control animals, compared to the *VTE* extract high dose(500mg/kg) treatment resulted the encouraging effects in the nitrosative stress conditions. The high ranges of plasma triglycerides, total cholesterol proved the significant dyslipidaemia in untreated diabetic rats [34]. Whereas *VTE* treated rats were showed reduction in these values. This extract with different concentrations seems to be producing beta cell protection by controlling oxidative and nitrosative stress. Based on the biochemical profiles in the pancreas and plasma biochemical parameters, it is evident that there is an increased glucose level which is mainly due to the destruction of pancreatic tissue.

CONCLUSION

The present study provided the information that leaf ethanolic extract of *Vitex trifolia.L* has a potential effect on glycaemic control, oxidative stress and antioxidant enzyme activities in STZ-induced diabetic rats by improving insulin secretion through β-cell restoration capacity. However, further studies are needed for the isolation and purification of bioactive constituents from this plant. Identification of the novel and potent bioactive compounds with specific chemical moieties will provide a new potential traditional approach for the treatment and management of diabetes and its associated complications.

ETHICAL APPROVAL

The experimental protocol was approved by the IAEC (Institutional Animal Ethical), BEA/B.Ph./ 193/2020-2021.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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