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Research Article

GLUCOSE HOMEOSTATIC AND PANCREAS PROTECTIVE POTENTIAL OF *TECOMELLA UNDULATA* ROOT EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The study was aimed to evaluate glucose homeostatic and pancreas protective potential of Tecomella undulata root extract in streptozotocin induced diabetic rats.

Methods: The ethanolic root extract was prepared by following standard soxhlation methods. The experimental design was divided in to control and treated groups for 28 days of comparative experimental schedule. The body and organ weights, serum biochemistry, histo-pathology, hematology and toxicity profiles were assayed by following standard methods and protocols.

Results: The treatment of ethanolic extract of root of *T. undulata* was significantly ($p \leq 0.001$) reduced glucose levels at 7day, 14day, 21day and 28 days in comparison to standard drug of metformin. Correspondingly, lipid profile i.e. total cholesterol, HDL, VLDL, LDL and triglyceride were also altered significantly. Whereas, body and organs weight and hematological parameters were not shown significant changes. Subsequently, toxicity profile i.e. hepatic and renal parameters were remained under normal ranges. Corresponding, the treatment of ethanolic root extract caused normalcy of histoarchitecture of pancreas in comparison to standard drugs.

Conclusion: The results of study illustrated that *Tecomella undulata* root extract possessing particular kind of phytocompounds which caused glucose homeostatic and pancreas protective potential in diabetic rats.

Keywords: Diabetes, Glucose homeostatic potential, Pancreasprotective, Histopathology, Glucose levels, *Ticomella undulata* root extract.

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INTRODUCTION

Diabetes is an endocrine, metabolic disease which is clinically characterized by chronic hyperglycemia due to poor insulin availability. The etiology of diabetes can be inducing pathological damage to the liver, kidneys, and pancreas, with characteristic abnormalities in the metabolism of carbohydrates, lipids, and proteins [1-3]. Whereas there are several kinds of therapeutic approaches for diabetes, but associated side effects pointed out to rethink. Behind this, the ancient Indian medical system of the Ayurveda having peculiar kind of therapeutic patterns which shown minimum drug-associated side effects those are mainly based on herbal formulation. Herbal formulations have been existed worldwide with long documented history and used in various civilizations, i.e., ancient Chinese, Greek, Egyptian, and Indian for several remedies determinations [4-11]. The World Health Organization estimated that 80% of the word's populations still depend on traditional medicines for their health problems. Whereas the Indian subcontinent is a renowned to be one of the major biodiversity center with about 45,000 plant varieties. In India, about 15,000 medicinal floras have been noted, and up to 7,000-7,500 plants are used by communities for therapeutics of various kinds of ailments [11,12].

In similar kind of view, western Rajasthan (Part of the Thar desert) having peculiar kind of medicinal plants which are used by local people from long back based on conventional wisdom for therapeutics of diabetes and other diseases [5,12,13]. The local desert plants having a huge and unique number of secondary metabolites and phytocompounds through the specific stress conditions of desert. Even Rajasthan state having their own state flower, i.e., *Tecomella undulate* is rich source of numerous valued bioactive phytocompounds. Whereas, the various parts of *T. undulata* are also used for various ailments. The root and bark possessing some peculiar kinds of phytocompounds, i.e., radermachol, lapachol, tecomaquinone-I, α-lapachone, β-lapachone, stigmasterol, β-sitosterol, oleanolic acid, ursolic acid, and betulinic acid which are prescribing in the Ayurveda practices and available in market [14,15]. The various parts of *T. undulate* have also been used in the curing of syphilis, painful swellings, antibacterial, hepatoprotective, immunomodulatory, and anti-inflammatory activities [16-20]. However, the root of this plant is main part which directly exposed to climatic stress conditions having an abundance of bioactive phytocompounds. Therefore, this effort was made to assess glucose homeostatic, and pancreas-protective potential of ethanolic root extract of *Tecomella undulate* through investigations of body and organs weights, serum biochemistry, histopathology of pancreas and hematology.

METHODS

Chemicals

Required chemicals and reagents were obtained from local Loba chemie suppliers, and test kits (Erba) were also used for biochemical analyses.

Collection and extraction of chosen plant material

The roots parts of *T. undulata* were collected from in and around university new campus which were authenticated by taxonomist of botany, department of botany.

The dried root was grinded and soxhalated in 70% ethanol for 18 hrs at 72°C temperature. The prepared extract was dried and used as per routine protocol [21].

Dose of extract and drug

The dose regime of extract and drug were allocated for oral administration, i.e., 500 mg/kg body weight for 28 days in comparison to metformin at the dose of 100 mg/kg/d.

Experimental animal
Adult albino rats (*Rattus norvegicus*) weighing of 170-250 **g** were obtained from the animal house of Indian Veterinary Research Institute, Briley, UP. The animals were kept under standard environmental situations, i.e., 12 hrs. light and dark cycles with optimal temperature conditions of 24-28°C. The feeding and diet were maintained as per recommendations of the Veterinarian advice with the proper proportion of nutrients and vitamins along with a supply of drinking water ad libitum. While animal experimental protocols were permitted by Institutional Animal Ethical Committee (Reg. No: 1646/GO/ERe/S/12/CPCSEA).

Diabetes induction
The induction diabetes by following our laboratory protocol as single IP inoculation of streptozotocin (STZ) (Sigma-Aldrich, Mumbai, India) dissolved in 0.01 M sodium citrate buffer (pH 4.5), and managed at a dose of 55 mg/kg in overnight fasted animals [22].

Experimental design
Diabetic animals were subjectively arranged into four groups and an individual group consisting of seven animals to control and experimental groups for respective treatment for 28 days.

- Group 1: Vehicle control.
- Group 2: Diabetic control.
- Group 3: Diabetic + *T. undulate* root extract (500 mg/kg b.w.) for 28 days.
- Group 4: Diabetic + metformin (100 mg/kg/d) for 28 days.

Serum biochemistry assessments
The glucose variations at different days, related lipid profile (triglyceride, total cholesterol, high-density-lipoprotein-cholesterol, low-density lipoprotein [LDL]-cholesterol, very LDL [VLDL] – cholesterol, and other indices) and toxicology profile, i.e., urea, uric acid, creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT), alkaline phosphatase, bilirubin, total protein, albumin, and globulin were evaluated from serum using commercial supplied biological Erba kits (glucose oxidase-peroxidase methods) [23-25].

Hematological analysis
Red blood corpuscle (RBC) counts, hemoglobin content, total leukocyte cells, platelets (thrombocytes) count, and other related parameters of hematology were calculated by standard methods [26].

Histopathological investigations
The histopathology specimens of the pancreas were made by following standard protocol of our laboratory. The paraffin sections were stained by hematoxylin-eosin and microphotography made by camera attached microscope to study pancreas-protective changes in histoarchitectures of pancreas in control and treated animals [27-29].

Statistical analysis
All values of biochemistry, hematology, body and organs weights were evaluated by one-way ANOVA, followed through Dunnett test using software Sigma-Sat trial version. In all the tests, the criteria of statistical significance were $p \leq 0.005$.

RESULTS

The treatment of root extract of *T. undulata* caused significant alterations in treated experimental groups in comparison to metformin treatment and diabetic control as shown in following results.

Body and organs weights
The root extract of *T. undulate* showed a non-significant reduction in body weight, pancreas, and liver. Whereas other morphological signs were shown fatigue and less activeness in diabetic rats in comparison to treated and vehicle control (Table 1).

Serum biochemistry

a. The treatment of root extract of *T. undulata* revealed significant ($p \leq 0.001$) gradual decreases in glucose levels in comparison to metformin at 7 days, 14 days, 21 days, and 28 days with diverse grades. However, significant raised glucose level was observed in diabetic rats up to 250-350 mg/dl (Fig. 1).
b. Accordingly, the lipid profile, i.e., total cholesterol, LDL- cholesterol, triglyceride, and VLDL-cholesterol were significantly ($p \leq 0.001$) decreased in treated animals as a comparison to diabetic control (Table 2).
c. Compassionately, toxicity profile, i.e., creatinine, urea, uric acid, SGOT, SGPT, alkaline phosphatase, bilirubin total protein, albumin, and globulin were endured under and around normal ranges (Fig. 2).

Haematology
RBC count, total leukocytes count, platelets count, and other related morphological parameters were not altered significantly in treated groups (Fig. 3).

Histopathology
The pancreatic histoarchitecture of vehicle control consisting of condensed, lobulated compounds of tubuloacinar gland of exocrine and endocrine part with distinguished symmetry. The islets, i.e., endocrine part exhibiting of diverse sizes and surrounded inside the exocrine tissue of pancreas with bright staining. There were three diverse categories of islets seen, i.e., small, medium, and large sized were distinguished. Individually, islet was consisting of alpha, beta, delta, and pancreatic polypeptide cells embedded in peripheral tissues of acini and vessels (Fig. 4). Whereas, diabetic pancreatic histology showed vacuolization and deterioration in islet cells with congestion of RBC. The diabetic pancreas correspondingly exhibited indiscretions in peripheral exocrine parts and vascular part (Fig. 5).

However, the treatment of root extract of *T. undulate* encouraged re-architecture of islet as well as the reorganization of peripheral tissues (Fig. 6). Correspondingly, the treatment of metformin revealed near to normalcy of islet with existing of cells and proper arrangement of peripheral tissues (Fig. 7).

DISCUSSION

Hyperglycemia and non-functional pancreatic β-cell are considered as a diabetic pathological condition from primary description [30,31]. Whereas there are several kinds of pharmacological approaches for

Table 1: Effect on body and organs weight of root extract of *T. undulata* in STZ-induced diabetic albino rats (mean of 5 values SEM)

Group	Mean body weight (gm)		Heart	Pancreas	Kidney	Liver
	Initial	Final	mg/100gm body weight			g/100 g body weight
Group I (vehicle control)	203.13±3.13	209.23±7.17	469.12±07.33	231.21±21.17	581.09±31.11	4.93±0.31
Group II (diabetic control)	199.11±7.01	193.11±10.02^{c}	453.17±13.11^{d}	178.17±31.10^{c}	511.31±22.07^{d}	4.13±0.23^{c}
Group III (*T. undulata* root extract treatment)	210.01±9.11	201.12±7.11^{d,h}	467.21±11.23^{d,h}	203.21±17.11^{c,g}	563.33±31.11^{d,h}	4.41±0.11^{d,h}
Group IV (metformin treatment)	211.7±7.01	203.01±9.21^{d,h}	471.70±19.01^{d,h}	201.19±19.13^{c,g}	577.23±17.31^{d,h}	4.89^{d,h}±0.21^{d,h}

Data are means±SEM. (n=7), $^{c}p \leq 0.001$, ^{d}non-significant as compared to the respective control values, $^{g}p \leq 0.001$, and ^{h}non-significant as compared to the respective values of the diabetic control group, *T. undulate: Tecomella undulate*, STZ: Streptozotocin

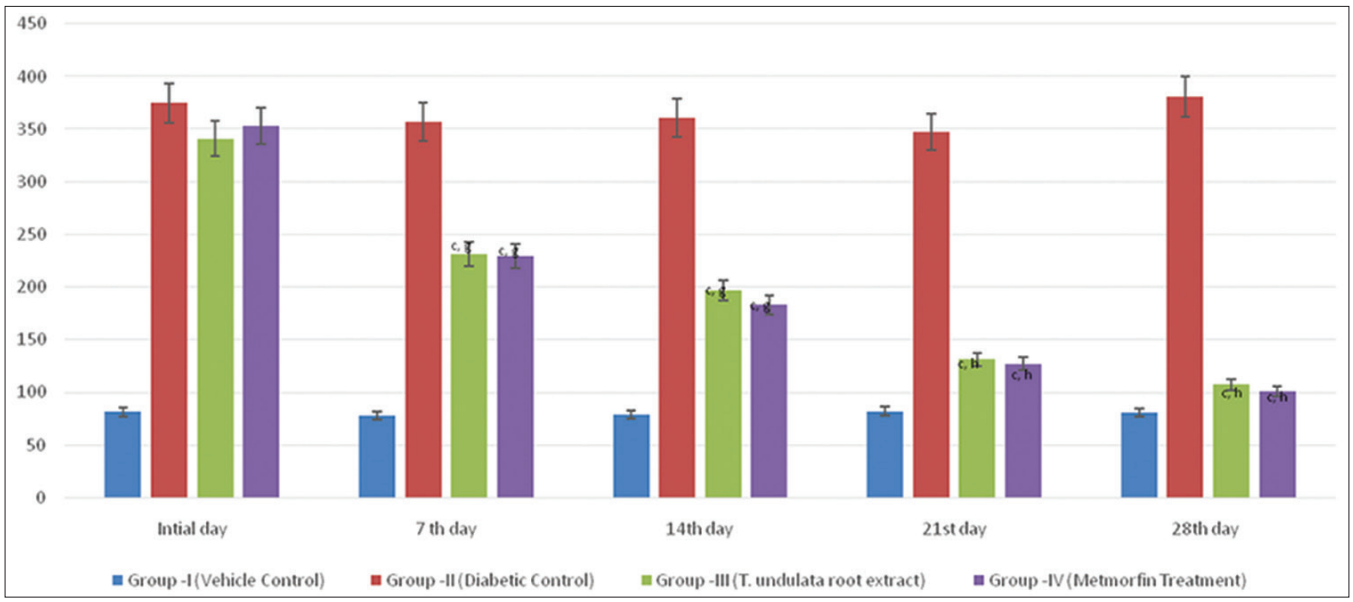

Fig. 1: Effect of *Tecomella undulate* root extract on glucose levels in streptozotocin induced diabetic albino rats. Data are Mean±standard error mean SEM (n=7), [c]p≤0.001, [d]non-significant as compared to the respective control values, [g]p≤0.001, and [h]non-significant as compared to the respective values of the diabetic control group

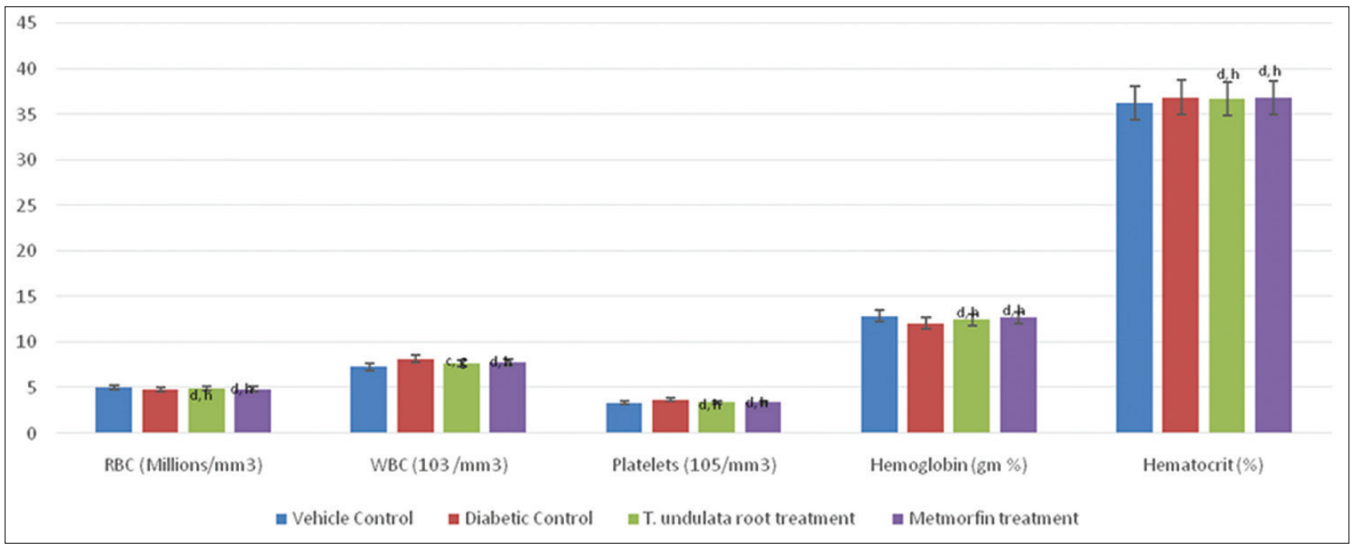

Fig. 2: Effect of *Tecomella undulate* root extract on renal and liver function indices in streptozotocin induced diabetic albino rats. Data are Mean±standard error mean. (n=7); [c]p≤0.001, d non-significant as compared to the respective control values, [g]p≤0.001, and [h]non-significant as compared to the respective values of the diabetic control group

Table 2: Effect of *T. undulata* root extracts on lipid profile in STZ induced diabetic albino rats

Groups	Cholesterol	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Triglyceride (mg/dl)	LDL/HDL
Group I (vehicle control)	106.01±7.23	30.11±2.10	52.29±3.16	23.61±1.96	118.03±09.31	1.74±0.02
Group II (diabetic control)	235.61±12.12[c]	29.89±2.23[d]	167.92±6.12[d]	37.80±3.16[d]	189.01±6.67[a]	5.62±0.32[c]
Group III (*T. undulata* root extract treatment)	126.58±09.73[c,g]	30.71±0.32[d,h]	73.33±0.59[c,g]	22.54±0.23[c,h]	112.7±0.62[c,f]	2.29±0.03[c,h]
Group IV (metformin treatment)	127.44±5.11[c,f]	30.17±0.63[d,h]	75.39±0.32[c,f]	22.08±1.07[c,h]	110.41±1.7[c,f]	2.57±0.17[c,h]

Data are means±SEM. (n=7), [c]p≤0.001, [d]non-significant as compared to the respective control values, [g]p≤0.001, and [h]non-significant as compared to the respective values of the diabetic control group, *T. undulate: Tecomella undulate*, STZ: Streptozotocin, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, [a]p≤0.05, [f]p≤0.01

caring diabetes and other diseases, but issues of side effects are still questionable. Although natural cures were used and still be used today with minimum side effects. There are many herbs with strong antidiabetic properties by following different mechanisms [32-37]. In the present study, the treatments of root extract of *T. undulata* performed hypoglycemic effect in gradual reductions in comparison to metformin

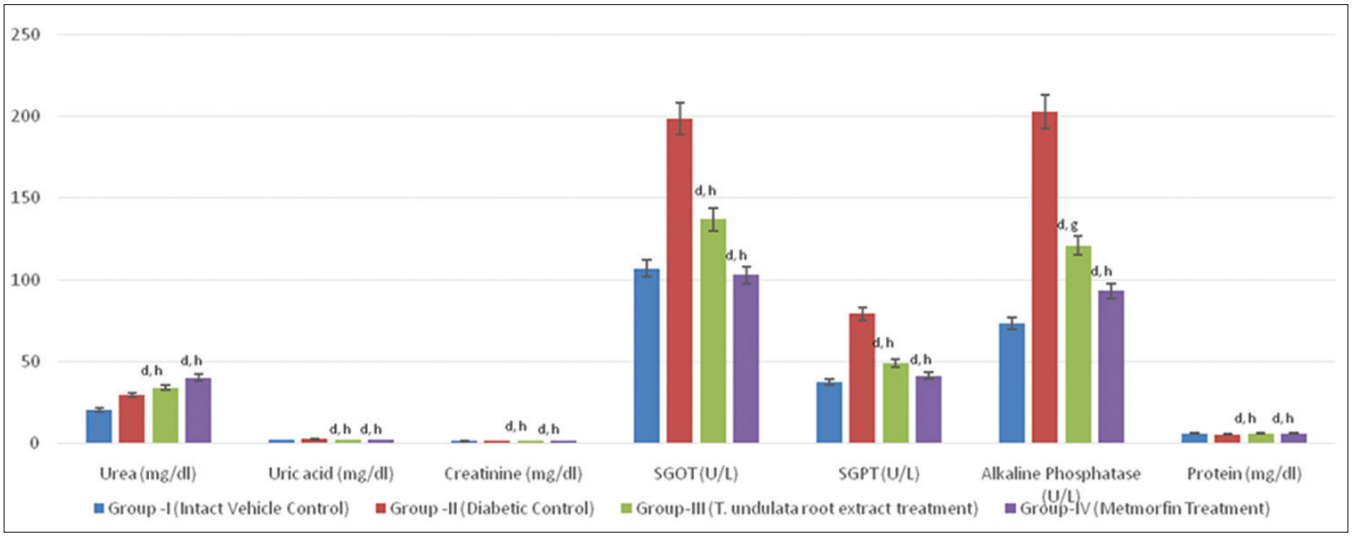

Fig. 3: Haematological assessments of *Tecomella undulate* root extract treatments in streptozotocin induced diabetic albino rats (mean of 5 value±standard error mean [SEM]). Data are means±SEM. (n=7), [c]p≤0.001, [d]non-significant as compared to the respective control values, [g]p≤0.001, and [h]non-significant as compared to the respective values of the diabetic control group

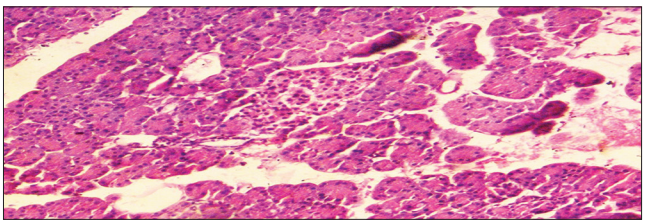

Fig. 4: Pancreatic histoarchitecture of intact vehicle control rats (H and E ×200) - the histoarchitecture of intact vehicle control pancreas exhibiting of islets of Langerhans with cluster of cells organized in asymmetrical departing and anastomosing strings detached by blood capillaries and subtle collagen fibres

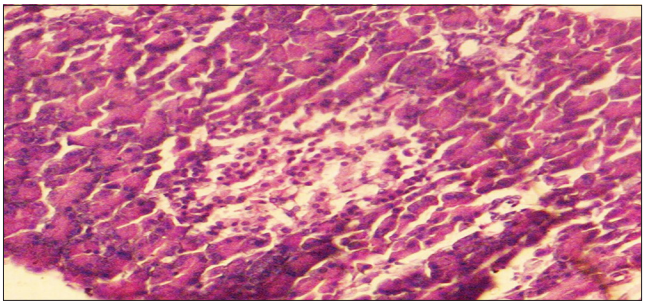

Fig. 5: Pancreatic histoarchitecture of diabetic control rats (H and E ×200) - diabetic pancreatic histoarchitecture containing of vacuolated islets of Langerhans and necrotic nuclei of islet cells with congested blood vessels

standard drug [13,20,38]. This type of activity may be conducted through alone or synchronic action of possessing bioactive compounds, i.e., oleanolic acid, ursolic acid, betulinic acid, triacontanol, cirsimaritin, cirilineol, pentariacontanol, 4,5-dihydroxy 3,6,8- trimethoxyflavones, and other phytocompounds as described by earlier researchers. The treatment caused non-significant and significant changes in body and organs weights. The changes in body and organs weights could be following STZ activities and ameliorative actions of phytocompounds against hyperglycemia correspondingly [35,39].

However, there are stated some hypothesis regarding hypoglycemic or insulin mimic effect of plant products as their effects on the activity of pancreatic beta cells, increase in the oppressive action against insulinase enzyme, establishment of the insulin sensitivity or the insulin-like effect of the phytocompounds and through other mechanisms [40]. However, other concept suggested that it might also be involved such as increase of peripheral consumption of glucose, intensification of synthesis of hepatic glycogen or reduction of glycogenolysis, inhibition of intestinal glucose absorption, decrease of glycemic index of glycemic content, and decrease of the consequence of glutathione. In parallel milieu, lipid parameters, i.e., total cholesterol, LDL -cholesterol, triglyceride, and other indices were also reduced which may be due to be lacking in changes of gluconeogenesis and unapproachability of peripheral excess glucose [41,42]. Subsequently, the STZ caused a substantial reduction in pancreatic cells and structural fluctuations as stated by some studies [1,16,42]. The introduction of STZ caused β-cell toxicity is rational through carboxylation of proteins, alkylation of DNA, release of free radicals (reactive oxygen species and reactive nitrogen species) and inhibition of O-GlcNAcse (glycoside hydrolase). These types of detrimental actions made by STZ are accountable for necrosis of pancreatic β-cells and introduction of experimental diabetes mellitus in laboratory animal models. Although the treatments of root extract of *T. undulata* caused various grades of a renaissance in pancreatic cells and endorsing numerous grade of re-histoarchitecture of pancreas. This kind of action of herbal products also stated by previous studies and illustrations that having bioactive compounds might consume congested more devastation of the enduring β-cells in the islet by mopping up the circulating reactive oxygen species generated through the STZ to devastate the β-cells and then permitting additional phytochemicals of the herbal to stimulate regenerative activities [1,43]. The administration of the herbal extract todiabetic animals provoked a decrease in aminotransferases in serum, i.e., SGOT, SGPT, and alkaline phosphatase, a result of experimental and toxicological importance quantified that changes in their actions are pinpointing of tissue impairment by contaminants or signs resultant to hepatic disorders. This declination of SGPT, SGOT, and alkaline phosphatase to their normal levels might be chosen by the absence of alkanes in the extract [43]. Correspondingly, other renal and hepatic parameters were also come to under or around regular ranges which suggested nontoxic nature of *T. undulata* extract. Compassionately, hematological parameters were also persisted under normal ranges which also proven least toxic nature of possessing phytocompounds [24,26,44]. Therefore, it is concluded that phytocompounds possessing in root extract of *T. undulate* having potential of glucose homeostatic and pancreatic protection which can be help in therapeutic strategies of diabetes.

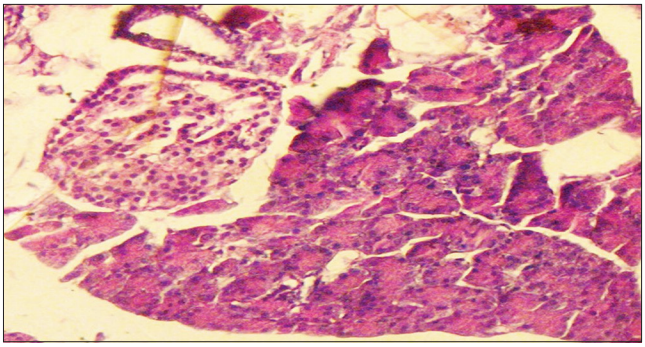

Fig. 6: Pancreatic histoarchitecture of *Tecomella undulate* root extract (500 mg/kg) treatment (H and E ×200) - the pancreatic histoarchitecture containing of acini and regenerated cellular portion of islets of Langerhans with appropriate distribution of blood vessels

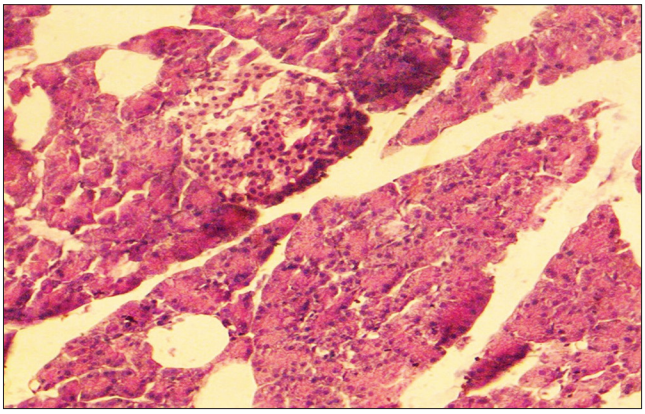

Fig. 7: Pancreatic histoarchitecture of metformin treatment (H and E ×200) - the pancreatic histoarchitecture comprising of acini and regenerated cellular portion of islets of Langerhans with appropriate distribution of blood vessel

CONCLUSION

From the finding of this study, it can be concluded that the root extract of *T. undulata* consisting numerous bioactive chemicals, which shows a glucose homeostatic and pancreas protective potential. This study will be beneficial in additional compounds level can be elaborate more utility of this plant product which can verify it as potent cost-effective antidiabetic agent from native resources using conventional wisdom.

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