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Research Article

**EVALUATION OF ANTIDIABETIC ACTIVITY OF LEAVES
EXTRACT OF *POPULUS DELTOIDES***Rajeev Jha^{1,*}, Nagalakshmi N.C.², Shiva Kumar Swamy³, Bishnu Adhikari⁴¹Division of pharmacology, Rajiv Gandhi University of Health Sciences, 4th 'T' Block,
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Anantapuramu, 515002 Andhra Pradesh India.**Abstract:****Aim:** The study was designed to evaluate the Antidiabetic activity of ethanolic leaves extract of *P. deltoides*.**Method:** The *P. deltoides* ethanolic leaves extract was tested for the presence of various phytoconstituents using standard procedure. The leaves extract was tested for its hypoglycaemic property in healthy albino rats and glucose loaded rats. The effect of extract was evaluated for its antidiabetic action in alloxan induced rats. The effect of extract on heart and kidney was determined by measuring biomarkers like total cholesterol, triglycerides, HDL, creatinine, BUN, CPK and protein in diabetic rats. The histopathology of pancreas was studied to support the experimental results.**Result:** The *P. deltoides* ethanolic leaves extract revealed the presence of variety of chemical constituents like glycosides, tannins, amino acids, alkaloids, protein, flavanoids, terpenes, phenols, saponins. The *P. deltoides* leaves extract exhibited significant reduction in blood glucose level in healthy albino rats and overloaded glucose levels. The extract exhibited significant reduction in blood glucose level in alloxan induced diabetic rats. The percentage of reduction was highest at 500 mg/kg dose of ethanolic extract at 48.55% at 7th day of the treatment. The extract protected experimental animals against cardiotoxicity and nephrotoxicity in diabetic and normal animals.**Conclusion:** The ethanolic extract of *P. deltoides* exhibited significant hypoglycaemic property in healthy and diabetic albino rats. The extract protected the diabetic albino rats against cardiac and nephrotoxicity.**Keywords:** alloxan, cardioprotective, hypoglycemia, *Populus deltoides*.**Corresponding author:****Rajeev Jha,**Division of pharmacology,
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INTRODUCTION:

Natural compounds with Antidiabetic activity, in descending frequency of occurrence, include complex carbohydrates, alkaloids, glycopeptides, terpenoids, peptides and amines, steroids, flavonoids, lipids, coumarins, sulfur compounds, inorganic ions and others[1]. Plants have always been a good source of drugs. About 800 plants that may possess antidiabetic potential as reported by ethno botanical information[2-4]. The beneficial uses of medicinal plants in traditional system of medicine of many cultures are extensively documented.

P. deltooides is an Indian traditional medicinal plant containing flavanoids in leaves used in the therapy of rheumatism, gout, scurvy, blood purifier, anticancer and pain. It is also used in case of urinary problems, thinner of bronchial secretions and tonic as diuretic, uric acid eliminator, antiinfectious[5]. Recent studies on diabetes claims that the flavanoids have antidiabetic activity[6]. However the literature reveals no scientific data on antihyperglycemic effect of *P. deltooides*. Many of the plants having antioxidant properties have been proved to possess antidiabetic property[7]. Hence the current research work focuses on evaluating the antidiabetic potential of a *P. deltooides* leaves extract in alloxan induced diabetic rats.

METHODS:

Plant materials

The leaves of *P. deltooides* were collected from the garden of Dehradun, Uttarakhand. The leaf was identified and authenticated by Dr. K. Ravikumar, senior botanist at FRLHT (Foundation for Revitalisation of Local Health Traditions) Jarakabande Kaval, post Attur, Yehalanka, Bengaluru (560106). A herbarium specimen was preserved in the college museum for future reference. The leaves were shade dried at room temperature for 15 days and pulverized.

Animals

For pharmacological experiments, male albino rats (180-220 g) were used. Animals were acclimatized and were maintained under standard condition in an animal house approved by the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Liveon Biolabs Private Limited, Tumkur. Reg. no. 1432/PO/a/11/CPCSEA.

Effect of *P. deltooides* leaves extract on blood glucose level on normal albino rats

The experiment was carried out to find out the effect of *P. deltooides* leaves extract on blood glucose level on healthy albino rats.

Experimental procedure

The normoglycemic study was carried out in the same group of animals which were used in part I. In this study the treatment as part I was continued by administering respective doses to each group everyday for next 28 days. Animals were fed with normal diet and water. The blood samples were collected from tail tip on 0, 7th, 14th, 21st, 28th, day respectively and were analysed for fasting blood glucose level by using a commercial glucometer.

The percentage reduction in blood glucose levels was calculated. The fasting blood glucose level and percentage reduction are expressed as mean \pm SEM in the **Table no. 5**, and graphically represented in **Fig no. 1**.

Experimental procedure

Group-I - Normal control rats were treated with normal saline only.

Group-II - Received glipizide (5 mg/kg p.o.) standard reference drug.

Group III - Received ethanolic extract of *P. deltooides* (250 mg/kg p.o.).

Group IV - Received ethanolic extract *P. deltooides* (500 mg/kg p.o.).

Effect of *P. deltooides* leaves extract on blood glucose level on glucose overloaded in healthy albino rats

Oral glucose tolerance test was studied in glucose overloaded male albino rats weighing between 180-220 g. The animals were divided into four groups containing six animals each and marked conveniently. The animals were fasted overnight before commencing of the experiment. During this period, rats were allowed to take adequate water. The rats of group-I were treated with vehicle, group-II received glipizide (5 mg/kg p.o.), the remaining two groups were treated orally with 250 mg/kg and 500 mg/kg ethanolic leaves extracts respectively. After 30 min of the drug treatment, the animal of all the groups were fed with glucose (4 g/kg p.o.) and blood glucose level was determined after 0.5, 1, 2, and 4 hours of the glucose load. Blood glucose level was estimated by using a commercial glucometer and a data of % reduction of blood glucose was recorded and tabulated in **table 6** and represented as the graph in **fig. 2**.

Evaluation of antidiabetic potential and secondary diabetic complications of plant extract of *P. deltooides* on alloxan induced diabetic rats

Preparation of diabetic rats

Male albino rats weighing between 180-220 grams were selected and fasted for 18 hours with water

ad-libitum. The rats were administered with alloxan monohydrate (120 mg/kg, p.o.) dissolved in normal saline (0.9% w/v of NaCl in distilled water) intraperitoneally. The rats were treated with 20% glucose solution intraperitoneally after 6 hours. They were kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia. The blood samples were collected after 24 hours by slicing tip of the tail and blood glucose levels were determined by using a commercial digital glucometer. Rats with blood sugar levels more than 300 mg/dl were considered to be diabetic. Animals were allowed to stabilize for 4 days and further employed in the study. The mortality rate of the rats after alloxan treatment was found to be 25%.

Experimental procedure

Thirty male diabetic albino rats weighing between 180-220 grams were selected and divided into 5 groups containing six animals each and marked conveniently. The animals were fasted for 18 hours before commencing the experiment. During this period, the rats were allowed to take adequate water. The fasting was continued till the completion of the experiment. Group-I: Normal control rats were treated with normal saline only. Group-II: Diabetic control rats alloxanized (120 mg/kg, B. W.) received only vehicle. Group-III: Received glipizide (5 mg/kg, p.o.) standard reference drug. Group IV: Received ethanolic extract of *P. deltoides* (250 mg/kg, p.o.). Group V: Received ethanolic extract *P. deltoides* (500 mg/kg, p.o.)

The experiment was continued by administering the fixed dose of treatment everyday for 28 days. Blood glucose was estimated by withdrawing blood samples from tail tip at 0, 7th, 14th, 21st and 28th of treatment. The data was recorded and percentage reduction of blood glucose level was calculated and noted in **table 7** and graphically shown in **Fig. 3**.

The body weight, food and fluid intake of all groups of animals was monitored on a daily basis for 28 days at regular time. Fixed amount of rat chow and fluid were given to each rat and replenished the next day.

At the end of 28th day, blood sample was withdrawn from retro orbital plexus into fresh centrifuge tubes and centrifuged at 3000 rpm for 10 min to obtain serum and plasma. Serum samples were stored at 4°C until utilized for further biochemical estimation parameters such as total cholesterol, total triglycerides, HDL, Creatinine, BUN, CPK Total, protein. The animals were sacrificed by cervical dislocation and the organ like pancreas was collected for histopathological investigation.

BIOCHEMICAL ESTIMATION:

The serum was stored at 4°C for the estimation of Total cholesterol, triglyceride, HDL, Creatinine, BUN, CPK TOTAL and protein. The estimations were performed using the standard procedures.

a) Estimation of serum Total Cholesterol-TC⁸ (CHOD-PAP: enzymatic photometric test)

Principle:

Determination of cholesterol after enzymatic hydrolysis and oxidation. The colorimetric indicator is quinoneimine which is generated from 4- aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction).

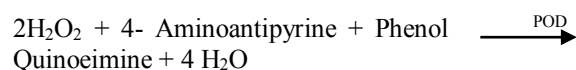
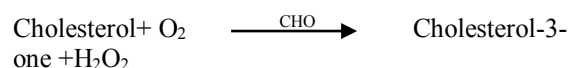
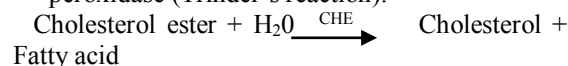


Table 1: Reagents used for the estimation of serum Total Cholesterol

Good buffer	pH 6.7	50 mmol/L
Phenol		5 mmol/L
4-Aminoantipyrine		0.3 mmol/L
Cholesterol esterase	(CHE)	≥200 U/L
Cholesterol oxidase	(CHO)	≥50 U/L
Peroxidase	(POD)	≥3k U/L

Standard: 200 mg/dl (5.2 mmol/L)

Procedure:

Pipetted out 1000µL reagent and 10µL sample or standard or blank, it was mixed and incubated at 20-25°C for 20minutes or 10 minutes at 37° C. The absorbance was recorded at 500nm within 60 minutes against reagent, blank.

Calculation:

$$\text{Cholesterol [mg/dl]} = \frac{\text{A Sample}}{\text{A Std}} \times \text{Conc. Std [mg/dl]}$$

Conversion factor:

$$\text{Cholesterol [mg/dl]} \times 0.02586 = \text{Cholesterol [mmol/L]}$$

b) Estimation of serum of triglycerides[8]

Span diagnostic kit was used for estimation of triglycerides, which followed end point colorimetry enzymatic test using glycerol-3-phosphate oxidase.

Principle: The enzyme, lipoprotein lipase catalyzes hydrolysis of TGs to glycerol and FAs. Glycerol then is phosphorylated in an ATP- requiring reaction catalyzed by glycerophosphate. The formed glycerophosphate is oxidized to

dihydroxyacetone and H₂O₂ in a glycerophosphate oxidase catalyzed reaction. H₂O₂ then reacts with 4 -AAP and 4 - chlorophenol under the catalytic influence of peroxidase to form coloured quinoneimine complex, the intensity of which was measured at 505nm.

Assay and Procedure: Fresh clear and dehaemolysed serum was used for the estimation. The reaction mixtures were mixed well and incubated for 10 min at 37°C. The absorbance of sample and standard were measured against reagent blank at 505 nm. The absorbance was measured by using a Shimadzu spectrophotometer (model 1601).

c) Estimation of serum high-density lipoprotein cholesterol (HDL-C):

Span diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method.

Principle: HDL-C is measured in the supernatant after the precipitation of the lipoproteins including chylomicrons, very low-density lipoproteins, low-density lipoproteins, intermediate-density lipoproteins directly from serum poly anions like phosphotungstic acid and along with MgCl₂ are added to an aliquot of serum an immediate heavy precipitation is formed. The precipitate then is sedimented by centrifugation and HDL cholesterol is measured in the clear supernatant, which is estimated by enzymatic method as described earlier in estimation serum of TC.

Assay & Procedure: Fresh clear and unhaemolysed serum was used for the estimation.

Standard: The concentration of standard glucose used was 50 mg/dl.

Reaction parameters:

Table 2: Reagents used for estimation of HDL-C

1	Reaction type	End point
2	Wavelength	505 nm
3	Optical path	1 cm
4	Temperature	37°C
5	Measurement	Against reagent blank

Standard of assay details:

1. 0.5ml of serum was taken into test tube and 0.5ml of precipitating reagent was added, mixed well and kept at room temperature for 15min.

2. Centrifuged for 15 min at 4000 rpm.

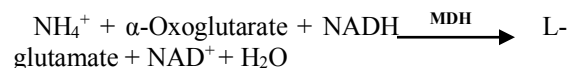
3. The clear supernatant was separated and immediately used to determine the cholesterol content as follows:

Pipetted in to test tube	Blank	Standard	Test
Reagent	1000 µl	1000 µl	1000 µl
Standard	-	100 µl	-
Supernatant from step3	-	-	100 µl

The reaction mixtures were mixed well and incubated for 10 min at 37°C. The absorbance of test and standards was measured against the reagent blank at 505 nm. The absorbance was measured by using a Shimadzu spectro photometer (model 1601).

d) Blood urea nitrogen[9]

Principle: Urea is hydrolysed in presence of urease to produce ammonia and CO₂ the ammonia produced combines with α-oxoglutarate and NADH in presence of GLDH to yield glutamate and NAD⁺.



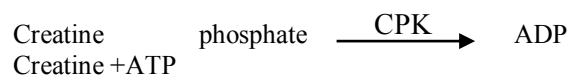
Procedure: Pipetted 1.0 ml of reagent into test tubes and allow reagent to attain 37°C. Added 0.01ml (10ul) of sample to test tube and immediately placed in the spectrophotometer. After thirty seconds absorbance was recorded. Sixty seconds after the first reading another reading was taken. The absorbance was recorded at 340 nm.

Calculation: Urea (mg/dl) = Abs. of test / Abs. of Std. X Conc. of Std.

e) Estimation of Creatine phosphokinase (CPK)[10]

Creatine phosphokinase (CPK) activity is greatest in striated muscle, heart tissue, and brain. The determination of CPK activity is a proven tool in the investigation of skeletal muscle disease (muscular dystrophy) and is also useful in the diagnosis of myocardial infarction (MI) and cerebrovascular accidents. Increased levels of CPK also can be found in viral myositis, polymyositis, and hypothyroidism. Following injury to the myocardium, such as occurs in acute MI, CK is released from the damaged myocardial cells.

Reaction:



Procedure: CK-MB activity in perfusate was estimated by using Kit method (Immuno inhibition method, Kinetic assay). The procedure involves the measurement of CK activity in the presence of an antibody to the CK-M monomer. This antibody completely inhibits the activity of CK-MM and half of the activity of CK-MB while not affecting the B subunit activity of CK-MB and CK-BB.

To 500 μ l of working reagent solution, 25 μ l of Anti CK-MM reagent (CK-MB LabKit, Span diagnostics) was added and mixed properly. 50 μ l of perfusate was added and mixed on a vortex mixer and aspirated immediately into Semi-Auto Analyzer (ARTOS) at 37°C and 600 seconds delay time. Increase in absorbance was measured at 340 nm every minute starting from 0 minute up to 3 minute and change in absorbance was calculated.

Calculation: CK-MB Activity = $\Delta E/340 \text{ nm} \times 3697.75$ (Kinetic factor) units/l, Where, $\Delta E/340 \text{ nm}$ = average change in absorbance at 340 nm

f) Estimation of Serum protein[11,12]

Principle: Biuret method was used for the estimation of serum protein. The principle of biuret method is a colorimetric procedure. The biuret reagent is based on a reaction between copper and molecules with at least 2 peptide bonds. The color intensity caused by this reaction is directly proportional to the number of peptide bonds, and therefore the amount of protein.

Table 3: Reagents: Preparation of biuret reagent.

Compound	amount	Instructions
Potassium sodium tartrate	4.5	Dissolve in 40 mL of 0.2 N NaOH
Copper sulfate	1.5	Added to above
Potassium iodide	0.5	Added to above Diluted the above to 500 mL using 0.2 N potassium iodide in 0.2 N NaOH

Calculations: Using the protein standard solution a five point standard curve was prepared by performing serial dilutions between a range of 0-150 mg/mL. Used these with the biuret reagent to quantify serum protein for the blood samples from incremental exercise to fatigue.

Procedure: Pipetted out 3 ml of Biuret reagent to an appropriate number of 12 x 75 mm glass tubes. Pipetted 50 μ l of each sample from your serial dilutions, from the incremental exercise samples, and for distilled water (blanks) into the tubes. Incubated the tubes at room temperature for 30 min. During incubation, turned on and warmed-up the spectrophotometer. Prepared to run all samples at 540 nm, and recorded the absorbance values for the standards and unknowns. Calculated the serum protein concentrations using the regression equation from standard curve.

HISTOPATHOLOGICAL STUDIES[13]

The animals were sacrificed and their pancreas were isolated. The isolated pancreas was cut into small pieces and preserved in 10% formalin for two days. Then the pancreas pieces were washed in running water for about 12 hours. This was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each and the final dehydration was done using absolute alcohol with about three changes for 12 hours each. Clearing was done by using chloroform with two changes for 15 to 20 minutes each. After clearing the organ pieces were subjected to paraffin infiltration in automatic tissue processing unit. After processing tissue were embedded in paraffin wax. Sections were cut at 5 μ thickness and stained with hematoxyline-Eosin for histochemical studies. After completion of staining sections were observed under microscope for histological change.

RESULTS:

The preliminary phytochemical study of 70% ethanolic extract of leaves of *P. deltoides* shows the presence of Alkaloids, saponins, flavanoids, phenolics, terpinoids, glycosides, proteins, amino acids and tannins.

Table 4: Evaluation of acute toxicity studies of 70% ethanolic leaves extracts *Populus deltoides*

Serial no.	Group(n=6)	Dose	Mortality	% Mortality
1	Group I	1000 mg/kg b. w	nil	0
2	Group II	2000 mg/kg b. w	nil	0
3	Group III	4000 mg/kg b. w	2	33.33
4	Group IV	5000 mg/kg b. w	3	50

Table 5: Effect of *P. deltoides* leaves extract on fasting blood glucose levels in healthy albino rats (NG)

Treatment Group	FBS (mg/dl)					% Reduction in blood sugar level			
	Days					Days			
	0.0	7	14	21	28	7	14	21	28
Normal control	91.66 ±1.45	96.5±0.42	98±2.01	95.33 ±0.66	91.5 ±1.1	-5.28	-6.91	-4.00	0.17
Glipizide 5mg/kg (standard)	96.66 ±0.61 *	50±1.36***	49.5±2.54** *	49 ±2.87** *	48.33±2.60** *	48.2 7	48.7 8	49.3 0	50
Ethanollic extract (250 mg/kg)	91.5 ±1.11	79.16±4.06 **	76.33±5.07* *	75.83 ±5.04**	75±5.37**	13.4 8	16.5 7	17.1 2	18.0 3
Ethanollic extract (500 mg/kg)	95 ±1.75	78±5.01**	75±5.41**	74.5 ±4.24**	73.66±2.94**	17.8 9	21.0 5	21.5 7	22.4 6

N = 6 animals. Values are expressed as Mean ± SEM. Values showing * superscript are $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 6: Effect of *P. deltoides* leaves extract on blood glucose level of glucose over loaded in healthy albino rats (OGTT)

Treatment Group	FBS (mg/dl)					% Reduction in blood sugar level			
	Time (in hours)					Time (in hours)			
	0.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0
Normal control	87±3.45	110±4.13	137.66±3.62	95±1.88	87.33±1.49	- 26.43	- 25.14	30.98	8.07
Glipizide 5mg/kg (standard)	92±1.31	127.33±5.15 **	102.66±8.11* **	64±2.79** *	49.66±2.12* **	- 38.40	19.37	37.65	46.02
Ethanollic Extract (250 mg/kg)	88±2.40	95±1.73*	100±2.23***	85.16±2.70*	78±3.15*	- 7.95	- 13.63	3.22	11.36
Ethanollic extract (500 mg/kg)	94±2.43	117±2.11	102±1.29***	84.33±1.58*	76±1.46**	- 24.46	- 8.51	10.28	19.14

N = 6 animals. Values are expressed as Mean ± SEM. Values showing * superscript are $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 7: Effect of *P. deltooides* leaves extract on fasting blood glucose levels in alloxan induced diabetic

Treatment Group	FBS (mg/dl)					% Reduction in blood sugar level			
	Days					Days			
	0.0	7	14	21	28	7	14	21	28
Normal control	91.6±1.45	96.5±0.42	98±2.01	95.3±0.66	91.5±1.11	-5.34	-6.98	-4.03	0.10
Diabetic control	396.833±8.79	341.33±46.11	337.33±45.17	303±51.92	301±53.30	13.98	14.99	23.64	24.14
Glipizide 5mg/kg (standard)	400.33±14.15	202.66±6.17**	178.16±4.91***	124.33±6.74** *	94±2.64***	49.37	55.49	68.94	76.51
Ethanollic extract (250 mg/kg)	390.66±21.53	214±4.34**	202.5±3.51**	160±5.59**	130±9.09***	45.22	48.16	59.04	66.72
Ethanollic extract (500 mg/kg)	392.5±16.77	205±3.34**	188±4.32***	138±3.03***	107±10.15***	47.77	52.10	64.84	72.73

rats.

N = 6 animals. Values are expressed as Mean ± SEM. Values showing * superscript are $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 8: Effect of *P. deltooides* leaves extract on biochemical parameters in alloxan induced diabetic rats.

Treatment group	Total cholesterol (mg/dl)	Total triglyceride (mg/dl)	HDL (mg/dl)	Protein (g/dl)	CPK (IU/L)	Creatinine (mg/dl)	BUN (mg/dl)
Normal control	53.00 ±0.57	52.6±1.85	24.76±0.88	5.9±0.10	640.3±20.51	0.233±0.03	52.19±2.1
Diabetic control	70.33 ±8.94	95.66±26.14	61.5±2.33	7±0.40	1335.33±165.89	0.7±0.03	71.5±12.59
Glipizide 5mg/kg (standard)	50.33±5.38	38.83±11.22*	58.33±5.60	6.2±0.29	758.66±23.16*	0.533±0.02	33.33±3.54*
Ethanollic extract (250 mg/kg)	60.33±3.83	74±2.03	31.33±12.65	6.5±0.18	851.66±158.64	0.633±0.02	68.33±6.67
Ethanollic extract (500 mg/kg)	51.33±1.33	39.33±12.69	47.33±1.22	6.3±0.78	819.66±211.30	0.6±0.10	40.33±11.16

N = 6 animals in each group. Values are expressed as Mean ± SEM. Values showing * superscript are $p < 0.01$

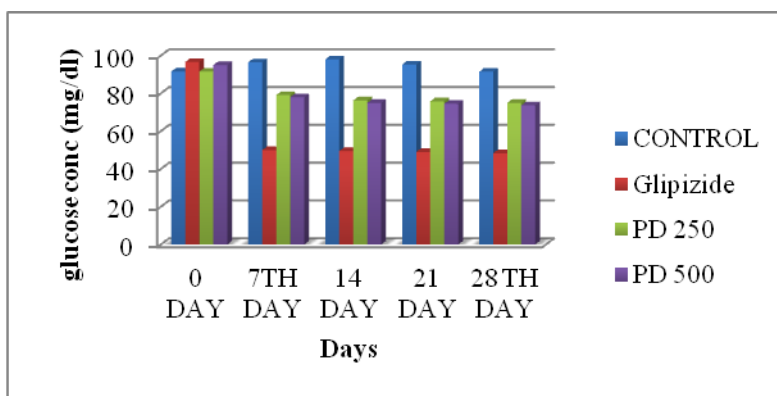


Fig 1(a): Figure showing the hypoglycaemic activity of extract of *P. deltoides* in healthy albino rats (NG)

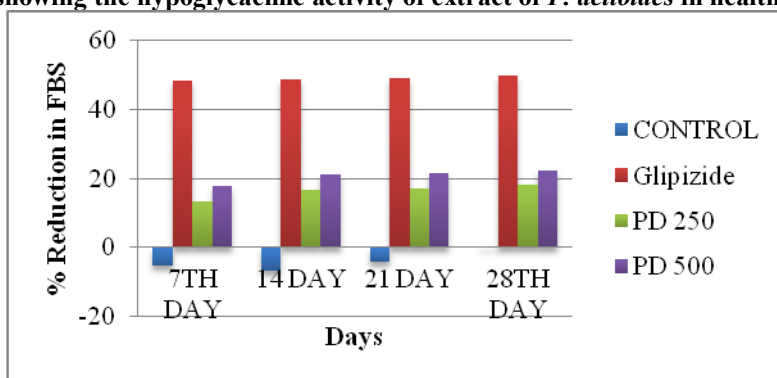


Fig 1(b): Figure showing the hypoglycaemic activity of extract of *P. deltoides* in healthy albino rats (NG)

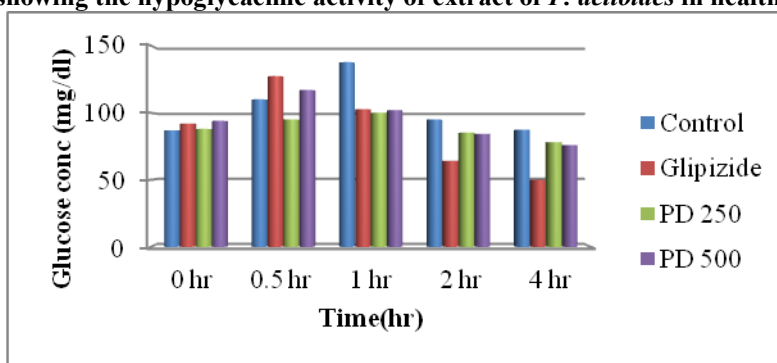


Fig 1(a): Showing the hypoglycaemic activity of extract of *P. deltoides* in healthy albino rats (OGTT)

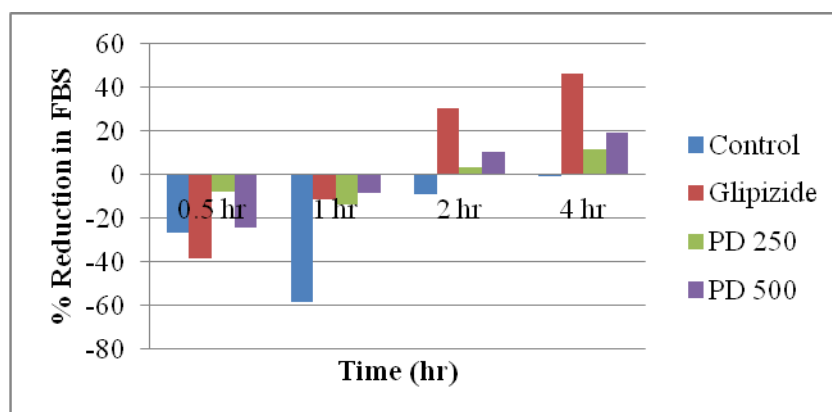


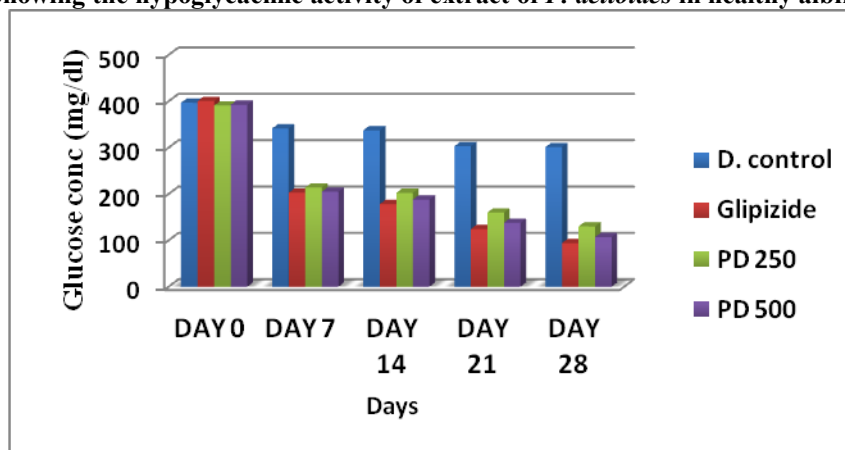
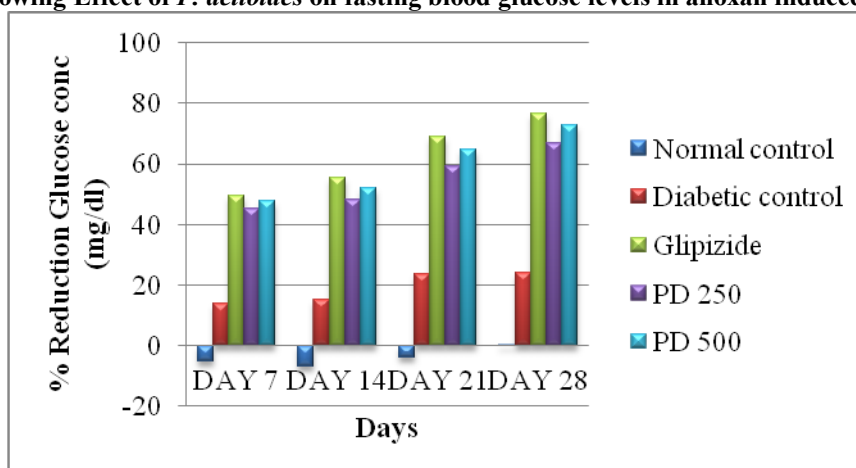
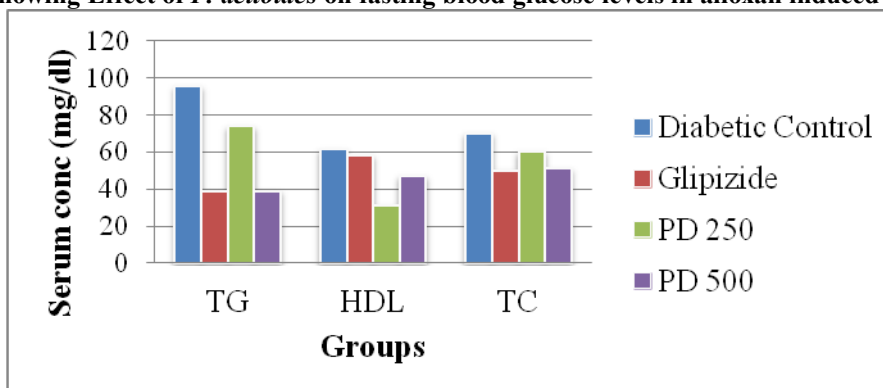
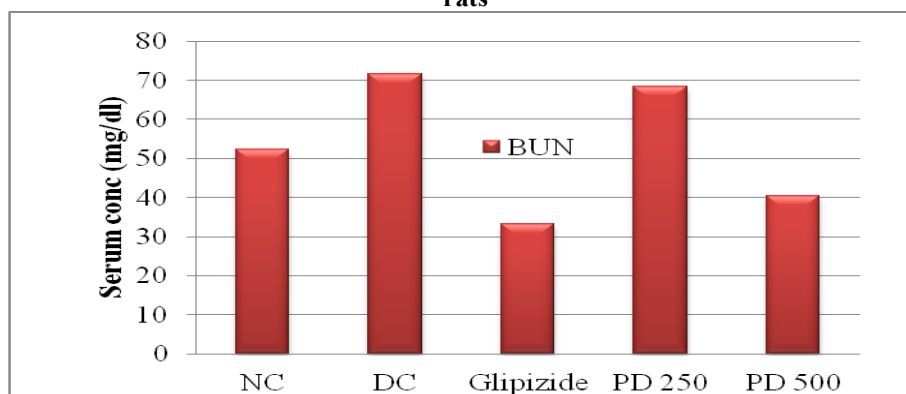
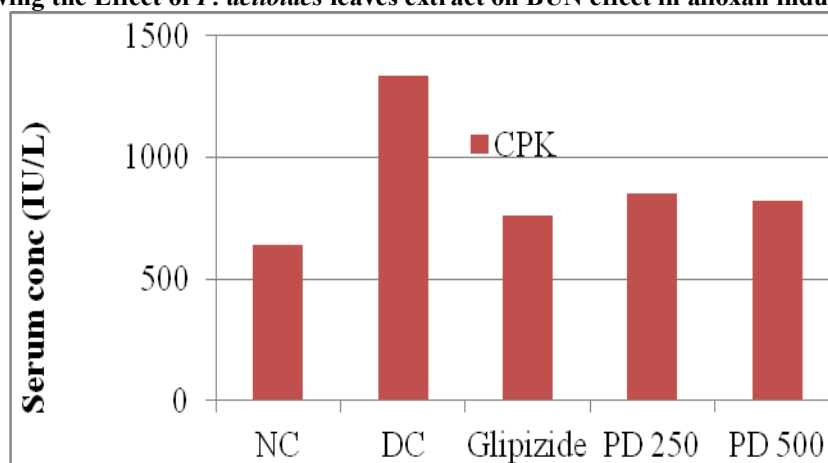
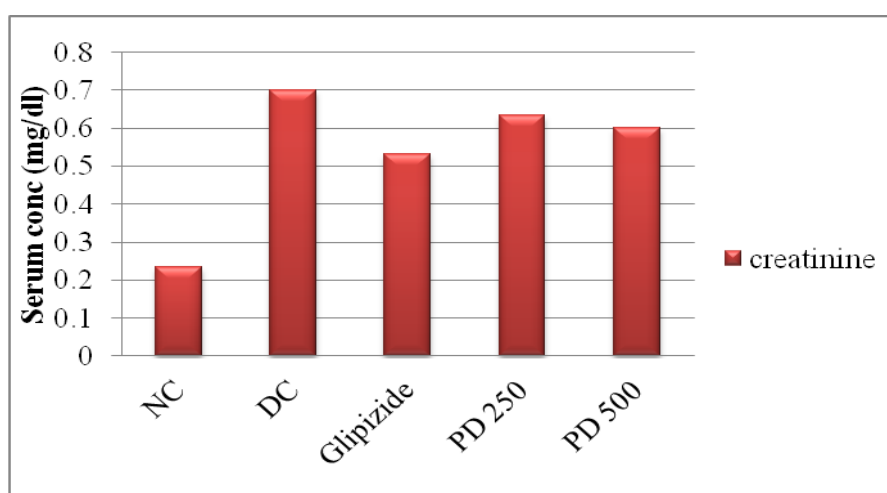
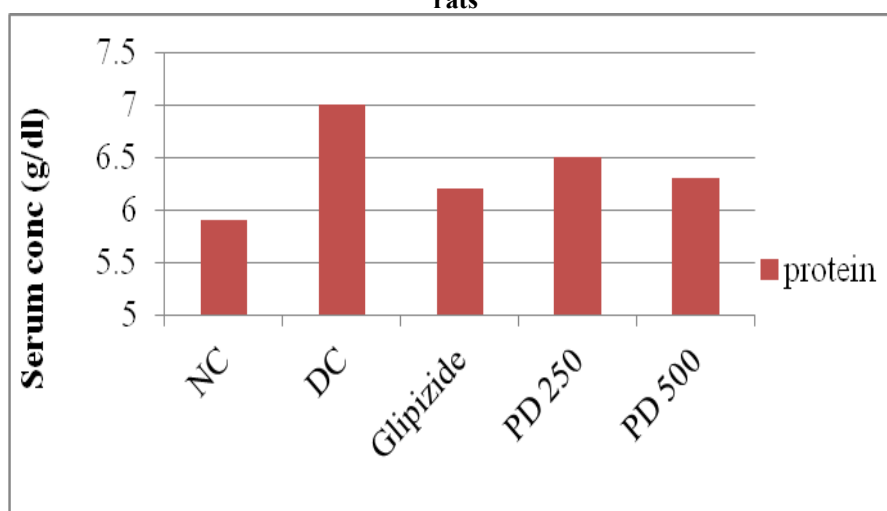
Figure 2(b): Showing the hypoglycaemic activity of extract of *P. deltooides* in healthy albino rats (OGTT)Fig 3(a): Showing Effect of *P. deltooides* on fasting blood glucose levels in alloxan induced diabetic ratsFig 3(b): Showing Effect of *P. deltooides* on fasting blood glucose levels in alloxan induced diabetic ratsFig 4(a): Showing the Effect of *P. deltooides* leaves extract on lipid profile effect in alloxan induced diabetic rats

Fig 4 (b): Showing the Effect of *P. deltoides* leaves extract on BUN effect in alloxan induced diabetic ratsFig 4 (c): Showing the Effect of *P. deltoides* leaves extract on CPK effect in alloxan induced diabetic ratsFig 4 (d): Showing the Effect of *P. deltoides* leaves extract on creatinine effect in alloxan induced diabetic ratsFig 4 (e): Showing the Effect of *P. deltoides* leaves extract on protein effect in alloxan induced diabetic rats

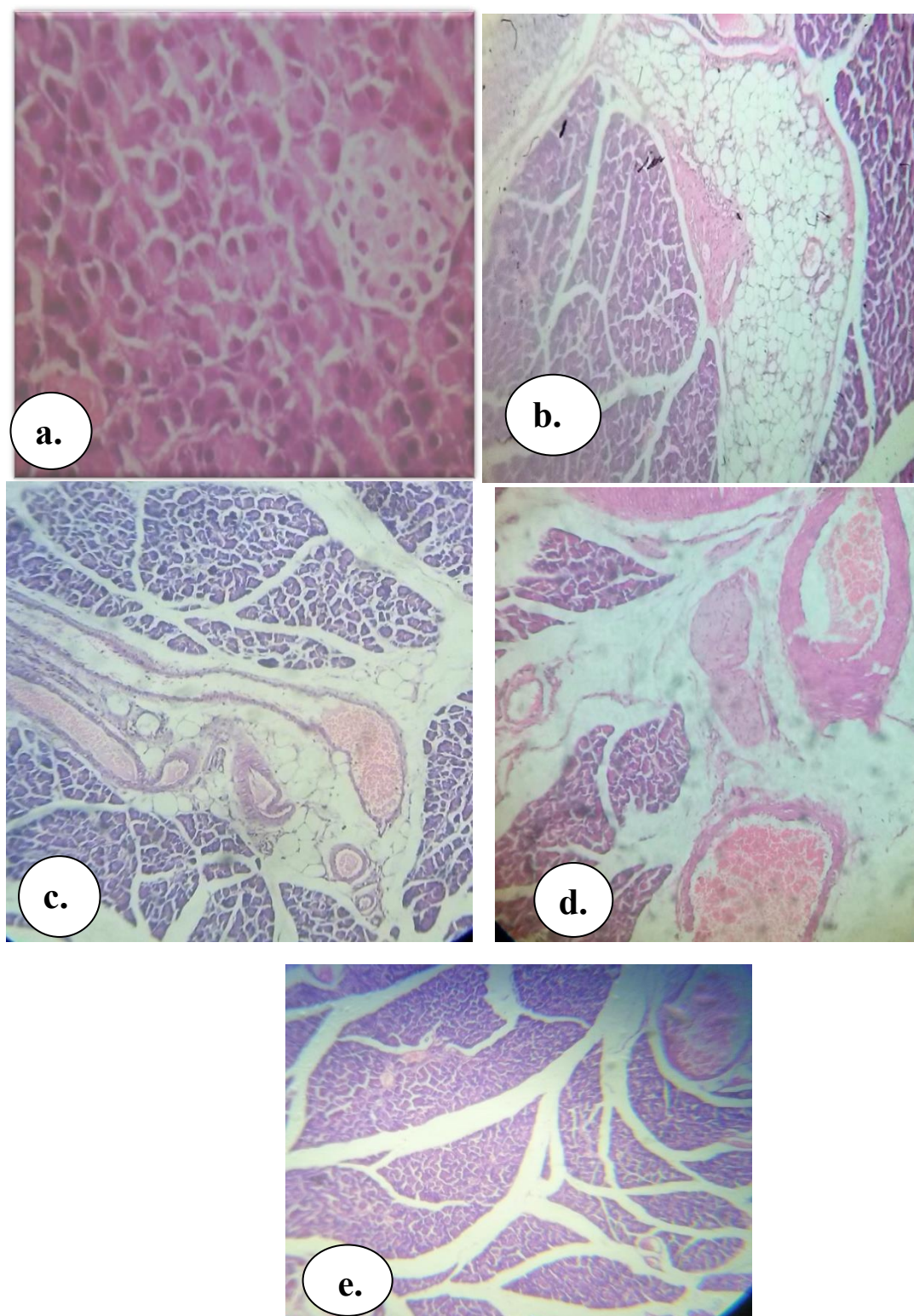


Fig 3: Histopathology of pancreas in alloxan induced diabetic rats. a) normal animal b) diabetic control, c) standard (glipizide treated), d) ethanolic extract 250 mg/k.g treated e) ethanolic extract 500 mg/kg treated

Phytochemical Analysis

The 70% ethanolic extract of *P. deltoides* was subjected to qualitative test to identify the presence of phytoconstituents. The result of phytochemical analysis confirmed the presence of amino acids,

glycosides, flavanoids, protein, alkaloids, phenols, terpenoid, tannin and saponins. The extract did not contain carbohydrates.

Acute toxicity study

The 50 % of animals died at the dose of 5000 mg/kg p.o. therefore 1/10th and 1/20th of this dose are taken as higher and lower dose respectively. The result of acute toxicity study are summarized in **table 4**.

Effect of *P. deltoides* leaves extract on normoglycemic rats (NG).

On observation of data in the **table 5** and **figure 1**, the results for the evaluation of *P. deltoides* extract on normal blood glucose revealed that, the animals in the normal group maintained around 95% mg/dl of blood glucose. The Glipizide 5 mg/kg p.o. reduced the blood glucose level significantly to 50, 49.5, 49 and 48.33 mg/dl on 7th, 14th, 21st and 28th day respectively. The *P. deltoides* ethanolic extract at 250 mg/kg, p.o. reduced the blood glucose level significantly on 7th, 14th, 21st, and 28th day of treatment. The *P. deltoides* ethanolic extract at 500 mg/kg p.o. also reduced the blood glucose significantly on all the tested days. The percentage reduction was at its peak on 28th day. The overall percentage of blood glucose reduction observed by *P. deltoides* ethanolic extract 500 mg/kg p.o was found to be better in comparison with *P. deltoides* ethanolic extract 250 mg/kg, p.o.

Effect of *P. deltoides* leaves extract on blood glucose level of glucose overloaded healthy albino rats.

The results of OGTT are summarized in **table 6** and **figure 2**. The standard drug glipizide 5 mg/kg, p.o. reduced blood glucose level 30.43 % at 2nd hr and 46.02 % at 4th hr. The ethanolic extract of *P. deltoides* 250 mg/kg, p.o. also reduced the blood glucose from 0.5th to 4th hour of treatment the highest being 11.36 % at 4th hour. The ethanolic extract of *P. deltoides* at 500 mg/kg reduced the blood glucose from 0.5th hour to 4th hour and 19.14 % was highest reduction observed at 4th hour.

Effect of *P. deltoides* leaves extracts on alloxan Induced Diabetic rats.**a) Blood sugar level**

The *P. deltoides* ethanolic extract at 500 mg/kg exhibited significant reduction of blood glucose level from 7th day to 28th day of the treatment 72.73 % of reduction was found to be the highest and was shown on 28th day with 500 mg/kg of ethanolic extract. The ethanolic extract at the dose of 250 mg/kg also significantly reduced the blood glucose level in diabetic rats. The percentage of reduction was highest at 66.72 % on 28th day. As compared to 500 mg/kg of ethanolic extract 250 mg/kg of ethanolic extract of *P. deltoides* showed nearly 6% less. And the results are presented in **table 7** and **figure 3**.

b) Biochemical parameters in alloxan induced diabetic rat.

The results of *P. deltoides* ethanolic extract on biochemical parameters in alloxan induced diabetic rats are presented in **Table 8** and **Figure 4**.

The alloxan induced diabetic rats showed a hypercholesterolemia, hypertriglyceridemia and increase in levels of HDL, protein, CPK, creatinine and BUN levels when it is compared with the normal control rats to 70.33 mg/dl, 95.66 mg/dl, 61.5 mg/dl, 7 g/dl, 1335.33 IU/L, 0.7 mg/dl and 71.5 mg/dl.

Treatment with *P. deltoides* ethanolic extract at the dose 500 mg/kg p.o. showed decrease in total cholesterol, triglyceride, HDL, protein, CPK, creatinine and BUN to 51.33 mg/dl, 39.33 mg/dl, 47.33 mg/dl, 6.3 g/dl, 819.66 IU/L, 0.6 mg/dl and 40.33 mg/dl levels respectively which showed the data closer to the normal readings. *P. deltoides* ethanolic extract at the dose of 250 mg/kg p.o. also reduced the levels of same biomarkers to 60.33 mg/dl, 74 mg/dl, 31.33 mg/dl, 6.5 g/dl, 851.66 IU/L, 0.633 mg/dl and 68.33 mg/dl as compared to diabetic control.

c) Histopathological investigation

The pancreas of albino rats of control group showed normal histology and appearance of Islet of Langerhans containing α , β and δ cells. The β -cells are the most abundant cells (**Figure 5 a**).

Pancreas of diabetic rats showed reduction in number of β cell and necrosis along with few surviving β -cells. Severe infiltration of inflammatory cells was also observed. It showed marked degeneration of the Islet of Langerhans and it also shows the fat deposition (**Figure 5 b**).

In the reference group, i.e., diabetic rats treated with glipizide, pancreas architecture was similar to that observed in control rat and it also showed slight regeneration of the beta cell, less damage to beta cells as compared with the diabetic rat (**Figure 5 c**).

The pancreas of the rats treated with the ethanolic extract of *P. deltoides* at the dose of 250 mg/kg p.o. showed reduction in the extent of necrosis, inflammation, increased in the number of islet cells of pancreas and less deposition of the fatty material as compared with the diabetic control (**Figure 5 d**).

Histopathological study of pancreas of diabetic rats treated with alcoholic extract of *P. deltoides* at the dose of 500 mg/kg p.o. body weight showed a significant improvement in number of β -cells with the diabetic rats. It was observed that it exhibited less damage to beta cells, improved beta cell regeneration and shows slight necrosis as compared to diabetic rat (**Figure 5 e**).

DISCUSSION:

The ethanolic leaves extract of *P. deltoides* were evaluated for its hypoglycaemic effect in healthy rats and alloxan induced diabetes in albino rats. The *P. deltoides* ethanolic extract significantly reduced the blood glucose in normal healthy rats.

The ethanolic extract of *P. deltooides* also reduced the blood glucose in glucose overloaded in healthy albino rats. This effect may be due to reduction of glucose absorption or due to increased insulin secretion. The effect on blood glucose was evaluated on alloxan induced diabetic rats, to record its action in pathophysiological condition. The ethanolic leaves extract of *P. deltooides* at 500 mg/kg dose significantly reduced blood glucose levels in diabetic rats. The extract at the dose of 250 and 500 mg/kg reduced 66.72 and 72.73 % of blood glucose respectively on 28th day of treatment. The results of histopathological studies revealed that the *P. deltooides* 250 mg/kg and *P. deltooides* 500 mg/kg have increased the number of β -cells and also decrease necrosis and inflammation in the pancreas. The antidiabetic activity of ethanolic leaves extract of *P. deltooides* may be due to reduction of necrosis and regeneration of β -cells leading to increase insulin release. Flavanoids might cause regeneration of β -cells¹⁴. The antioxidant property of phenolic glycosides found in *P. deltooides* has already reported. Antioxidant property of *Populus species* may also play a role in its antidiabetic property¹⁵. The lipid profile, BUN, creatinine, CPK and protein were also reduced but statistically not significant compared to standard glipizide. Antidiabetic activity of *P. deltooides* might be due to the presence of glycosides, flavanoids but exact molecular mechanism by which it produced the hypoglycemic activity could not be drawn from the present study. Further study has to be conducted to know the molecular mechanism of the extract.

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