EVALUATION OF ANTI-DIABETIC AND ANTI-HYPERLIPIDEMIC ACTIVITY OF PRUNUSDULCIS SEED EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RAT

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ABSTRACT
Amygdaline is naturally occurring chemical compound, it also known as laetrile or vitamin B17 Best known for being falsely promoted as a cancer cure. Amygdaline is a bitter substance and most found in the seed of apples, apricots, peaches, bitter almond and plums. In Indian cultural system of medicine the herbal remedies are prescribed for the treatment of various diseases including diabetes mellitus. In recent year plant are being effectively tried in a variety of pathophysiological state, Bitter Almond is one of them. PDWA extract is prepared by sox halation extraction process with 50% of ethanol and characterized by High Performance Thin Layer Chromatography and Infra Red Spectroscopy. Non insulin dependent diabetes mellitus was induced in Wistar albino rats by intraperitoneal administration of streptozotocin (60mg/kg). At the end of experiment period of 21 days reduction in the fasting blood glucose level, serum insulin, serum lipid parameter and renal function biomarker were estimated in the controlled and treated rats. Histopathological examination of liver, kidney, and pancreas were also carried out.PDWA extract were given (250mg/kg and 500mg/kg) orally for the duration of 21 days as per protocol of treatment. Blood glucose level and various biochemical parameters were measured by glucometer and respective diagnostic kit. Such as cholesterol, triglyceride, LDL, HDL, VLDL and antioxidant parameter by using diagnostic kitsOn administration of PDWA seed extract, studied blood glucose level of animal showed a significant decrease ($P<0.001$) in elevated blood glucose level along with biochemical parameter it shows significant to antihyperlipidemic activity and Antioxidant effects. The result showed promising effect of PDWA seed extract treatment as compared to treatment with the standard drug Metformin for Antidiabetic.

Keywords: Antidiabetic, Antihyperlipidemic, Antioxidant, Amygdaline, PDWA.

1. INTRODUCTION

The term diabetes mellitus is impairment to structural and functional proteins due to which it fails to maintain blood glucose homoeostasis .Many research work and evidence state that oxidative stress play a pivotal role as carrier of Diabetes mellitus and its related complications, also it effective for both insulin action and insulin secretion[1]. After cardiovascular and cancer the diabetes is third most life threaten disorder to mankind. Therefore it's necessary to go through various dependent complications in patient with type 2 diabetes.

Nowadays in the field of herbal treatment and nutraceuticals plants play a very effective role for treatment of hyperglycemic activity till now about 800 plants are found to be helpful in diabetes mellitus[2]. Among this Bitter Almond is found to be miraculous to hyperglycemic activity and may possess potential to antidiabetic the presence of Amygdaline or vitamin B17 as a main constituent is a reason for supporting this plant as boom for antidiabetic activity. Bitter almond contains cynogenic glycoside.[3], [4] There are many fruit which contain cynogenic glycoside and on chewing, digestion there is release of hydrogen cyanide[5].

In some families of plant mostly cynogenic glycoside are found, such as Rosaceae, fabaceae, leguminosae, linaceac, and compositae and also it is mostly useful tool for informative taxonomic markers. There are about 25 known cynogenic glycoside which are mainly found in various edible part of plant such as Almond, plums, cherries, bamboo shoot, chick peas, cashew, cassava etc. Amygdaline is naturally occurrence chemical compound. Mainly it present in plant such as bitter almond, apricot, plum, peaches, black cherry and Nanking cherry[6], [7].

It classified as cynogenic glycoside. It include nitrile group by the action of a beta glycosidase it can release pestilential cyanide anion[8]. Amygdalus plant belonging to family Roseaceae. It contain flavonoids, phenols, antioxidant properties, glycosides and many more phytochemical, vitamins, minerals, unsaturated fatty acid, fiber which help to reduce the risk of chronic disease[9], [10]. A study by much researcher state that it is milestone helpful in many disease and disorder.It posesas memory enhancer and promotes change in dementia, provide antioxidant properties to reduce oxidative stress and elevating free radical formation[11], [12], antiatherogenic, inhibition of renal

interstitial fibrosis, lung injury, antitumor, antinflammatory, and antiulcer, to reduced glutathione (GSH), total protein and malondialdehyde (MDA) level of liver and kidney tissue[13], [14].

Amygdalus may act as a potential drug to treat type 2 diabetes and its complications. The study shows that amygdalus has anti-hyperglycemic action, the extract of plant additional put through to bioactive guided discovery of drug to isolate the lead molecule and work for respective activity and to explore the likely mechanism of action at cellular and sub cellular levels.[15], [16]

2. MATERIAL AND METHOD

2.1 Collection of plant

The seed of plant is collected from the local market of Prayagraj, (U.P).India in the month of July, august and it was authenticated from botanical survey of India, Prayagraj by taxonomist of their institute (Botanical survey of India with the voucher accession No – 104271)

2.2 Extract preparation

The collected seed are peeled off then with the help of grinder we make a fine powder then about 200gm of powder was subjected for defatting with the help of petroleum ether, and then we filtered it with the help of muslin cloth and then filtered material is dried at 50 for 10 minute in hot air oven for removing the moisture from it and make it dry. Now we take defatted powder and fit it into soxhlet apparatus for further extraction process by using 40%of methanol +20%of ethanol+40% water and the resulting obtained solution was placed on water bath at 45ºC temperature. After obtain extract we collect it in air tight container.

2.3 Chemical used.

Streptozotocin (STZ) Mol.wt 265.22 min assay 98%purchased from central drug House pvt ltd New Delhi. Standard drug metformin was obtained from HCL,KOH,Fecl,iodine,nitric acid, H_2SO_4, Fehling solution A and B, Potassium paramagnet,n-Hexane, formic acid,NaOH,CuSO4 obtained from geetraj corporation and chemical laboratory, acetic acid, ethyl acetate,picricacid,chloroform,potassiummercuriciodide ,potassiumdihydrogen,orthophosphate obtained from united institute of pharmacy NainiPrayagraj. Biuret reagent obtained from central drug house (CDH) Delhi, alpha-naphtol, Millons reagent, acetone, formaldehyde, obtained from Fizmerk India chemicals

2.4 Persistence of PDWA by HPTLC

About 2gm of PDWA was mix in 25ml of CH_3OH and use for the estimation of HPTLC. The sample plate form in Ethyl acetate: formic acid: Glacial acetic acid: water (100:11:11:10). After that the practical was done under colorimetric beam and derived with sample developed plate under UV radiation at 254 nm the figure are estimated by the help of visible light. At the various wave length at 254 nm the plate was scanned, the R_f and the R.P value are recorded in every peak of area of drug extract mentioned and further calculate

2.5 Characterization of PDWA by IR

The compatibility research work on PDWA plant extract mainly utilized to formulate for Anti-hyperglycemic activity which was intent by IR spectroscopy. By the help of spectra the analyses of plant extract which contain amygdaline was investigated through IR and along with this investigation the various other chemical compounds are also analyzed by the help of IR which present in plant extract. The study of IR was compiled by **Perkin Eirner Spectrum IR ES** and the frequency range drawn from **2000 -400 cm^{-1}**

2.6 *In – vitro* Activity

Hydrogen peroxide scavenging (H_2O_2) assay:

Mainly environment is responsible for the indirectly exposure of H_2O_2 to the human body. Just about 0.32mg/kg/day mostly taken from leafs and crops. It also takes up in body of human by help of inhalation of mist, vapour and also by coming in contact through skin and eye. In oxygen and water it is readily decomposed hydrogen peroxide and formed hydroxyl radicals (OH-) and it commence the lipid per oxidation which genesis to damage the DNA of body. Scavenge hydrogen peroxide capacity to extract of plant was approximate or evaluate by the H_2O_2 Solution (40mM) which put together in phosphate buffer (50mM maintain PH 7.4). Hydrogen peroxide concentration was arbitrated by using 230nm absorbance by spectrophotometer. About (20-60µg/ml) of plant extract taken with distilled water and sum up to hydrogen peroxide then at 230nm the absorbance after 10 minute was done or recorded in opposition to blank sample which contain phosphate buffer and there is absence of H_2O_2 in that. The calculation for hydrogen scavenging of percentage is done as follow.

$$\% \text{ scavenged } (H_2O_2) = [(A_i - A_t)/A_i] \times 100$$

Which represent A_i as the absorbance control and A_t as test absorbance reported by **(Alam et al; 2013).**

2.7 Pharmacological Studies

2.7.1 Experimentalanimals

Young Wistar albino rats (male) weight 140 -200gm received from central drug research institute animal house Lucknow. Cage of polypropylene is used for animal to keep in. Where the temperature of about 25±2ºC and 12 hr dark light are to be maintained the animal are kept on*ad*libitium during or before the experiment. An animal was kept in quarantine area for at least 2 week for awareing to animal house condition before the experiment. I.A.E.C has approved according the protocol of experiment (Reg.No. UIP/IAEC/Nov – 2019/01)

2.7.2 Induction of diabetes

The freshly prepared soln., of STZ (60mg/kg body weight) which dissolved in citrate buffer amount 0.01M whose pH should be maintain4.5 and given by I.P. streptozotocin administered to Wistar albino rat or experimental animal tested after 48 hrs they contain large glycosuria and hyperglycemia. FBG with BG.L>200 mg/dL measured with diabetes and further continue with our research work. All animal were allowed to access tap water and pellet diet and maintained at room temperature in polypropylene cages.

2.7.3 Experimental design

Five groupof Wistar albino rat were involve in this research with the outcome of 50% ethanolic extract of *prunusdulcis*varamara. Each group contains six experimental animals.

Following are the activity which involved by group.

Group 1: positive control group, which received saline solution as a vehicle

Group 2: negative control group which received saline solution use as a vehicle

Group 3: chemically induced Diabetic rat, which were given with standard drug, Metformin at 100mg/kg as per body weight.

Group 4: chemically induced diabetic rat, which were given with PDWA 250mg/kg body wt.

Group 5: chemically induced diabetic rat, which was given with PDWA 500mg/kg body wt.

The drug and vehicles were administered orally by the help of oral gavages tube for the periods of about three weeks. By the help of retro orbital puncture the blood of experimental animal were taken on 0^{th} day, 7^{th} day, 14^{th} day and 21^{st} day to measure blood glucose level by Dr. Morepen BG-03 glucometer. The various biochemical parameters such as Triglyceride, Total cholesterol, HDL, LDL, VLDL were estimated by the help of diagnostic kits.

2.7.4 Histopathological studies

All the sample of tissue were obtained from the similar portion at the right side of Kidney, liver, pancreas which were placed in 10% buffered formalin and further embedded in a paraffin wax as per protocols and by the help of microtome the section were cut into 5µm thick, staining is done with the help of hemotoxylin as per protocol and examined by the help of Bx50 Olympus at 250x photomicroscope by the pathological method and examination were recorded with the help of light microscopy. (**Mishra *et* al., 2011)**

2.7.5 Analysis of statistical data

The evaluation of statistical data was performing by the one way ANOVA in which Newman keuls method was used. This analysis was done by G.P.P of version 8.4.3 window software.

3. RESULT:

3.1 Determination of PDWA by TLC

The different constituent present in sample for more efficiency are the variety of mobile phase of different composition is used, the solvent system included are chloroform : methanol : water : (5 : 1 : 15 :), butanone : acetone : water : (15 : 5 : 3), n-butanol : ethanol : water : (40 : 11 : 14), n- butanol : acetic acid : formic acid : (12 : 3 : 5)and spots were identified under daylight, then it observed under shorter wave length and longer wave length of UV and it determined different spot with the help of n-butanol: acetic acid: formic acid (12:3:5) mobile phase.

Table-1: TLC analysis of ethanolic extract of PDWA showing different R_f value:

S. No	Solvent system	R_f
1.	Chloroform + methanol + water	0.25
2.	Butanone + acetone + water	0.38
3.	n-butanol + ethanol + water	0.43
4.	n-butanol + acetic acid +water	0.65

3.2 HPTLC ANALYSIS

Mentioned result in table 2 shows the no. of peaks, maximum % R_f value, maximum Rf value, area and total% of area mentioned. PDWA shows 09 peaks in 100-1000 nm range (spectra). In PDWA the maximum % area 38.25% was surround by peak NO. 6 (R_f value 0.57). This procedure maydefine for very well and good qualitative analysis and determination of the formulation containing PDWA plant seed extract .

Table-2: HPTLC analysis of ethanolic extract of (Prunusdulcis (MILL.) D.A. Webb var.amara (DC.)

Peak	Max. R_f	Max%
1.	0.08	2.83
2.	0.14	2.12
3.	0.22	2.17
4.	0.27	2.51
5.	0.38	17.37
6.	0.57	38.25
7.	0.66	27.92
8.	0.80	3.18
9.	0.83	3.64

HPTLC of PDWA extract gives a number of peaks with density at 354 nm. Most prominent R_f value are **0.08, 0.14, 0.22, 0.27, 0.38, 0.57, 0.66, 0.80, 0.83.**

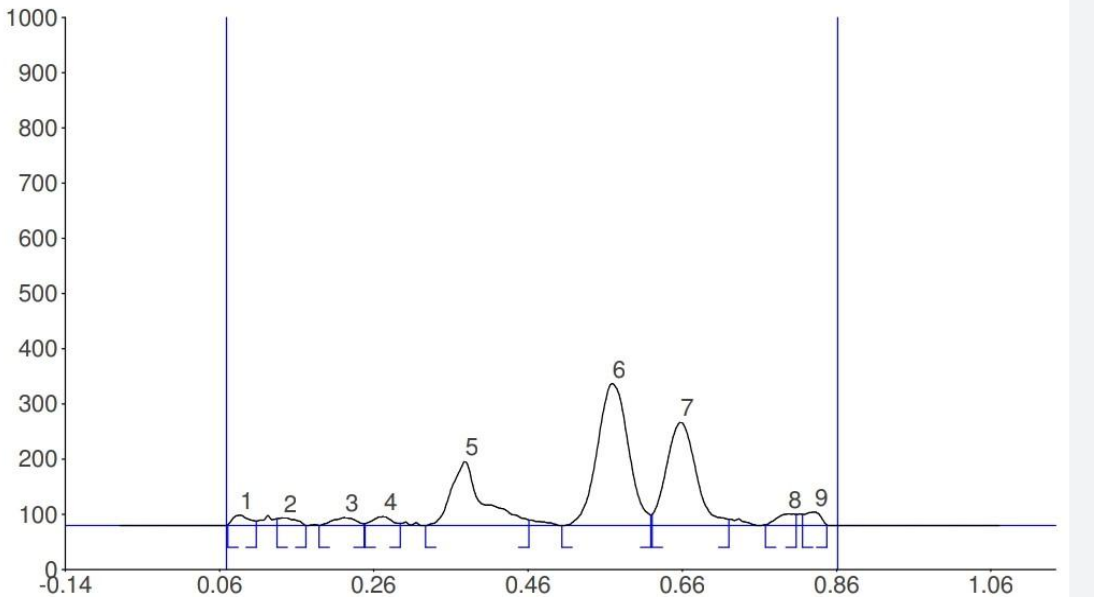

Figure-1: HPTLC spectra of ethanolic extract of PDWA.

3.3 INFRA RED SPECTROSCOPY STUDIES:

IR spectroscopy was executed for the compatibility study of PDWA plant extract. Which contain amygdaline as a molecule and plant extract has various chemical compound which shows its own peak to represent, in fig 2 Plant extract has specific peak at 1710cm^{-1} C=C (Aromatic) stretch vibration from the benzene ring, 1185cm^{-1} shows C-O bond stretching from ether and hydroxyl group, peak at 750cm^{-1} shows intense absorption bond and C-H bending vibration allocate to plane, where peak at 1510cm^{-1} state cyclohexane group and the raised peak to 2000cm^{-1} shows the possibilities of CN (cyanide) group in stunted anticipate intensity.

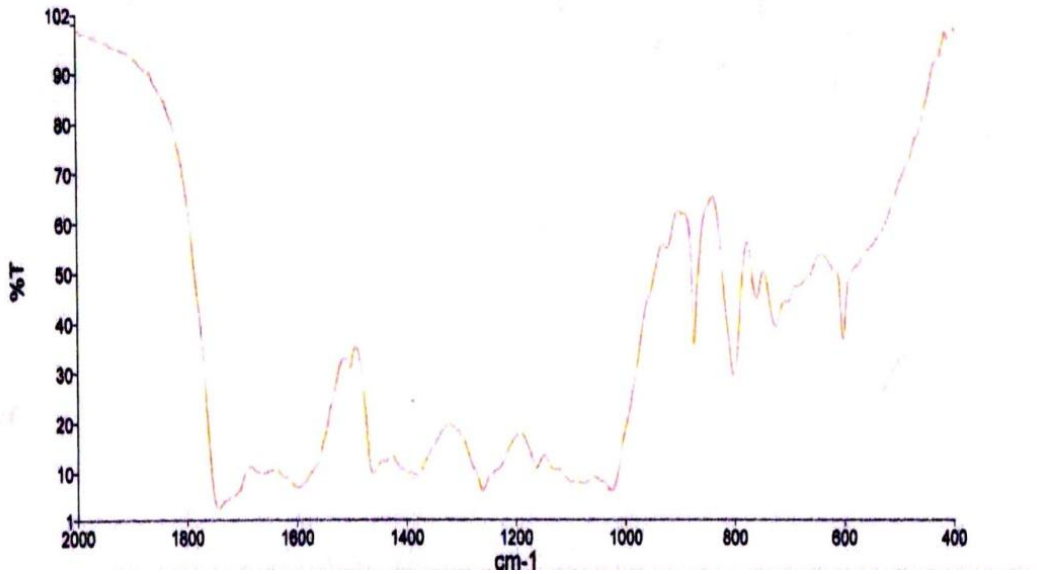

Fig-2: IR spectra of PDWA plant extract

3.4 ANTI-OXIDANT ACTIVITY:

Estimating antioxidant activity, hydrogen peroxide scavenging (H_2O_2) assay examined in below mention table: 3showsFree radical scavenging activity of ethanolic extract of PDWA seed extract. It shows inhibition of hydrogen peroxide scavenging assay by PDWA as a good dose dependent action. As compared to standard BHT the ethanolic extract shows good scavenging action or ability, as resultant the ethanolic extract and BHT at different concentration shows different percentage of inhibition such as PDWA at concentration of (25µg/ml-56.14), (50µg/ml-63.85), (100µg/ml-68.49) and BHT at concentration of (25µg/ml-70.25), (50µg/ml-72.65), and (100µg/ml-74.32) of % inhibition.

Table-3: Free radical scavenging activity of ethanolic extract of PDWA seed extract.

Drug	Concentration (µg/ml)	Hydrogen peroxide scavenging (H_2O_2) assay (% inhibition)
PDWA	25	56.14
PDWA	50	63.85
PDWA	100	68.49
BHT	25	70.25
BHT	50	72.65
BHT	100	74.32

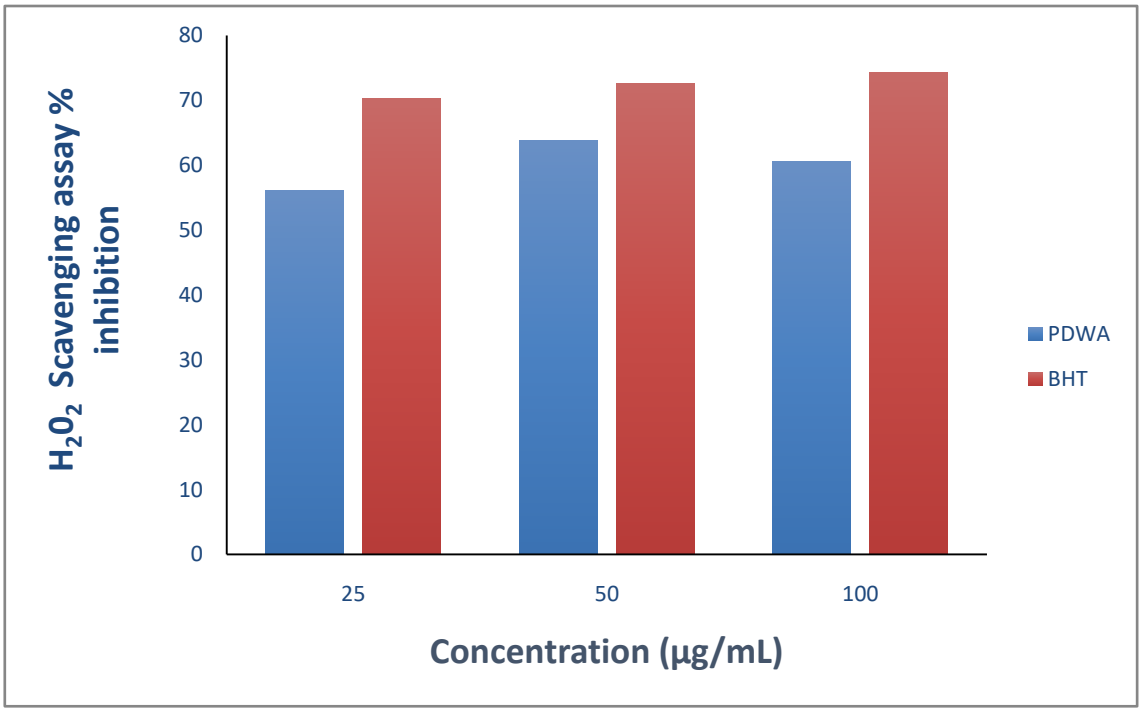

Fig-3: Graph represents scavenging of hydrogen peroxide by PDWA and BHT

3.5 Anti- hyperglycemic activity:

STZ induced diabetic rats were treated orally with crude extract at a dose of 250mg/kg and 500mg/kg for the period of 21 days. After completion of experimental duration it shows reduction in blood glucose level in comparison to diabetic control rats. Whereas the oral administration of crude extract at a dose of 250mg/kg and 500mg/kg cause to leads dose dependent fall in blood glucose are recorded as 222.67±13.59mg/dL – 127.33±6.49mg/dL and 222.67±13.59mg/dL – 110.6±7.62mg/dL respectively. Whereas the diabetic rat treated with Metformin use as standard drug at a dose of 100mg/kg the result recorded is 222.67mg/dL – 105.66±6.01mg/dL

Table-4: Effect of ethanolic extract of PDWA plant seed extract on BGL in STZ – induced experimental animals (Mean±SD).

Treatment (dose)	'0' day (mg/dl)	'7th' day (mg/dl)	'14th' day (mg/dl)	'21th day' (mg/dl)
Normal Control	99.33±6.11	97.83±2.58	97.66±3.32	96.16±5.02
Diabetic Control	249.67±33.70^{z}	261.33±23.5^{z}	230.67±8.87^{z}	222.67±13.59^{z}
Metformin 100mg/kg	242.5±7.86	198.33±2.42***	163±7.44***	105.66±6.01***
PDWA 250mg/kg	244±11.01	217.6±11.06***	176.66±5.99***	127.33±6.49***
PDWA 500mg/kg	252.2±6.14	199.67±10.37***	166.33±7.72***	110.6±7.62***

The inputs or data may be allow to interpreted the standard deviation for experimental 6 animals in per group .^{z}p<0.001 as evaluate normal; ***p<0.001 as evaluate to diabetic control.

3.6 Effect on Biochemical parameters:

The present study shows the levels of various biochemical variables such as Triglyceride, Total cholesterol, HDL, LDL, VLDL in respective groups. By duration of 21 days treatment with crude extract of PDWA it shows the reduction in Triglyceride and Total cholesterols variables by treating there is significantly improvement in HDL level and there are significant changes recorded with LDL and VLDL it improved by treating with PDWA crude aqueous extract asTable: 5 represent compared to diabetic control group. The levels of biochemical parameters such as Triglyceride, Total cholesterol, HDL, LDL, VLDL are recorded in respective group 1 are 73.33±7.16mg/dL, 143.66±10.29mg/dL, 47.5±4.80mg/dL, 36.5±4.71, 19.33±5.54mg/dL. whereas in diabetic control group the parameters are recorded respectively Such as Triglyceride (73.33±7.16 – 189.66±16.95), Total cholesterol (143.66±10.29 – 227.5±15.04), HDL (47.5±4.80 - 29±5.85), LDL (36.5±4.71 – 87.33±3.61), VLDL (19.33±5.54 – 31.83±3.35) where (P<0.001) the result recorded during study state that the PDWA crude extract consist significant Antihyperglycaemic activity which help to control and maintain the lipid level in experimental animal

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Table-5: The effect of orally administered of ethanolic extract of PDWA of (250 and 500mg/kg of body wt.) (Mean ± SD)

Treatment (dose)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Normal Control	73.33±7.16	143.66±10.29	47.5±4.80	36.5±4.71	19.33±5.54
Diabetic Control	189.66±16.95^{z}	227.5±15.04^{z}	29±5.85	87.33±3.61^{z}	31.83±3.35^{z}
Metformin (100mg/kg)	132.5±3.95	130.33±12.09***	46.83±5.84	45.5±3.65***	21.0±5.45***

PDWA (250mg/kg)	162.5±3.24	149.66±4.49***	42.33±2.18	70.16±6.55***	26.0±2.87***
PDWA (500mg/kg)	143.83±4.54	129.33±13.33***	47.5±2.91	57.83±4.25***	24.66±2.60***

The data can also represent the mean ± SD for 6 experimental animals in each group.
$^{z}p<0.001$ to indicate as normal, $^{***}p<0.001$ to estimate as Diabetic control.

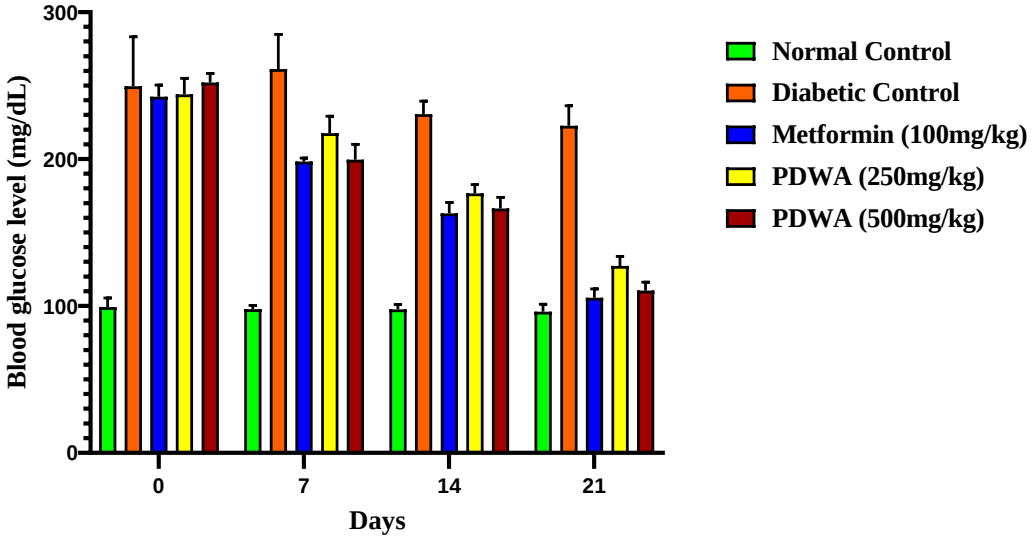

Fig: 4 Graph representing BGL in experimental animal 21 days by inducing streptozotocin

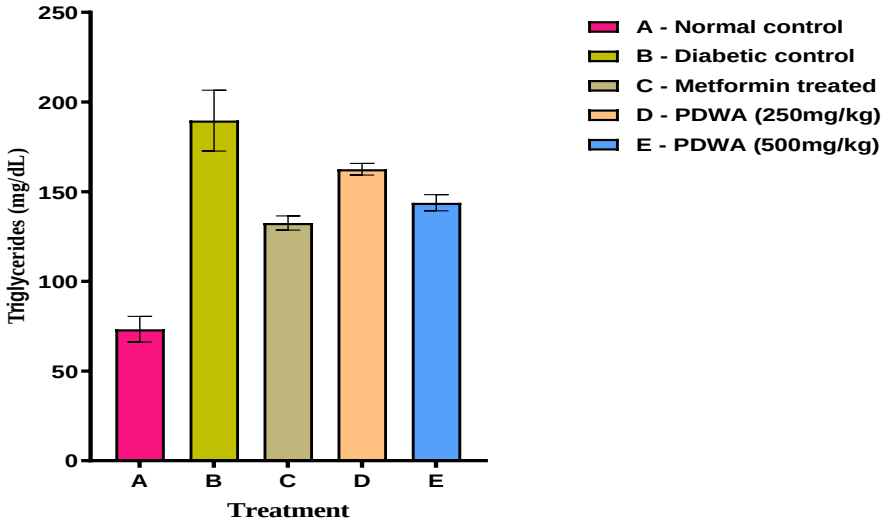

Fig: 5 Graph represents TG level in streptozotocin induced in experimental animal.

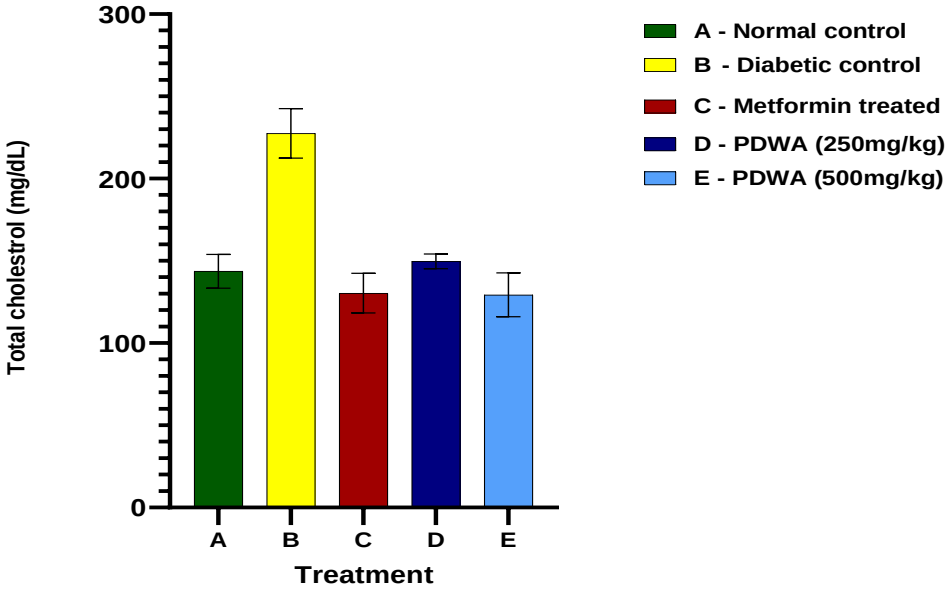

Fig: 6 Graph represents TC level in experimental animal by inducing streptozotocin.

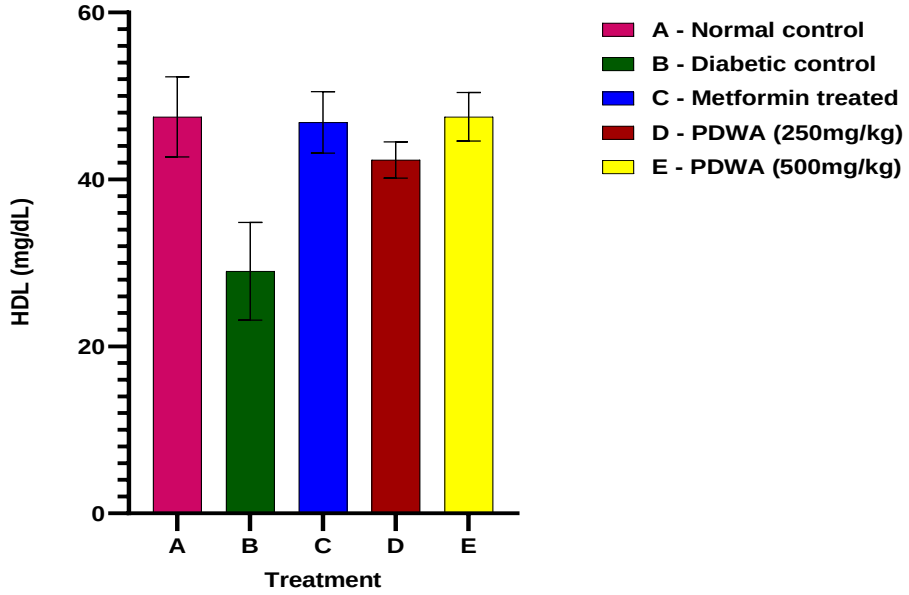

Fig: 7 Graph representing HDL level in experimental animal by inducing Streptozotocin

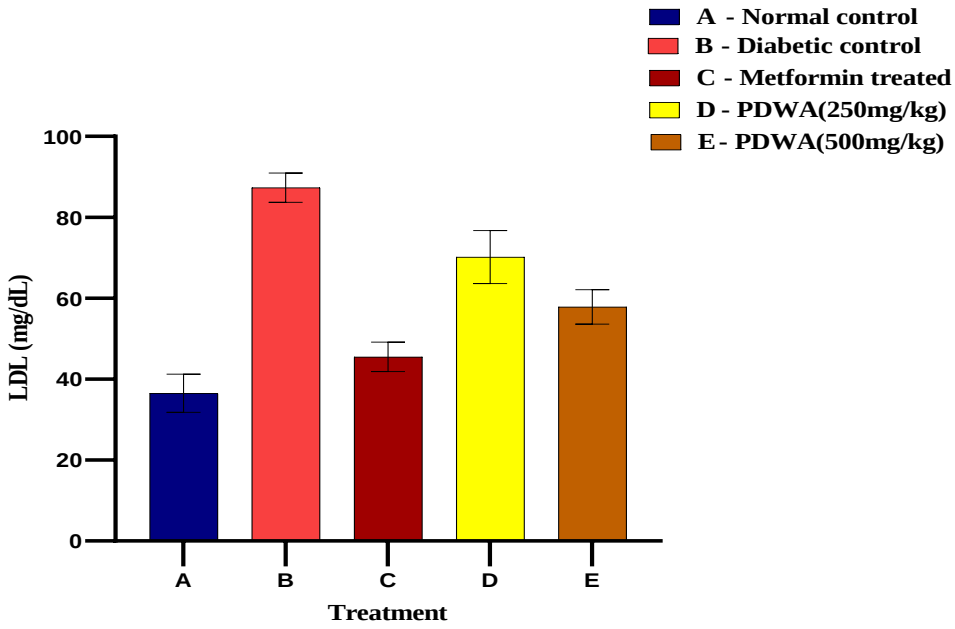

Fig: 8 Graph representing LDL level in experimental animal by inducing streptozotocin

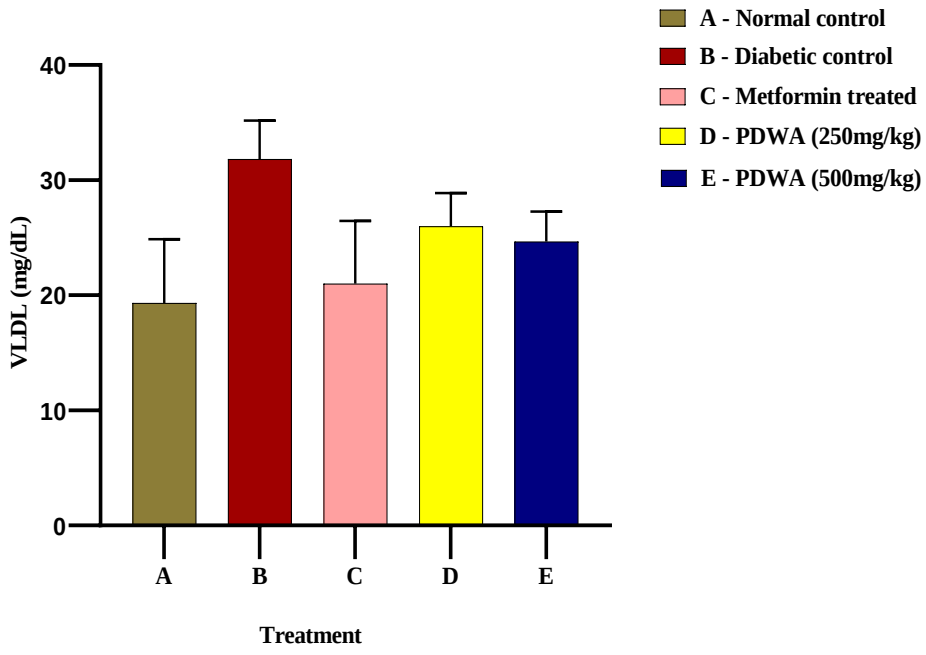

Fig: 9 Graph representing VLDL level in experimental animal by inducing Streptozotocin

3.7 Histopathology estimation of Kidney, Liver & Pancreas

3.7.1 Kidney:

In fig 10 group 1 state as control group tubules stoma and glomerulli shows normal appearance.There is increment in glomerullimessengial cellularity. In negative control group, the report shows focal lymphoplasmacytic infiltrate and in tubule there is single layer of cuboidal cell appeared and observed. Group 3 known as standard drug group there is no significant sign are observed. Group 4 detailed and minutely study of kidney section state inapplicable change in matrix and messengial cellularity growth.

3.7.2 Liver:

In fig 11 group 1 the histopathological inspection in the section of liver, state normal view of hepatocyte portal trials and central veins are set in expected pattern around to oval nuclei with moderate cytoplasm which appeared in hepatocyte. Group 2 in section of liver shows demolition in structure of liver. Portal trial, central veins indicate sympathetic periportal inflammation along lymphocytes; steatosis and perivenular necrosis seems to look. Group 3 it shows normal glomerulli structure with mild disarray and cause tenderness at portal trial site. Group 4 sections microscopically there are hepatocyte cytoplasmic moderate change seen. The central vein portals trials are seen with few normal arrange structure. Group 5 of liver section minutely there is low change in liver fat with gentle inflammation. There is no appearance of intermodal cross bridging, its means the drug is hepatoprotective and have no risk to cause hepatic change in the state of diabetes.

3.7.3 Pancreas:

In fig 12Group 1 which is normal control group shows normal architecture with usual epithelial cells and acni of serous, no fibrosis and no inflammation is recorded. Group 2, its shows demolition of acni of serous epithelial cells and tenderness or inflammation and also the inflammation of focal lymphocytic is seen. Group 3 of pancreatic section, its shows acini along with usual architecture, Group 4 indicate demolition structure with scattered lymphocyte invade in stoma. Group 5 section of pancreas its shows mild demolition in cells of acinar and there is no evidence of inflammation and there is recovery seen in detach pancreas Langer Hans cells.

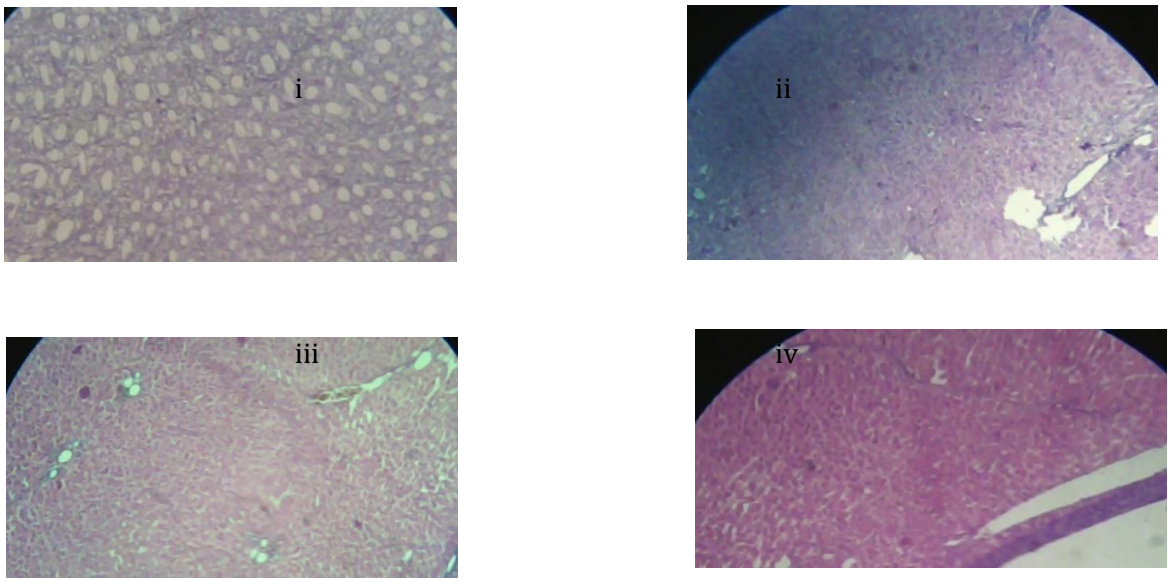

Fig: 10 Histopathology estimation of kidney portion (i) Normal rat (ii) Metformin (100mg/kg) (iii) PDWA (250mg/kg) (iv) PDWA (500mg/kg).

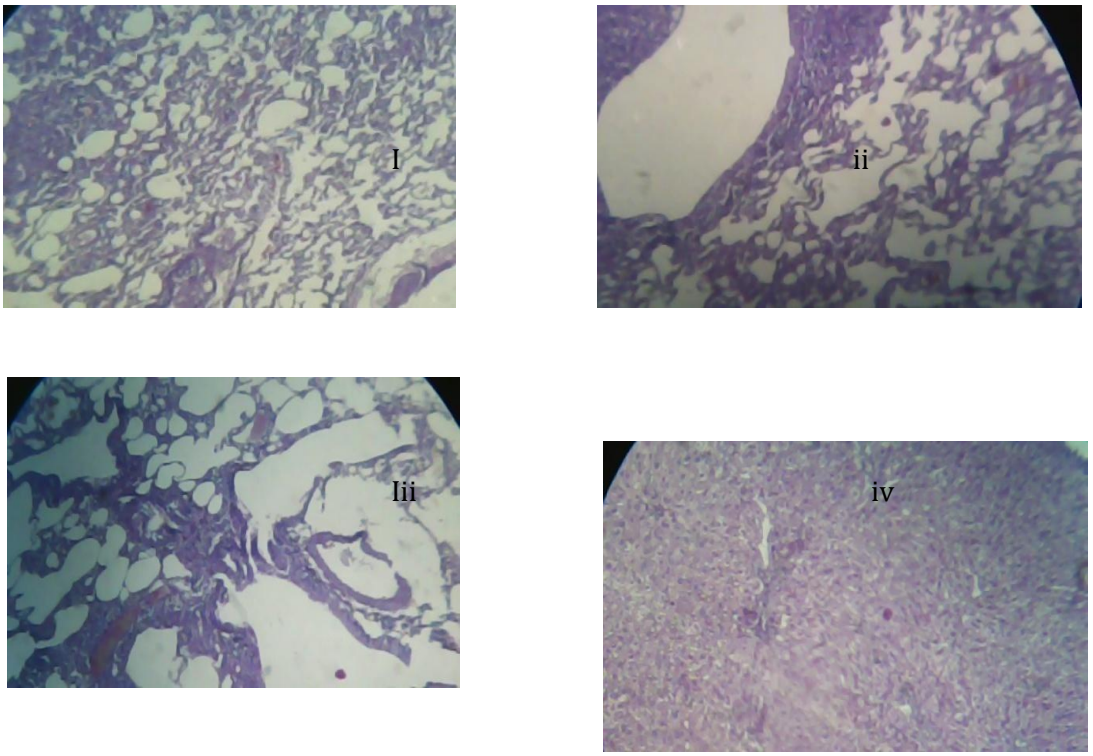

Fig: 11 Histopathology of estimation of liver (i) Normal rat (ii) Metformin (100mg/kg)(iii) PDWA (250mg/kg) (iv) PDWA (500mg/kg)

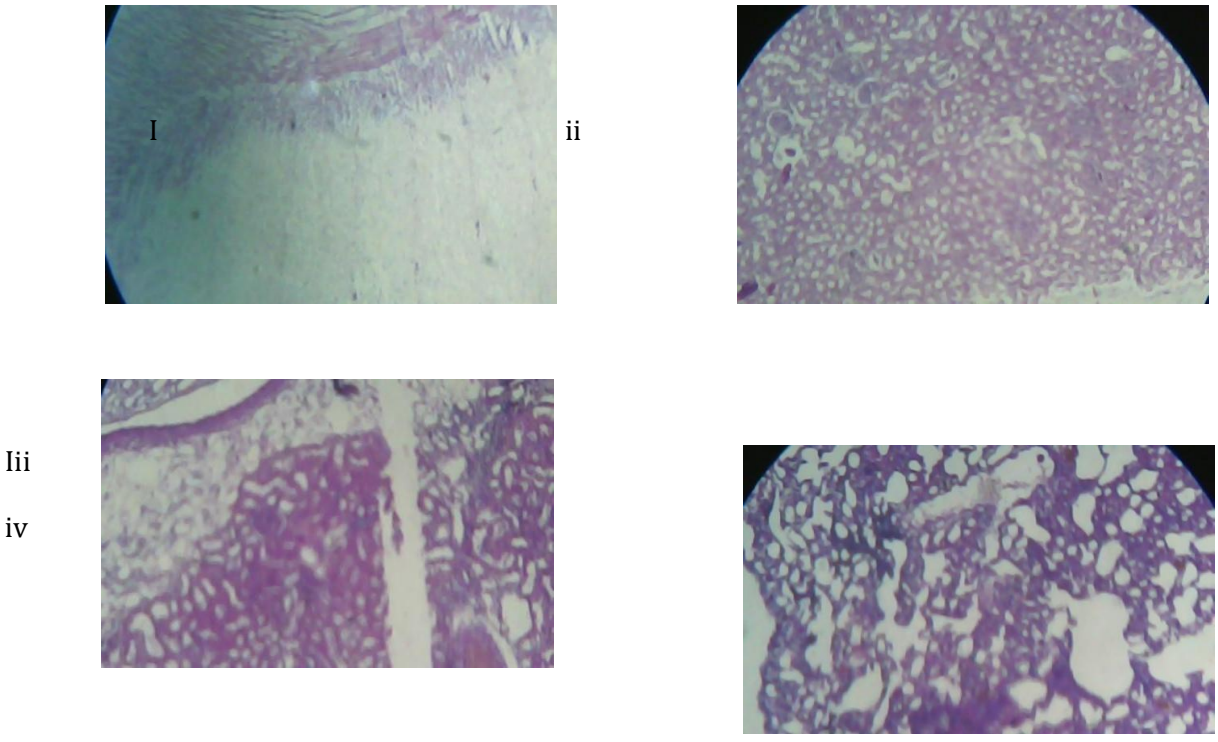

Fig: 12 Histopathology estimation of Pancreas (i) Normal rat (ii) Metformin (100mg/kg)(iii) PDWA (250mg/kg) (iv) PDWA (500mg/kg)

4. DISCUSSION:

Diabetes mellitus is a metabolic disorder which is characterized by insensitivity in insulin which causes insulin resistance, lower the production of insulin and the failure of pancreatic beta cell. Which cause decrease in the transportation of glucose in liver, fat cells and muscles cells; it lead to increase in fat with hyperglycemia breakdown [17], [18]. The presence of various bioactive constituent and compound such as amino acid, amygdaline, alkaloids, carbohydrates, flavonoids, phenolic, and steroids give us idea for the valuable and inherent treatment of miscellaneous disease. Some scientist and analyst had reported that the flavonoids, phenolic compound and steroidal glycosides act as antidiabetic bioactive factor or constituent throughout the research work. [19], [20]I perceive anti-hyperglycemic, antihyperlipedimic and antioxidant activity of plant seed extract. It allocate the extant of mentioned bioactive ingredient and there beneficial combined effect.To examine the qualitative and quantitative analysis the extract was characterized by TLC and HPTLC the extract gives a peak with density at 254 nm. Where the extract max % area 38.25% by peak no. 6 whose Rfvalue is 0.57. Infra Redspectroscopy of the seed extract exhibit the peak from 400 cm-1 to 2000 cm-1 region was performed to identify the presence of the various chemical compound in PDWA seed extract and to know the possibilities of presence of amygdaline in extract.[21]

In-vitro antioxidant and free radical scavenging activity, the extract of PDWA contain various Phytochemical constituent such as flavonoids, glycosides, phenolic compound which is helpful in preventing oxidative stress and also show effective result in treatment.[22]

PDWA produce anti- hyperglycemic effect and it improve the glucose level in normal rat. Crude extract of plant seed contain glycoside, omega 3faaty acid, amygdaline and myrestin. Due to stimulant of GLUT4 transportation activity in adipose tissue the crude plant seed extract show the hypoglycemic activity. STZ is used for inducing diabetes in experimental animal due to which the beta-cells cytotoxicity occur and it also interference with the metabolic oxidative mechanism[23].The executed animal experiment recorded that PDWA significantly reduced the raised blood glucose level by single dose of crude extract plant seed for 21 days to estimate anti-hyperglycemic analysis

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5. Conclusion:

It comes to conclude that an oral dose of PDWA 500mg/kg shows a therapeutic effect against diabetes and shows a beneficiary effect in diabetes type II, The biochemical studies has done and according the studies and data it conclude that PDWA drug extract shows alteration in both biochemical parameter, blood glucose level and also PDWA extract improved the antioxidant level in diabetes induced rats.

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