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EVALUATION OF ANTIDIABETIC ACTIVITY OF *PICRORHIZA KURROA* ROYLE EX. BENTH. – *IN-VITRO* AND *IN-VIVO*

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ABSTRACT: During the past few years, diabetes has been one of the leading metabolic disorders. It occurs when the body does not produce enough insulin or the body cells are not efficient enough to take up the insulin. This increases blood glucose levels that affect the body system, particularly blood vessels and nerves. Due to the side effects of allopathic medicines, individuals are searching the other alternatives with lesser side effects and more efficacy. Such properties are present in plant-based product. *Picrorhiza kurroa* Royle ex. Benth. belonging to the family Plantaginaceae/Scrophulariaceae distributed in Himalayan regions of China, Pakistan, India, Bhutan and Nepal. The tribes of North–Eastern Himalayan regions of India use this herb as for controlling diabetes. The present study was conducted to evaluate the antidiabetic activity of ethanolic extract of *P. kurroa in-vitro* and *in-vivo*. *In-vitro* antidiabetic activity was done by inhibiting the alpha-amylase enzyme, which is hydrolyzing enzyme for the starch by plant extract. *In-vivo* antidiabetic activity was done by injecting alloxan monohydrate (120 mg/kg bw) to male Wistar rats and which were given plant extract (60 mg/kg bw) orally for 14 consecutive days. The treatment shows a significant reduction in blood glucose levels and also in lipid profile levels. The IC_{50} value for plant extract was reported to be 73.52 µg/ml, and for positive control, it was 184.54µg/ml. The results showed that plant extract of *P. kurroa* shows promising results *in-vitro* and *in-vivo* for the treatment of diabetes.

INTRODUCTION: Diabetes mellitus (DM) is a metabolic disorder described by hyperglycemia caused because of deformities in insulin secretion, insulin activity, or both. This never-ending hyperglycemia prompts different full-scale and miniaturized scale vascular difficulties prompting harm, brokenness and disappointment of different organs, particularly the eyes, kidneys, nerves, heart and veins (American Diabetes Association 2008).

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In 2011, an estimated 366 million individuals (8.3% of grown-ups) suffered from diabetes all over the world. This figure was expected to go up to 552 million individuals by 2030. Around the world, the percentage of type 2 diabetes is greater than 90% [1]. Therefore, it is important to discover new ways to manage this well-being challenge.

One objective of treatment for diabetic patients, especially type 2, is the support of ordinary postprandial blood glucose levels. Postprandial hyperglycemia (glucose level) plays a vital role in improving type 2 diabetes and complications related to this disease. The main defect found in type 2 diabetes is that cells become insulin resistant or less responsive to it, which brings about weakness in the insulin flagging pathway and

reduced glucose uptake in target tissues like muscles and fats. At present, treatments to treat diabetes include incorporating insulin and different oral antidiabetic operators, such as sulfonylureas, biguanides, and glinides. Despite the impressive development in the treatment of diabetes by oral hypoglycemic operators, there is a need for new medications since existing medications have a few impediments what's more destructive impacts. It has been accounted for that treatment with plant extracts encourages actuation of β-cells, regranulation, and insulinogenic impacts [2].

Picrorhiza kurroa (family Scrophulariaceae) is a perennial herb which grows wild in alpine regions in Himalayas. It commonly grows in rock crevices and also in organic soils. The leaves of *P. kurroa* are basal and alternate, approximately 5-10 cm long and possess a thick, subcylindrical, straight-elongated rhizome that may be curved to some extent. This plant flowers from June to September produce pale-blue, showy flowers arranged in dense terminal racemes **Fig. 1**. The root powder decoction is used twice a day for the treatment of diabetes.

FIG. 1: *PICRORHIZA KURROA* ROYLE EX BENTH.

MATERIALS AND METHODS: Field trips were undertaken to collect the plant material during its flowering season *i.e.* between May-September. Local herbal healers, elderly individuals, shepherds and vaids were contacted to gather the information related to the various antidiabetic plants of the region. The information gathered includes the local name of the plant, part of the plant used, mode of preparation and consumption of the drug.

The plant was collected from Jagatsukhin Kullu district of Himachal Pradesh. The plants were first thoroughly washed with tap water and then with distilled water to prepare the plant extract. The washed plants were shade dried for 4 days and then powdered in a grinder. The powdered plant material was stored in air-tight plastic bags at room temperature, away from the light. Twenty-five grams of powder was added to 50 ml of 70% ethanol and kept on a shaker for 24 h. After that, the extract was filtered through Whatman filter paper and the filtrate was evaporated at room temperature. The remaining residue was stored at 4 °C for future utilization. This filtrate was used for the investigation of its antioxidant and antidiabetic potential.

Preliminary Phytochemical Screening: The phytochemical screening of *P. kurroa* was done by the given procedure of Trease and Evans [3]; Siddiqui and Ali [4].

***In-vitro* Study of Antidiabetic Activity:** The alpha-amylase inhibition assay was adopted and modified from Conforti*et al* [5]. The enzyme solution was prepared fresh by dissolving 0.001 g of alpha-amylase in 100 mL of 20 mM sodium phosphate buffer (pH 6.9).

Animal Ethical Clearance: The research proposal was approved by the Institutional Animals Ethics Committee (IAEC), Central Animal House, Panjab University, Chandigarh (Regd.No.45/99/CPCSEA; Dated: March 11, 1999), and all the protocols for the investigation was also approved by IAEC.

Animal Procurement: 8-10 weeks older male Wistar rats were used for *in-vivo* study. The rats were obtained from Central Animal House Panjab University Chandigarh (45/99/CPCSEA). The rats were employed to assess the antidiabetic activity, and the lipid profile was checked. Rats were housed in a clean cage.

Experimental Induction: The rats fasted for 16-18h before the induction of diabetes [6].

Animals were made diabetic with a single intraperitoneal injection (i.p) of 160 mg/kg body weight of alloxan. Rats with fasting blood glucose levels of more than 200mg/dl were considered diabetic rats and taken for experimentation [7, 8]. The body weight and blood glucose level of each rat was measured on the 1st, 7th, and 14th day of treatment. Blood glucose level was determined by code free Glucometer.

Experimental Design and Preparation of Dose: Four groups of rats, 5 in each group, were given the following treatment.

Group 1: Normal control.

Group 2: Diabetic control (Alloxan 160mg/kg bw).

Group 3: Alloxan (160mg/kg bw) + plant extract (60mg/kg bw).

Group 4: Alloxan (160 mg/kg bw) + standard drug, glibenclamide (5mg/kg bw).

Change in body weight % and glycemic change % after 14th days were calculated by the formula [9].

Biochemical Studies: At the end of the 14th day of the experiment, blood samples were collected, and serum was separated and used further for biochemical estimation. The level of total cholesterol [10], Triglyceride [11], HDL [12], VLDL and LDL [13] was measured.

Histopathological Observations: Pancreas were collected in 10% of formalin solution, and the sections were stained with hematoxylin-eosin stain, and further observations were made.

Statistical Analysis: The values for body weight, blood glucose level, and biochemical estimations were expressed as Mean±SEM of five replicates and analyzed by one-way ANOVA using SPSS version 16.0 followed by Dunnet's test. The values when P≤0.05, were considered statistically significant.

RESULTS:

***In-vitro* Antidiabetic Activity:** The alpha-amylase inhibitory activity by ethanolic rhizome extract of *P. kurroa* and acarbose investigated at different concentrations *i.e* 50, 100, 150, 200 and 250 µg/ml. The IC_{50} value for ethanolic extract of *P. kurroa* was found 73.52 µg/ml. Our positive control *i.e* acarbose showed an IC_{50} value at concentration of 184.54 µg/ml as shown in **Table 1** and **Fig. 2.**

TABLE 1: PERCENTAGE OF ALPHA-AMYLASE INHIBITION AND IC_{50} VALUES BY ETHANOLIC RHIZOME EXTRACT OF *P. KURROA* AT VARIOUS CONCENTRATION

Concentration (µg/ml)	% inhibition by *P. kurroa*	IC_{50} (µg/ml) of *P. kurroa*	% inhibition by Acarbose	IC_{50} of Acarbose
50	41.78±2.11	73.52±2.81	13.35±2.00	184.54±6.43
100	75.83±1.18		17.12±3.01	
150	86.06±1.93		40.84±2.10	
200	91.85±2.04		55.73±2.10	
250	92.51±1.49		62.60±1.98	

Values are expressed as Mean± SEM of three replicates.

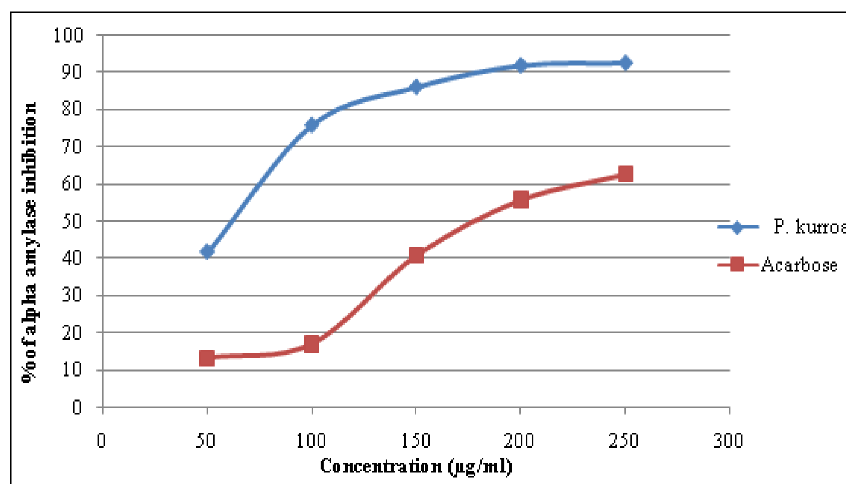


FIG. 2: % INHIBITION OF ALPHA-AMYLASE BY ETHANOLIC RHIZOME EXTRACT OF *P. KURROA*

***In-vivo* Antidiabetic Activity:** Effect on average body weight: *P. kurroa* treated group showed an increase of 2.94 % after the extract treatment. The diabetic untreated group showed a reduction in the body weight %, which was 11.33% **Table 2** and **Fig. 3.**

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *P. KURROA* ON AVERAGE BODY WEIGHT OF ALLOXAN-INDUCED DIABETIC RATS

Group	Average body weight (gm)			
	Day 1	Day 7	Day 14	Change in body weight %
Normal control	218±3.74	230^{bcd}±4.47	240^{bcd}±4.47	10.09
Diabetic control (Alloxan 160mg/kg bw)	203^{a}±2.00	189^{acd}±4.00	180^{acd}±3.54	-11.33
Alloxan (160mg/kg bw)+ EtPE (60mg/kg) of *P. kurroa*	204^{a}±1.87	208^{ab}±1.22	210^{ab}±0.75	2.94
Alloxan (160mg/kg bw)+Glibenclamide(5mg/kg)	204^{a}±2.45	205^{ab}±1.87	206^{ab}±1.97	0.98

bw- body weight; EtPE-Ethanolic Plant Extract. [a] P≤0.05 when compared with normal control, [b]P≤0.05 when compared with diabetic control, [c]P≤0.05 when compared with alloxan-induced diabetic rats treated with ethanolic extract of *P. kurroa.* [d]P≤0.05 when compared with alloxan-induced diabetic rats treated with standard drug glibenclamide.

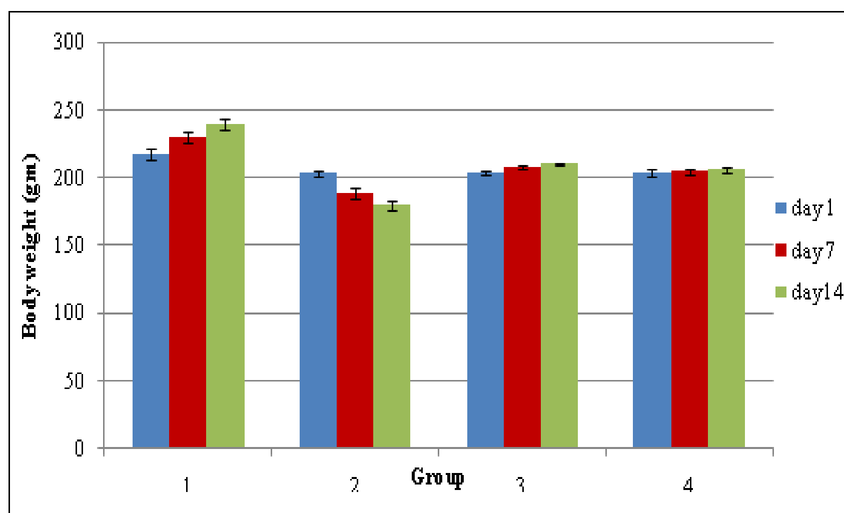


FIG. 3: DIFFERENCE IN THE AVERAGE BODY WEIGHT BETWEEN AND WITHIN THE GROUP IN ALLOXAN-INDUCED DIABETIC RATS TREATED WITH ETHANOLIC *P. KURROA* EXTRACT. Group1- Normal control, Group 2- Diabetic control, Group 3- Alloxan+ *P. kurroa*, Group 4- Alloxan + Glibenclamide.

Effect on Average Blood Glucose Level: In diabetic control untreated group the % of glycaemic change was increased by 22.87 and in *P. kurroa* extract, it was reported to be reduced by 53.47 **Table 3** and **Fig. 4.**

TABLE 3: EFFECT OF ETHANOLIC PLANT EXTRACT OF *P. KURROA* ON AVERAGE BLOOD GLUCOSE LEVEL OF ALLOXAN-INDUCED DIABETIC RATS

Group	Average blood glucose level (mg/dl)			
	Day 1	Day 7	Day 14	Glycaemic change%
Normal control	122±1.79	124.80±1.28	124±1.22	1.64
Diabetic control (Alloxan 160mg/kg bw)	411^{a}±7.48	450^{acd}±7.07	505^{acd}±2.34	22.87
Alloxan(160mg/kgbw)+EtPE (60mg/kg bw) of *P. kurroa*	404^{a}±7.48	298^{abd}±3.74	188^{ab}±2.55	-53.47
Alloxan(160mg/kgbw)+Glibenclamide (5mg/kg bw)	402^{a}±6.63	304^{abc}±2.45	190^{ab}±3.54	-52.73

bw- body weight; EtPE-Ethanolic Plant Extract. [a] P≤0.05 when compared with normal control, [b]P≤0.05 when compared with diabetic control, [c]P≤0.05 when compared with alloxan-induced diabetic rats treated with ethanolic extract of *P. kurroa.*, [d]P≤0.05 when compared with alloxan-induced diabetic rats treated with standard drug glibenclamide. Each value represents as mean± SEM of 5 observations (one way ANOVA followed by Dunnett's T test) where values are statistically significant at P≤0.05.

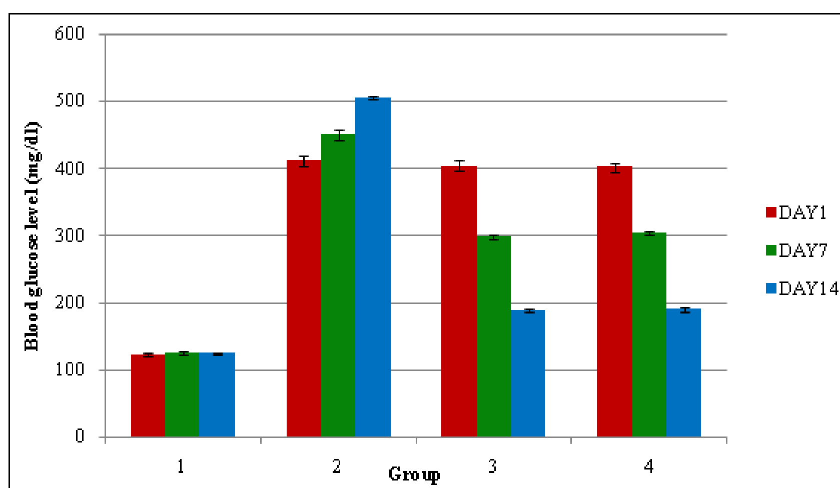


FIG. 4: DIFFERENCE IN THE BLOOD GLUCOSE LEVEL WITHIN AND AMONG THE GROUPS WHEN TREATED WITH *P. KURROA*ETHANOLIC EXTRACT. Group 1 – Normal control, Group 2- Diabetic control (alloxan alone), Group 3- Alloxan + *P. kurroa*, Group 4- Alloxan+ Glibenclamide, Each value represents as mean ± SEM of 5 observations.

Effect on Serum Lipid Profile and Creatinine Level: The level of TC, TG, VLDL, LDL, TC/HDL and LDL and Creatinine get reduced after the treatment with the plant extract. The level of HDL get reduced after the treatment as shown in **Table 4** and **Fig. 5.**

TABLE 4: SERUM LIPID PROFILE AND CREATININE LEVEL OF *P. KURROA*

Group	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	TC/HDL (mg/dl)	Creatinine (mg/dl)
Normal control	92.35^{b} ±1.46	85.54^{bd} ±1.62	32.29^{b} ±0.73	17.10^{bd} ±0.32	42.94^{b} ±1.78	2.83^{b} ±0.08	0.56^{bcd} ±0.01
Diabetic control	127.62^{acd} ±1.14	145.53^{acd} ±2.84	23.26^{acd} ±1.18	29.10^{acd} ±0.57	75.26^{acd} ±1.74	5.51^{acd} ±0.25	2.00^{acd} ±0.02
Alloxan +plant extract (*P. kurroa*)	93.22^{b} ±1.40	85.00^{bd} ±1.47	30.69^{b} ±0.50	17.00^{bd} ±0.29	45.53^{b} ±1.61	3.01^{b} ±0.07	0.62^{ab} ±0.01
Alloxan +glibenclamide	93.36^{b} ±1.32	91.95^{abc} ±0.85	30.25^{b} ±0.45	18.39^{abc} ±0.17	45.73^{b} ±1.50	3.10^{b} ±0.10	0.61^{ab} ±0.01

TC -Total cholesterol, TG-Triglycerides, HDL- High-Density Lipoprotein, VLDL- Very Low-Density Lipoprotein, LDL- Low Density Lipoprotein, TC/HDL- Total cholesterol/ High-Density Lipoprotein. [a] P≤0.05 when compared with normal control, [b]P≤0.05 when compared with diabetic control, [c]P≤0.05 when compared with alloxan-induced diabetic rats treated with ethanolic extract of *P. kurroa*, [d]P≤0.05 when compared with alloxan-induced diabetic rats treated with standard drug glibenclamide. Each value represents Mean±SEM of 5 observations (one way ANOVA followed by Dunnett's T test) where values are statistically significant at P≤0.05.

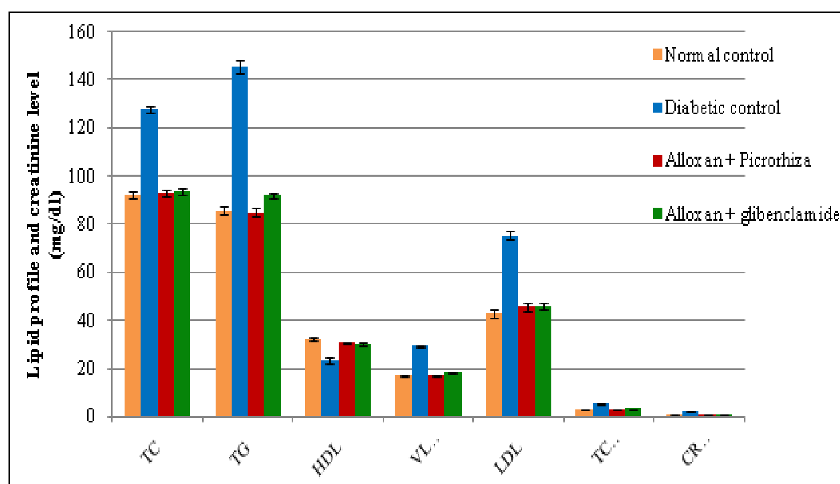
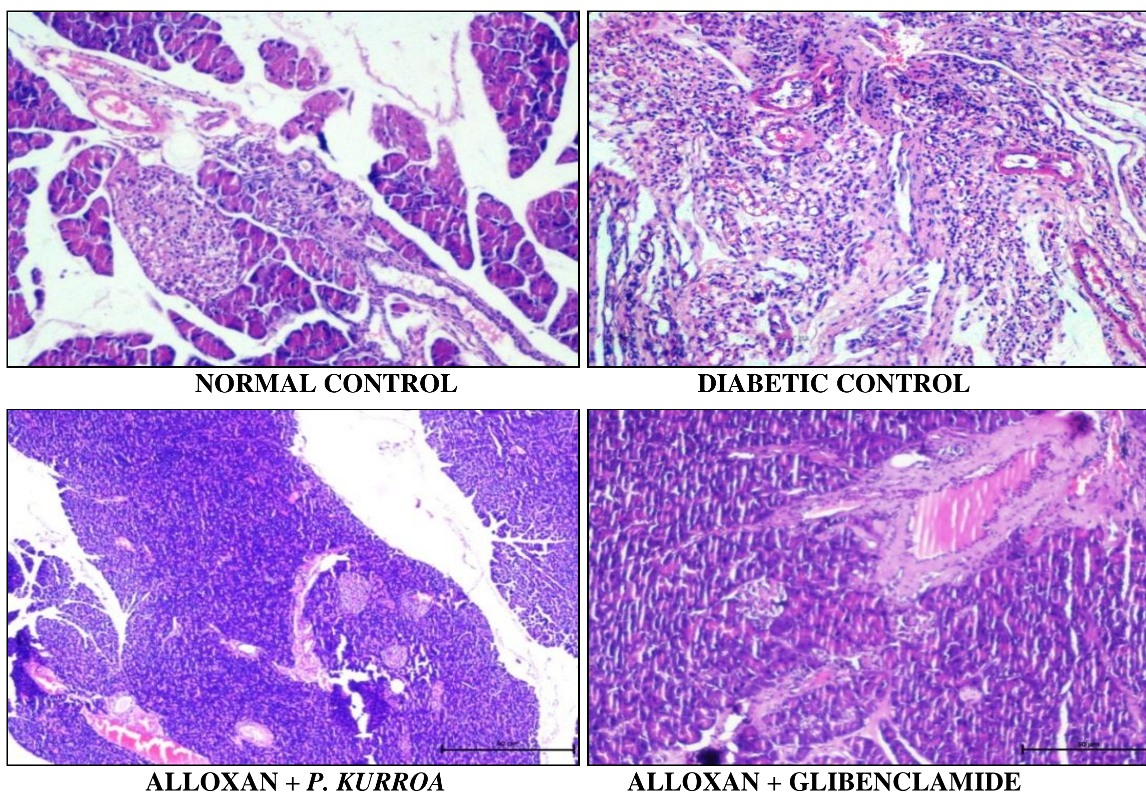


FIG. 5: DIFFERENCE IN THE SERUM LIPID PROFILE LEVEL AND CREATININE LEVEL IN ALLOXAN-INDUCED DIABETIC RATS WHEN TREATED WITH ETHANOLIC EXTRACT OF *P. KURROA*. Each value represents 5 observations of mean± SEM.

Histopathological Study: The pancreas of the normal control group does not show any abnormality. Islets of Langerhans are embedded in the acinar cell. The pancreas of diabetic control shows large area of inflammation. The acinar cells are separated by inflammation. The pancreas of alloxan-induced diabetic rats treated with plant extract shows good recovery and less inflammation (**Fig. 6).**



A-ACINAR CELL, B-BLOOD VESSEL, I- ISLETS OF LANGERHANS, LI- LYMPHOCYTIC INFILTRATION
FIG. 6: HISTOPATHOLOGICAL STUDY OF RAT PANCREAS TREATED WITH *P. KURROA* EXTRACT.

DISCUSSION: The present study was conducted to find out the possible antidiabetic potential of ethanolic extract of *P. kurroa*as; this plant is used by the local people of Kullu district of Himachal Pradesh. The ethanolic extract of *P. kurroa* extract was reported to have an inhibitory effect on alpha-amylase, an enzyme that breaks down starch into more simple sugars. The alpha amylase inhibitors can postpone the starch absorption and diminish the rate of glucose retention [14]. Thusly, they can diminish the weakened postprandial plasma glucose levels and enhance glucose resilience in diabetic patients. The percentage of alpha-amylase inhibition by the plant extract was reported to be 41.78% and for acarbose (positive control) it was reported to be 13.35 %. At all the concentrations, the plant extract shows more inhibition as compared to the control. The IC_{50} value for *P. kurroa* was found to be 73.52 µg/ml, and for acarbose it was 184.54 µg/ml, which demonstrates it to be a more effective antidiabetic. Alpha-amylase inhibitors may be of great value as novel therapeutic agents [15]. The plant extract of *P. kurroa*has can regenerate the beta cells of pancreas, increase the production of insulin, and hence help reduce blood glucose levels. The enzyme α-amylase is responsible for hydrolyzing dietary starch into maltose, breaking down to glucose prior to absorption. Beta-amylase inhibition significantly reduces postprandial glucose level in diabetic and borderline patients. The delay in carbohydrate absorption with a plant-based alpha-amylase inhibitor provides a therapeutic approach for the treatment of type 2 diabetes [16]. The inhibition of the alpha amylase can be an important strategy for the treatment of diabetes [17, 18]. The blood glucose level in *P. kurroa* treated extract was significantly reduced compared to the diabetic control groups, which were alloxan-only treated groups. In type-2 diabetes, there is a marked imbalance in lipid metabolism [19]. Diabetic dyslipidemia is characterized by low HDL-C and elevated level of

TG and LDL-C particles [20, 21]. A significant increase in plasma cholesterol and triglycerides and a significant decrease in HDL-C were observed in diabetic rats in the present study. The plant extract-treated group shows a reduction in cholesterol and triglyceride levels. Our results resemble the work done by Husain *et al.* [22]. *Picrorhiza kurroa* forms an important ingredient in a few commercial herbal products such as Ojamin (manufactured by Tates Remedies) and *Madhumehari granules* (formulated by Baidyanath) used for controlling diabetes.

CONCLUSION: Current study revealed a fresh method and scientific mechanism for the use of plant extract of *P. kurroa* as preventive and alternative medicine in the treatment of diabetes mellitus because of its hypoglycemic, antioxidant, hypolepidemic and insulin secretagogue effects. Further analyses are required in order to arrive at the optimal formulations.

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