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Evaluation Of Antidiabetic Potential Of Berberine On Albino Wistar Rats

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Abstract:
Plant-derived secondary metabolites are small molecules or macromolecules biosynthesized in plants including steroids, alkaloids, phenolic, lignans, carbohydrates and glycosides, etc. that possess a diversity of biological properties beneficial to humans, such as their antiallergic, anticancer, antimicrobial, anti-inflammatory, antidiabetic and antioxidant activities Diabetes mellitus is a chronic disease result of metabolic disorders in pancreas β-cells that have hyperglycemia. Hyperglycemia can be caused by a deficiency of insulin production by pancreatic (Type 1 diabetes mellitus) or insufficiency of insulin production in the face of insulin resistance (Type 2 diabetes mellitus). Chemical compounds derived from plants have been used since the origin of human beings to counteract a number of diseases. The alkaloid has drawn extensive attention as therapeutics against a number of diseases including hyperlipidemia, metabolic syndrome and polycystic ovary syndrome. In present study an attempt has been made to evaluate the antidiabetic activity of berberine in different doses.
Keywords: *Antidiabetic activity, Berberine, Natural products*

INTRODUCTION:

Diabetes mellitus is a chronic disease result of metabolic disorders in pancreas β-cells that have hyperglycemia. Hyperglycemia can be caused by a deficiency of insulin production by pancreatic (Type 1 diabetes mellitus) or insufficiency of insulin production in the face of insulin resistance (Type 2 diabetes mellitus) [1, 2].

The current medications of diabetes mellitus focus on controlling and lowering blood glucose levels in the vessel to a normal level. However, most modern drugs have many side effects causing some serious medical problems during a period of treating. Therefore, traditional medicines have been used for a long time and play an important role as alternative medicines. Moreover, during the past few years, some of the new bioactive drugs isolated from plants showed antidiabetic activity with more efficacy than oral hypoglycemic agents used in clinical therapy. Traditional medicine performed a good clinical practice and is showing a bright future in the therapy of diabetes mellitus [3, 4].

World Health Organization has pointed out this prevention of diabetes and its complications is not only a major challenge for the future, but essential if health for all is to be attained. Ayurveda and other traditional medicinal systems for the treatment of diabetes describe numeral plants used as herbal medicines. Because of low side effects and low cost, they play an important role as an alternative drug. Hyperglycemia is associated with the etiology of progression of diabetic complications. Hypoglycemic drugs increase insulin secretion, increase the uptake of glucose by adipose or muscle tissues and inhibit the absorption and production of glu- cose from intestine and liver. Insulin and oral hypogly- cemic agents such as biguanides and sulphonylureas are still the key players in the management but are seeking to develop more effective anti-diabetic agents [5, 6].

The current research efforts have made significant contributions in developing medicinal plant-based therapeutic strate- gies that can effectively reduce or prevent diabetes-induced complications. Most of the reported data and patent studies have used the whole extract as a remedy. Whereas it is considered that the antidiabetic potentialities of various medicinal plants-based extracts are due to the presence of several bioactive compounds such as glycosides, alkaloids, steroids, guanidine, polyphenols, polysaccharides, galactomannan gum, carbohydrates, terpenoids, peptidoglycan, hypoglycin, glycopeptides, amino acids and inorganic ions, etc. The above pointed bioactive constituents can affect metabolic cascades and ultimately affect the bold glucose level in the human body, directly or indirectly. However, to evaluate the individual and combine effect of each bioactive compounds, future prospective research should screen for individual constituents that possess health-promoting properties in various medicinal plants-based extracts [7-10].

Clinical trials involving compounds with a higher efficacy against abnormal carbohydrate metabolism linked with high blood glucose level, hormonal irregulation at pancreatic cells, and target organ insensitivity to secreted insulin, which are considerable causes of DM, when used in combination with standard one might pave the way to treat DM successfully. This will also give a deep insight to understand the exact relationship between the medicinal plant species and their health benefits with special reference to antidiabetic potentialities. Also, the effect of short or long-term consumption of medicinal plants-based extracts as natural herbal remedies on human health needs to be further evaluated in future studies. Such in-depth studies are highly requi- site because the role of the gut microbiota in degrading the available polyphenolic constituents in the medicinal plants or extracts has not been considered yet. The aspects mentioned above should be put forward to establish or develop new standardized therapeutic strategies [11-11]. In present study an attempt has been made to evaluate the antidiabetic activity of berberine in different doses.

Antidiabetic activity

Isolation of berberine from Berberis aristata stem

100 g of the powdered Berberis aristata stem stem was extracted with methanol by using a Soxhlet apparatus. The extract was concentrated under reduced pressure using rotary flash evaporator; 2% HCl was used to acidify 10 g of extract. The acidic solution was then filtered and extracted with diethyl ether to remove neutral materials and used sodium bicarbonate (Na2CO3) to make it basic, then extracted with chloroform. The chloroform extract was subjected to column chromatography and same fractions which gave single spot on TLC plate were pooled. The crystals of the isolated compound thus obtained was powdered and stored in the airtight amber coloured bottle away from light and moisture. The characterization of the isolated berberine was carried out by HPLC, 1 HNMR, and MASS spectral studies.

Animals: Animals wistar rats weighing between 180-200 gm were selected. The animals were housed in cages at 25 ± 2°C, and relative humidity (50 ± 5%) with 12 h light, and 12 h dark cycle. All the animals were acclimatized to laboratory environment for a week before the experiment. They were provided with free access to food and water ad libitum. The animals were cared and used in accordance with the CPCSEA guidelines and experimental protocols approved by institutional animal ethics committee.

Acute Toxicity Studies:

The objective of this Acute toxicity Study on rats was to assess the toxicological profile of the test drug when given intraperitoneally once (single dose) to the test system and monitor the vital signs for 14 days. This study will provide information on the possible health hazards likely to arise from single oral exposure of the extracts of the plant. Plants extracts administered in the dose of 50mg/kg, 100mg/kg and 500mg/kg and observed for 14 days. Animals were observed for autonomic or behavioural response during this period. The body weight was also observed. Mortality was observed up to 14 days. In acute oral toxicity study, extracts at the dose of 2000 mg/kg neither showed visible signs of toxicity nor mortality during the study and observations and measurements did not indicate any evidences of substance-related toxicity.

Induction of Type 2 Diabetes in rats: Administration of STZ causes alteration in blood insulin and glucose concentration. Hyperglycemia was observed after two hours of STZ administration with an associated drop in blood insulin. Later after six hours hypoglycemia occurred with high concentration of plasma insulin, gradually hyperglycemia developed and blood insulin level decreased

After acclimatization, the animals designated as controls were fed with a normal rat pellet diet and water ad libitum for four weeks, while the diabetic experimental animals were fed with a normal rat food and 30% fructose solution ad libitum for four weeks. After a 12 h fasting period, the experimental animals were injected intraperitoneally with 40 mg/kg of STZ dissolved in cold citrate buffer (0.1 M, pH 4.5), while the control groups were injected with same quantity of citrate buffer. Three days later, Blood glucose was determined by taking blood, with a lancet, from the tail and measured using test strips (Accu-Chek glucometer) 10 days after the STZ injection. Rats with a fasting glucose level >250 mg dL−1 were classified as diabetic Animals with fasting blood glucose (FBG) level above 250 mg/dL with typical diabetic symptoms of polyuria, polydipsia and polyphagia were selected for the study. The animals were subsequently divided into the following groups (n =6)

Oral glucose tolerance test: OGTT was evaluated in all the animals after an overnight fast. Briefly, the animals were dosed with 2 g/kg of glucose solution and blood samples were collected by tail pricking method at five time point (0, 30, 60, 90 and 120 mins) after glucose load for estimation of blood glucose level. Glucose is the main source of energy in our body; oral glucose tolerance test measures the body's ability to use glucose effectively. Berberine was suspended in CMC, were administered orally using an intragastric tube to different groups.

Experimental design for Oral glucose tolerance test

Normal Control:	Rats gavaged with normal saline
Standard treated:	Rat gavaged with Glibenclamide5 mg/kg b.w
Berberine 5 mg/kg:	Rat gavaged with Berberine 5mg/kg
Berberine 10 mg/kg:	Rat gavaged with Berberine 10 mg/kg
Berberine 20 mg/kg:	Rat gavaged with Berberine 20 mg/kg
Berberine 50 mg/kg:	Rat gavaged with Berberine 50 mg/kg

Experimental design for anti-diabetic study

Normal Control:	Control rats gavaged with normal saline
Diabetic Control:	Diabetic control rats gavaged with normal saline.
Standard treated:	Diabetic rat gavaged with Glibenclamide 5mg/kg b.w
Berberine 5 mg/kg:	Diabetic rat gavaged with Berberine 5mg/kg
Berberine 10 mg/kg:	Diabetic rat gavaged with Berberine 10mg/kg
Berberine 20 mg/kg:	Diabetic rat gavaged with Berberine 20 mg/kg
Berberine 50 mg/kg:	Diabetic rat gavaged with Berberine 50 mg/kg

All the groups received their treatment by oral route once a day. In addition, the blood glucose level, body weight, food and water intake were measured periodically throughout the treatment period.

Test solutions were prepared by dissolving 1 g of Berberine in 100ml of distilled water. Glibenclamide was used as a standard antidiabetic drug in STZ-induced diabetes to compare the antidiabetic properties of a variety of hypoglycemic compounds. A standard dug solution was prepared by glibenclamide 5mg/kg b.w. suspended in 100 ml distilled water, which will give 0.5mg/ml of glibenclamide solution as a standard. Antidiabetic study of Berberine was conducted by taking albino rats.

Overnight fasted animals were divide into groups to determine the antidiabetic activity of berberine. The experimental rats were divided into groups consisting of six rats per group. The Berberine, Glibenclamide, and normal saline were orally administered once every day for eight weeks.

On 0, 7th, 14th, 21stand 28th day of extract administration, the fasting glucose levels were determined. During the experimental tenure, weights of rats were taken daily and mean change was calculated. Blood samples were collected by tail prick method. Body weights and fasting blood glucose levels were measured on a weekly basis. At termination, the rats were fasted overnight and sacrificed by cervical dislocation.

Body weight assessment

The body weight of the normal rats before and after completion of experiment remained unchanged, but a significant reduction in body weight of STZ-induced diabetic rats was noted and maximum reduction was observed on 28th day.

Estimation of insulin level

After 28th day of treatment blood samples were withdrawn by in order to examine the insulin levels. Serum insulin was measured using a Glazyme Insulin-eia test.

Insulin was estimated in plasma samples by radioimmunoassay kit (Estradiol RIA Kits,) according to method of The radioimmunoassay method is based upon the competition of unlabeled insulin in the standard or samples and radio iodinated (125I) insulin for the limited binding sites on a specific antibody. At the end of incubation, antibody bound and free insulin is separated by the second antibody and poly ethyleneglycol (PEG) aided separation method. Insulin concentration of samples is quantitated by measuring the radio activity associated with the bound fraction of sample and standards.

The decrease in serum insulin level indicates hyperglycemia in STZ-induced diabetic rats. The STZ-induced diabetic rats exhibited maximum decrease in serum insulin levels on 28th day.

Pancreatic weights assessment:

After sacrifice pancreas, was dissected and rapidly stored in cold saline until further use. Pancreas was isolated and store in ice cold saline, blotted and weighted. There was a remarkable reduction in the pancreatic weights in diabetic control animals compared to Normal Control animals. There were maximum decreased in the pancreatic weight in diabetic control animals [13].

Histopathological evaluation of pancreas: After sacrificing the all experimental animals, part of the pancreatic tissuewas removed and fixed in 10 % formalin saline. Formalin fixed tissues were processed according to the standard histological tissue processing. These processing steps include dehydration of tissues by subjecting to gradual increasing concentration of alcohol final step of dehydration is done with 100%(absolute alcohol). After subjecting the tissues for xylene clearing step, paraffin wax infiltration step was performed and paraffin embedded blocks were prepared. These blocks were cut into 5 μm thin sections with microtome (Leica, Germany) and stainedwith hematoxylin and eosin (H&E). Specimens were observed under light microscope for histological changes. The number pancreatic beta cells and their sizes were evaluated from each slide and quantification was done [14].

RESULTS AND DISCUSSION

Acute Toxicity studies of berberin as per OECD: Acute toxicity studies were performed for berberin according to the toxic classic method as per guidelines 423 prescribed by OECD. Acute toxicity studies of berberin were performed in animals at dose levels of 100, 300 and 500 mg/kg as per OECD guide lines. No mortality was observed in all doses (p.o). The treated animals did not demonstrate any significant changes in behavioral pattern and exhibited normal activity. Also there were no clinical signs of tremors, convulsions, exophthalmos, salivation, diarrhea and lethargy. There was no significant difference in the mean body weights between treated groups and control group and the rats exhibited normal body weight gain during the study. No lethal effects or mortality was observed in animals throughout the test period following single oral administration at all selected dose levels of all extracts. The animals were examined for long term toxicity (14 days). None of these extracts showed any mortality even at the dose of 2000mg/kg. From the results of acute toxicity studies 1/10th, 1/20th doses were selected for the experimental study.

Oral glucose tolerance test: OGTT was evaluated in all the animals after an overnight fast. Briefly, the animals were dosed with 2 g/kg of glucose solution and blood samples were collected by tail pricking method at five time point (0, 30, 60, 90 and 120 mins) after glucose load for estimation of blood glucose level. Glucose is the main source of energy in our body; oral glucose tolerance test measures the body's ability to use glucose effectively.

Table 1: Effect of Berberine on Oral glucose tolerance test

Treatment	Blood glucose level (mg/dL)				
	0 min	30 min	60 min	90 min	120 min
Normal Control	79.30± 1.21	148.23± 1.18	154.23± 1.17	148.21± 1.19	104.17± 1.15
Standard treated	80.27±1.72	141.93± 1.24	138.14± 1.14	110.20± 1.31	88.98± 0.94**
Berberine 5 mg/kg	80.23± 1.05	149.63± 1.16	147.26± 1.49	121.31± 1.76	99.41± 1.29*
Berberine 10 mg/kg	81.82± 0.94	146.53± 1.37	142± 1.64	116± 0.89	9.42 ± 1.71**
Berberine 20 mg/kg	81.73± 1.67	144.63± 1.31	140± 1.29	113± 1.62	95.12± 1.58*
Berberine 50 mg/kg	81.53± 1.15	142.53± 1.46	138± 1.53	111± 1.73	94.3± 1.49**

Values are mean ± SEM (n=6); *P <0.05, **P <0.01 compared to respective control group

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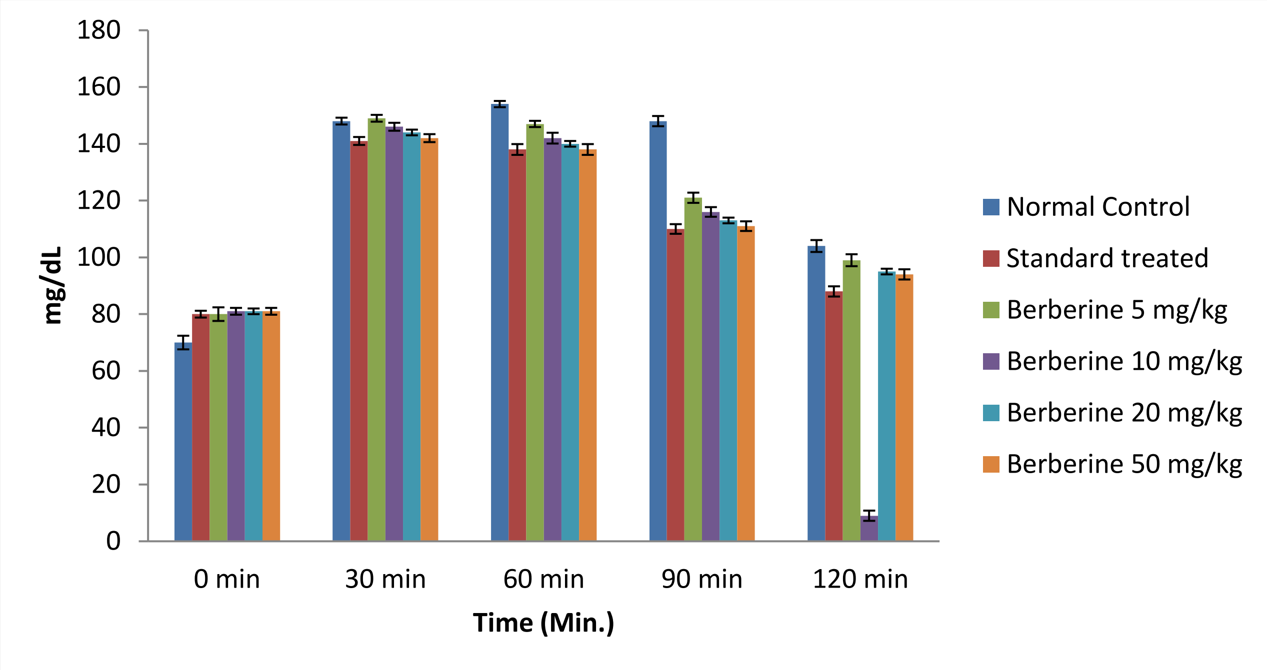

Figure 1: Effect of Berberine on Oral glucose tolerance test

It was observed that in normal control group average blood glucose was found to be 80 mg/dL. The glucose levels have not brought down to normal levels following 90 Min, surprisingly, the glucose levels have diminished in Berberine 50 mg/kg treated rats. Berberine 50 mg/kg and 20 mg/kg administration has resulted significant effects on glucose profiles with dose dependent improve in glucose transfer direct. Berberine at low dose 50mg/kg have no effect. High dose of Berberine has normalized the glucose intolerance close to normal control animals. Similar kind of improved glucose tolerance was found in glibencamide treated groups. It was found that Berberine 50 mg/kg and 20 mg/kg cause significant reduction of the elevated blood glucose levels.

Antidiabetic activity

Rats with a fasting glucose level >250 mg dL−1 were classified as diabetic Animals with fasting blood glucose (FBG) level above 250 mg/dL with typical diabetic symptoms of polyuria, polydipsia and polyphagia were selected for the study.

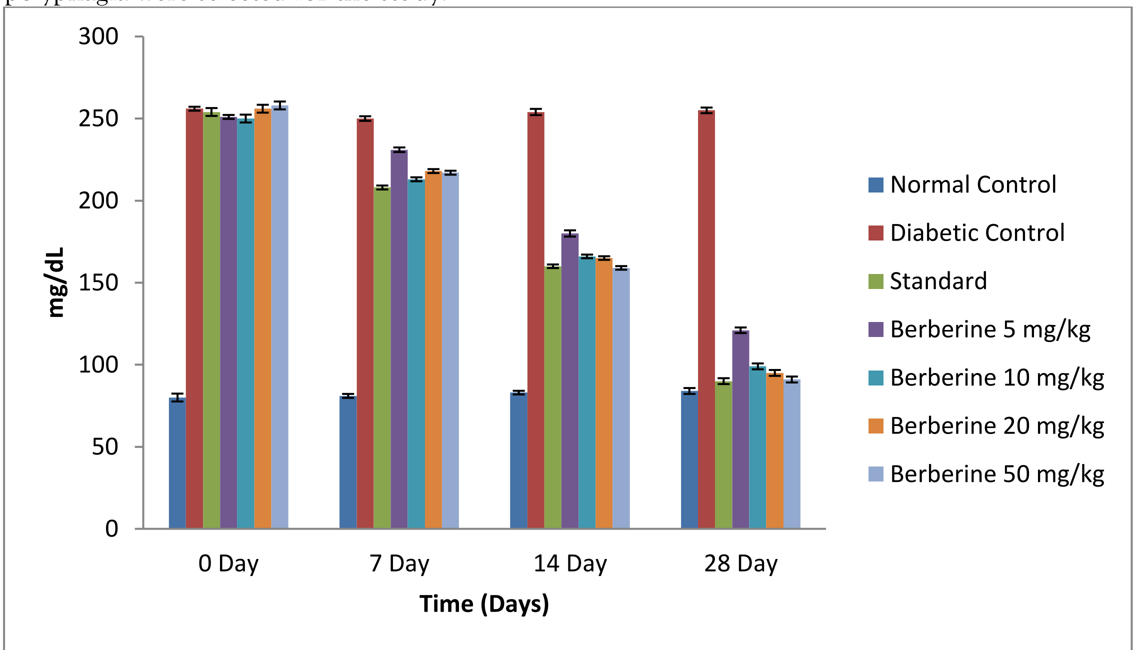

Figure 2: Effect of Berberine on the blood glucose levels in diabetic rats

The Antidiabetic activity of Berberine was evaluated and the findings are presented and the blood glucose level of diabetic rat significantly enhanced compared to normal rats. The administration of Berberine at different doses (10 mg/kg, 20 mg/kg and 50 mg/kg) to STZ-induced diabetic rats caused significant reduction of blood glucose levels. The Antidiabetic activity of Berberine depends upon the dose and duration of the treatment. The maximum reduction of blood glucose in rats was observed on 28th day.

Body weight assessment

The body weight of the normal rats before and after completion of experiment remained unchanged, but a significant reduction in body weight of STZ-induced diabetic rats was noted and maximum reduction was observed on 28th day. The administration of Berberine at different doses (10 mg/kg, 20 mg/kg and 50 mg/kg) and Glibenclamide treated rat showed no significant reduction in body weight on 28th day.

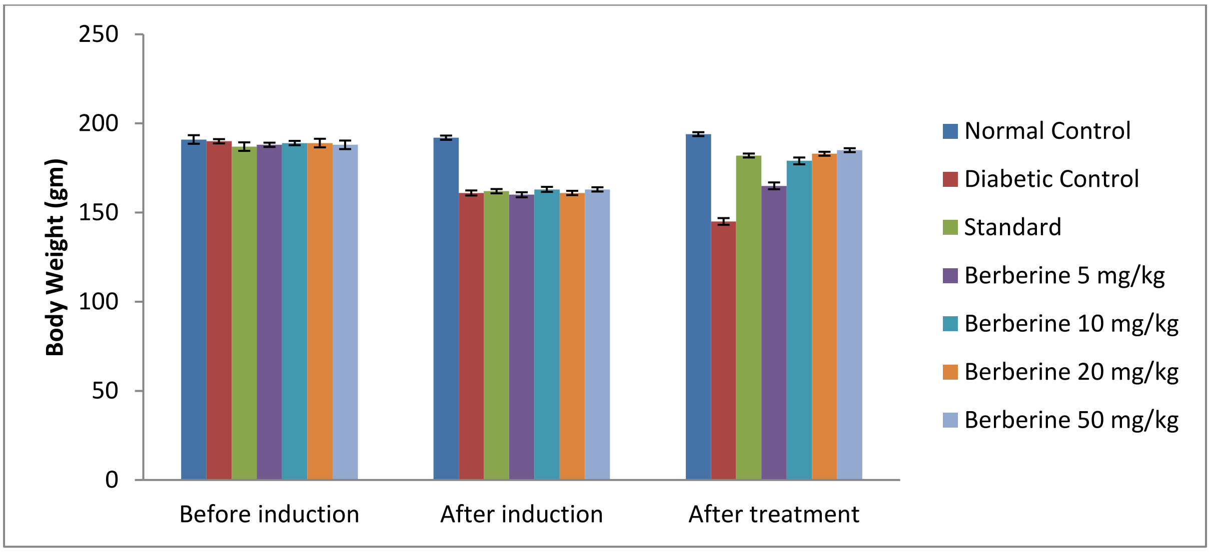

Figure 3: Effect of Berberine in change on body weight of animals

Estimation of insulin level

After 28th day of treatment blood samples were withdrawn by in order to examine the insulin levels. Serum insulin was measured

The decrease in serum insulin level indicates hyperglycemia in STZ-induced diabetic rats. The STZ-induced diabetic rats exhibited maximum decrease in serum insulin levels on 28th day. The administration of Berberine at different doses (10 mg/kg, 20 mg/kg and 50 mg/kg) and Glibenclamide to diabetic rats for 28th day resulted in significant increase in serum insulin levels were observed.

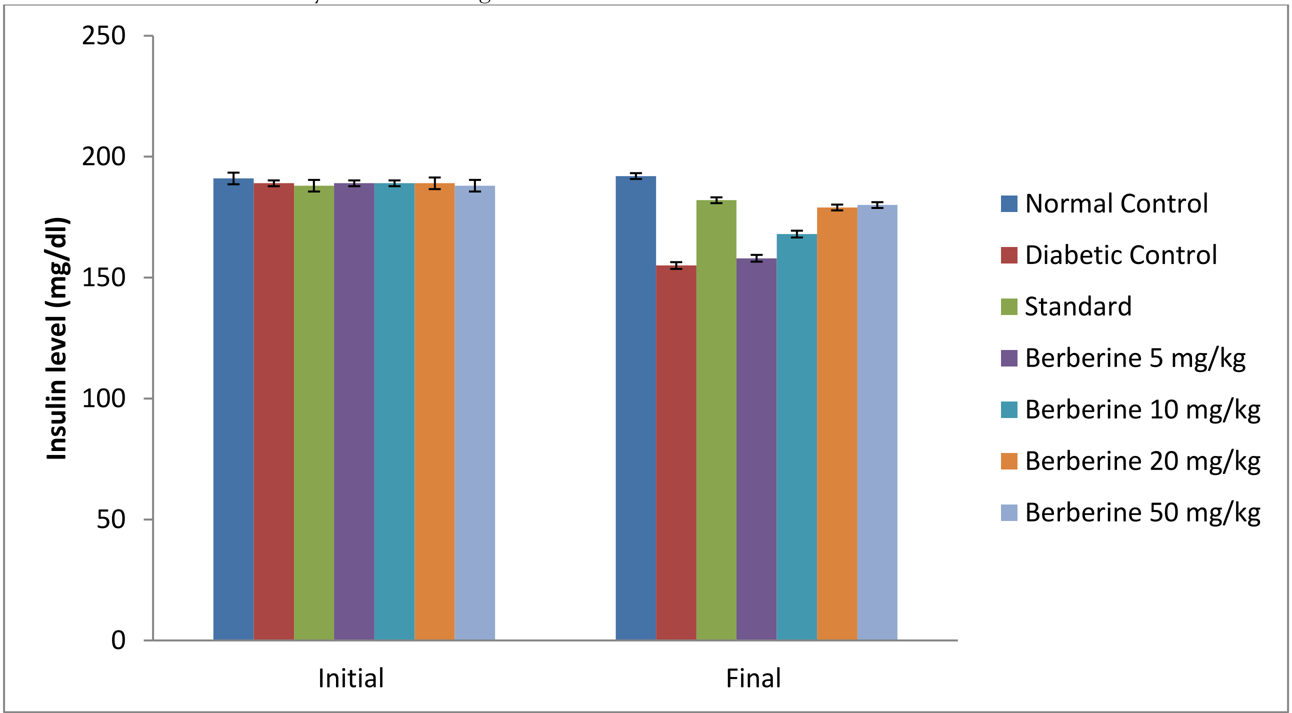

Figure 4: Effect of Berberine on insulin level of animals

Pancreatic weights assessment:

After sacrifice pancreas, was dissected and rapidly stored in cold saline until further use. Pancreas was isolated and store in ice cold saline, blotted and weighted.

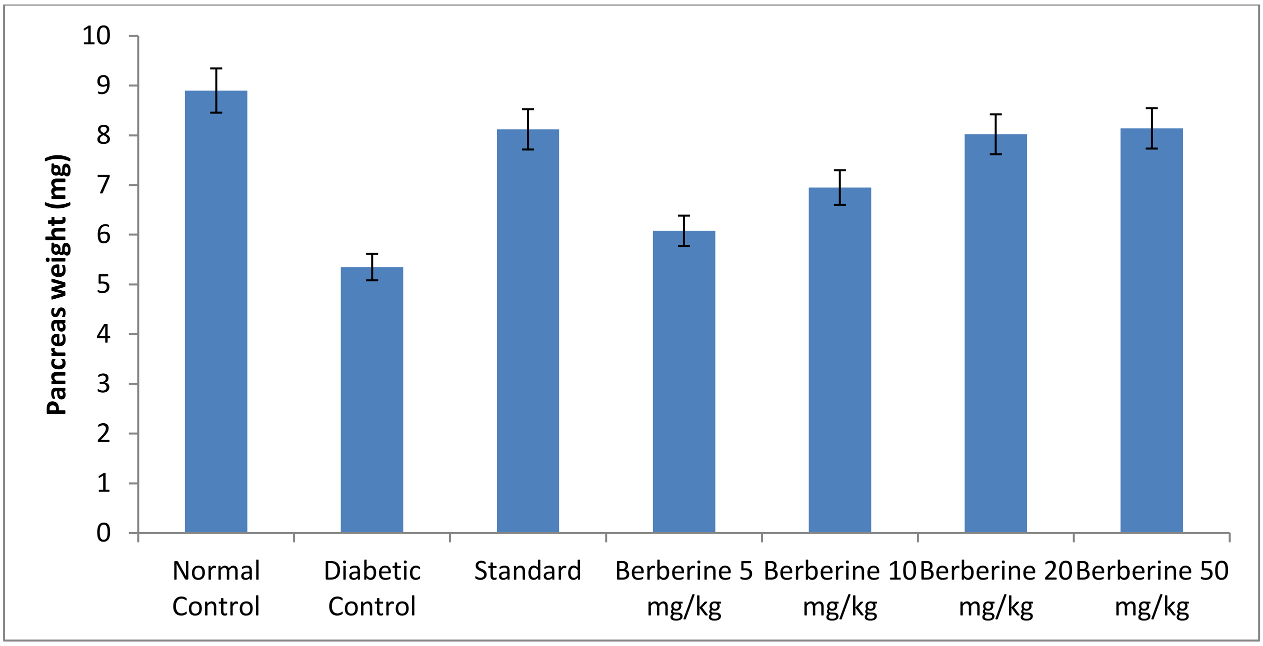

Figure 5: Effect of Berberine on Pancreas weight

There was a remarkable reduction in the pancreatic weights in diabetic control animals compared to Normal Control animals. Their were maximum decreased in the pancreatic weight in diabetic control animals. Treatment with Berberine at different doses (10 mg/kg, 20 mg/kg and 50 mg/kg) showed increase in pancreatic weight dose dependently.

Histopathological evaluation of pancreas: After sacrificing the all experimental animals, part of the pancreatic tissue was removed and fixed in 10 % formalin saline. Formalin fixed tissues were processed according to the standard histological tissue processing.

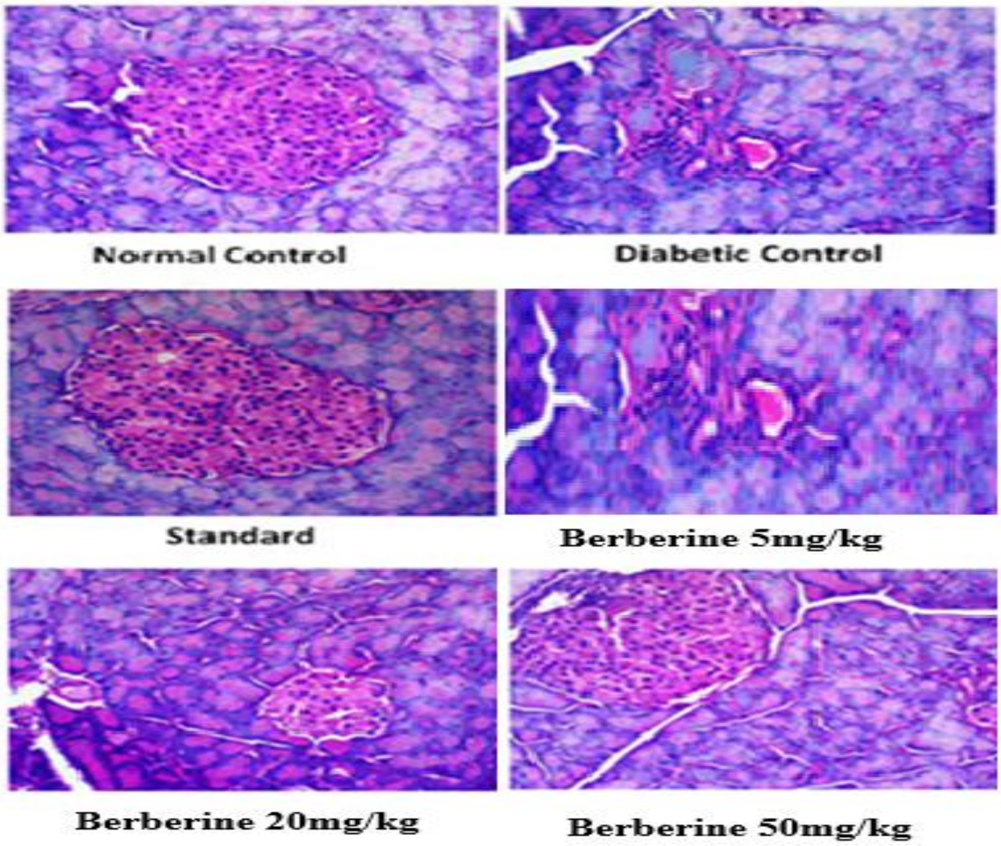

Figure 6: Histopathological evaluation of pancreas

Comparison with STZ treated pancreatic tissues; non diabetic animals demonstrated clearly normal pancreatic beta cell and acinar cell histological features with no confirmation of beta cell degeneration and inflammation. In diabetic control groups, the pancreatic histology showed expanded ductular fibrosis close by increase of ductular epithelial cells.

Treatment with berberine made dose dependant improvement of damaged histological features. In treatment groups, there was no evidence of pancreatic beta cell degeneration watched. Similar kind of clear and normal histological discoveries were seen on standard antidiabetic drug metformin treated animals. The number of pancreatic beta cells and the average size of beta cell and pancreatic cells area were extremely decreased in STZ animals contrasted to non-diabetic control pancreas. Treatment with berberine showed progressive and dose dependent improvement in the pancreatic beta cell number and beta cell sizes.

CONCLUSION:

Berberine is a nonbasic, quaternary benzylisoquinoline plant alkaloid with a proven medicinal history in Ayurvedic and Chinese medicinal systems. Berberine occurs as an active constituent in the root, rhizome and stem bark of many medicinally important plants, including Hydrastis canadensis (goldenseal), Coptis chinensis (Coptis or goldenthread), Berberis aquifolium (Oregon grape), Berberis vulgaris (barberry), and an Indian species Berberis aristata (Tree turmeric). B. aristata, (fam. Berberidaceae) known by common names such as "Daruhaldh, Daruharidra, Kashmal, Chitra" is a spinous shrub of upto 3 m that grows at an altitude of 2000–3000 m with a wide distribution in the Himalayan region and Nilgiri hills in South India. The Antidiabetic activity of Berberine was evaluated and the findings are presented and the different parameters of diabetic rat significantly improved compared to normal rats. The low dose of Berberine (5 mg/kg) do not produce any effect, while high dose found effective in diabetes treatment. The administration of Berberine at different doses (10 mg/kg, 20 mg/kg and 50 mg/kg) to STZ-induced diabetic rats caused significant reduction of blood glucose levels. The Antidiabetic activity of Berberine depends upon the dose and duration of the treatment.

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