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Research Paper

Antidiabetic activity of roots of *Boerhaevia diffusa* against streptozotocin induced diabetic rats

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ABSTRACT

The present study was carried out to evaluate the antidiabetic activity potential of *Boerhavia diffusa* roots against streptozotocin (STZ) induced diabetic rats. STZ is the most common method for chemically inducing diabetes in animal models widely. *Boerhavia diffusa* is used in the indigenous system of medicine as an antioxidant, pain reliever and hepatoprotective agent. How ever there are fewer or no reports available in literature on its hydroalcoholic root extract being effective in the treatment of diabetes. The blood glucose was determined on 0, 7th, 14th and 21st day after STZ administration. An increase in serum glucose was observed after the oral administration of streptozotocin when compared to normal group.

Glibenclamide was used as the standard drug. Different doses (200mg/kg and 400 mg/kg) of the hydroalcoholic root extract of the plant treated groups have shown a significant decrease in the blood sugar levels when compared to the control group. This decrease in blood sugar was observed from 7th day after continuous administration of extract. The hydroalcoholic root extract of *Boerhaavia diffusa* was found to reduce the blood glucose levels as well as its effect on the serum total cholesterol, lipid profile, low density lipoproteins, very low-density lipoproteins, high density lipoproteins was also measured in both test and control groups. Reduced levels of total cholesterol, LDL, VLDL and HDL cholesterol in diabetic rats was reported. This resulted in the indication that the plant extract possesses antidiabetic activity.

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1. INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterised by increased fasting and post prandial blood sugar levels. It occurs significantly due to insufficient insulin production or insulin dysfunction i.e. the inability of insulin to metabolise its own sugar occurs when there is less insulin produced from pancreas or the body cannot use it effectively[1]. Diabetes is a chronic metabolic disorder with long term impairment and failure of various organs (heart, blood vessels, kidneys etc) causing hyperglycaemia. Mainly the symptoms include obesity, weight loss, hyperlipidaemia, numbness, fatigue, polyurea, blurry vision. Diabetes is of two types, Type I (T1DM) and Type II (T2DM). T2DM occurs widely and also called non-insulin dependent This type of diabetes is characterized by two main insulin-related anomalies: insulin resistance and β-cell dysfunction leading to decreased insulin production[2]. On the other hand T1DM occurs mostly in children and adolescents which is characterised by autoimmune destruction of pancreatic beta cells leading to insufficient insulin production and hyperglycaemia. Most of the causes include obesity, sedentary lifestyle, unhealthy diet, family history, smoking. According to the world health organisation report Globally, an estimated 422 million adults were living

with diabetes in 2014, compared to 108 million in 1980[3]. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. This reflects an increase in associated risk factors such as being overweight or obese.

Over the past decade, diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries[4]

Efforts are being made to explore an alternate to the conventional drug therapy with less side effects, economic and effective at low concentration. The categorisation of natural plants for health research is due to the various phytoconstituents such as flavonoids, alkaloids, saponins, carotenoids, glycosides and terpenoids which may possess antidiabetic activity[5]

2.MATERIALS AND METHODS

Collection of plant material

The plant *Boerhaavia diffusa* used in the present study is found worldwide. It is a perennial herb characterised by stout root and stem stock. It belongs to the family Nyctaginaceae. It is known for its healing properties and also called as "punarnawa". It possesses wide variety of therapeutic activities useful in the treatment of hyperglycaemia, inflammation, anxiety, infections, hepatoprotective activities. The major active principle in *Boerhavia diffusa* is an alkaloid in nature called boeravinone[6]. The whole pant especially roots have shown antidiabetic and anti-hyperlipidaemic activity. The ethnopharmacological studies has shown that the plant leaf extract possessed antidiabetic activity. How ever no such studies against diabetes were reported with its hydroalcoholic root extract. There-fore the present study has been carried out to explore the antidiabetic activity in the hydroalcoholic root extract of *Boerhavia diffusa.* The plant *Boerhavia diffusa* was collected and authenticated by Dr Madhav chetty, botanist, tirupati by carrying out macroscopic and microscopic evaluation.

Preparation of the Extract

After collection of the plant material, the roots of the plant *Boerhavia diffusa* were thoroughly washed and shade dried under normal environmental conditions. The roots were then coarsely grinded to obtain a coarse granule and passed through 40 mesh sieve to obtain 1300gm of powdered drug. The dried roots approximately (250gm) were extracted with petroleum ether followed by hydroalcoholic (30:70) extraction using hot continuous percolation in Soxhlet apparatus. The marc left after pet ether extraction was extracted with double distilled water and evaporated over a water bath to yield total hydroalcoholic root extract. The extracts were concentrated by heating over a water bath. The extracts were stored at 4 °C for further use[7].

Drugs and Chemicals

Ethanol (90%), petroleum ether, Tris-buffer, Distilled water and all others were obtained from Sigma-Aldrich, Bangalore. Streptozotocin (98% pure) was purchased from Sigma Aldrich pvt.ltd and tablet Glibenclamide (Daonil) was purchased from the Sanofi-Aventis PVT. Ltd. and all were of analytical grade. The parameters evaluated in the present study were estimated using their respective kits obtained from Erba Diagnostics Ltd. India i.e., Total cholesterol, HDL and Triglyceride estimation kits.

3. EXPERIMENTAL SETUP

We housed (30) healthy young albino rats weighing about 180-200 g in an animal facility at Institute of Pharmacy.The experiment was conducted according to approved methods of institutional committee[8]. The animals were kept for two weeks as acclimatization period prior to the start of experiment and received normal diet and water *ad libitum.* After adaptation period, rats were randomly divided into following groups.

Group I Control group kept on normal routine diet

Group II Positive (+ve) control, STZ diabetic group received normal diet.

Group III standard, diabetic rats treated with glibenclamide

Group IV Treated I, diabetic rats treated with Hydro- alcoholic root extract (200 mg/kg b.w)

Group V Treated II, diabetic rats treated with Hydro-alcoholic root extract (400 mg/kg b.w)

Induction of diabetes

Rats were induced diabetes by using single intraperitoneal injection of STZ monohydrate (50mg/kg body weight) dissolved in normal saline[10]. As severe hypoglycaemia has been reported immediately after STZ induction due to massive release of insulin from pancreatic beta cells, so rats were administered 50% dextrose-saline within 12 hours of STZ injection to lessen/avoid death rate. Random blood glucose level of rats was measured in the morning on day 0 (after STZ dose), on day 3 and day 7 by using commercially available glucometer. After seven days of STZ induction, graded doses of Hydro-alcoholic root extract were administered through intragastric tube[11].

Biochemical analysis

Fasting Blood Glucose (mg/dL)

Commercially available glucometer On.Call ® *Ez II* (SN 303S0014E09) was used for the determination of fasting blood glucose from the tail vein of rats.

Serum Glucose (mg/dL)

Serum glucose was analyzed by using commercially available kit (Flutiest ® GLU- Analyticon diagnostic kit) consisting of following reagents.

Lipid Profile

Serum total cholesterol and triglycerides levels were determined by DiaSys Diagnostic Systems USA reagent kit method.

HDL-Cholesterol (mg/dL)

HDL-Cholesterol was determined by reagent kit method.

VLDL-Cholesterol (g/dL)

VLDL-cholesterol of each sample was calculated by using serum triglycerides and serum cholesterol.

Histopathalogical Examination

Histopathology was performed on collected tissue samples of pancreas

Fixation of Tissue

Tissue samples were placed in 10% neutral buffered formalin (NBF) immediately after slaughtering and remained in that solution for about 10-15 days at 20-25°C to prevent the autopsical changes. The tissue texture got rigid in NBF that is essential for histopathological analysis[13].

Post Fixation Treatment

Tissue samples placed in NBF were sliced into 1cm^2 pieces and rinsed with tap water for overnight to remove NBF. After overnight washing, tissue samples were placed in different concentrations of ethanol, xylene and paraffin for dehydration, clearing and infiltration.

Embedding

After infiltration, the tissue samples were fixed in paraffin wax to form the homogenous mass.

Sectioning

After embedding, the tissue samples were sliced with microtome into transparent and thin sections, placed on glass slide and stained.

Mounting

The tissue sections were mounted on the glass slides with the help of a gummy mixture (Meyers egg albumin).

Staining

Tissues were stained with eosin and hematoxylin

Statistical Analysis

The data thus obtained was expressed as Mean ± S.E.M. The Statistical significance observed between more than two groups was analysed using one way ANOVA followed by the Tukey's test..

4. RESULT

Phytochemical analysis was performed according to standard methods. The presence of various phytoconstituents like alkaloids, saponins, terpenoids, glycosides, phenols, tannins and flavonoids were reported in the root extract of the plant.

Effect on physical parameters.

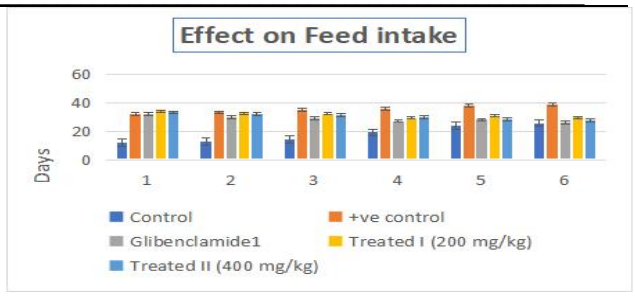

Figure no :1 Effects of treatments on Feed intake

Effect on Weight (grams)

Mean body weight of positive control, Glibenclamide and plant extract treated groups I, II and III was significantly reduced (P≤0.01) after induction of diabetes. In Glibenclamide treated group, mean body weight of rats was gradually increased from days 0 to 21 days. Moreover, treatment of diabetic rats with 200mg/kg, 400mg/kg in treated groups I, II and III respectively significantly increased (P≤0.01) the mean body weight of diabetic rats from days 0 to 21 days in dose dependent manner.

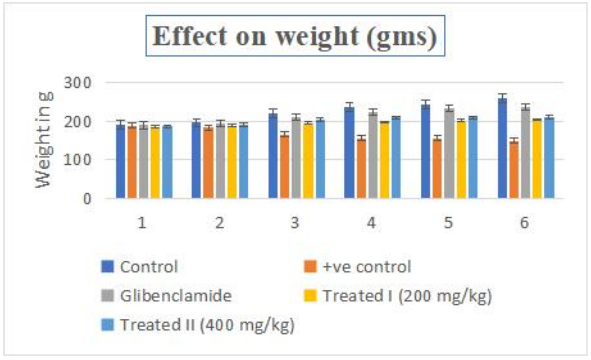

Figure no :2 Effects of treatment on Body weight

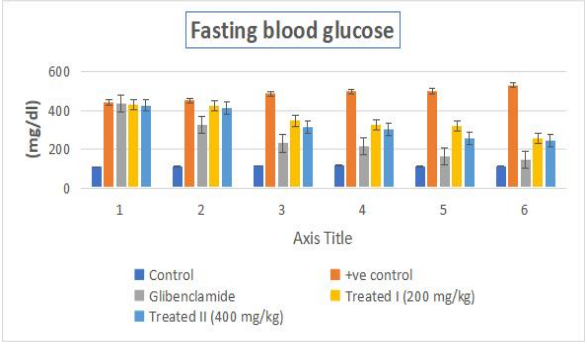

Figure no :3 Effects of treatments on Fasting blood glucose

Mean fasting blood glucose level was significantly (P≤0.01) increased in STZ induced diabetic groups at week 0. Use of Glibenclamide significantly (P≤0.01) reduced the mean fasting blood glucose level compared to +ve control. However, treatment of diabetic rats with herbal formulation significantly (P≤0.01) restored mean fasting blood glucose level in treated groups I, II and III from 0-21 days in dose dependent manner.

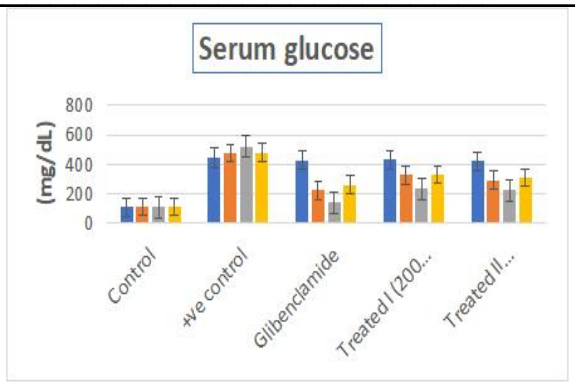

Figure no :4 Effects of treatments on serum glucose

Treatment of diabetic rats with Hydro-alcoholic root extract at 200, 400 mg/kg showed improvement by significantly (P≤0.01) reducing the mean serum glucose level in treated groups I and II. Results have also indicated insignificant (P>0.05) difference in mean serum glucose level between treated groups. Moreover, overall mean serum glucose level did not differ significantly (P≤0.01) between treated groups I and II

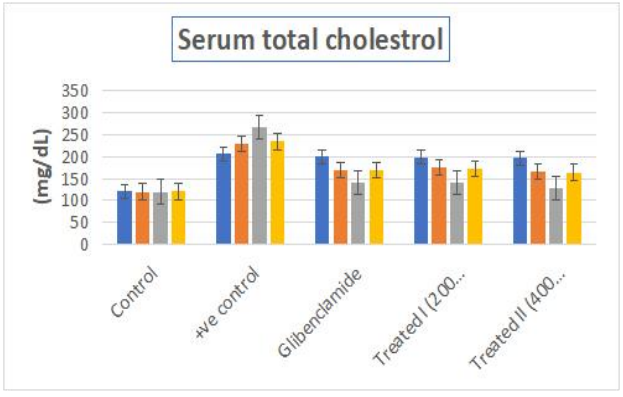

Figure no :5 Effects of treatments on Serum total cholesterol

Serum Total Cholesterol (mg/dL)

Statistical analysis has shown that mean serum total cholesterol level was significantly (P≤0.01) high in STZ treated groups compared to control group. Glibenclamide tended to reduce (P≤0.01) the mean serum total cholesterol level as observed. Statistical analysis also revealed nonsignificant (P>0.05) difference in mean serum total cholesterol level between glibenclamide and lowest dose of extract (200mg/kg) treated group at 21 days of experiment. Moreover, extract treatment significantly (P≤0.01) restored mean serum total cholesterol level in treated groups at 200 and 400mg/kg respectively compared to +ve control.

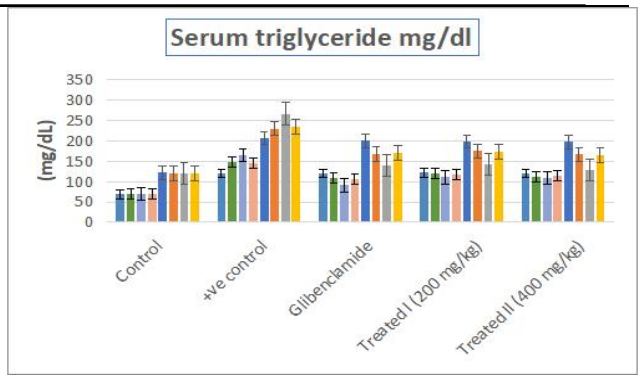

Figure no :6 Effect of various drug treatment on serum triglycerides (mg/dl)

Serum triglycerides level was significantly (P≤0.01) increased in +ve control, glibenclaminde, and plant extract treated groups at day 0 compared to control group. Glibenclamide significantly reduced (P≤0.01) mean serum triglycerides level compared to +ve control. Results have also exhibited that use of 200mg/kg of plant extract in diabetic rats of experiment nonsignificantly (P>0.05) resotored the mean serum triglycerides level.

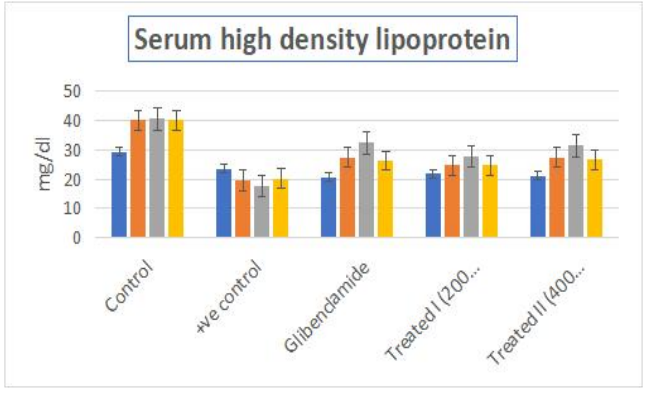

Figure no. 7: Effect of various treatment on serum high density lipoproteins (mg/dl)

Statistical analysis has revealed that mean serum HDL-C level was significantly (P≤0.01) decreased in +ve control, glibenclamide and plant extract treated groups I and II after induction of diabetes at day 0. Glibenclamide treated rats showed significant (P≤0.01) improvement in mean HDL-C level particularly at 21 days of experiment.

Administration of graded doses (200mg/kg and 400mg/kg) of polyherbal formulation in treated groups I, II significantly (P≤0.01) reversed the mean HDL-C level in dose dependent manner particularly at 21 day of treatment compared to +ve control. However, overall mean serum HDL level varied non significantly (P>0.05) between glibenclamide and treated group II at 400mg/kg dose of herbal formulation.

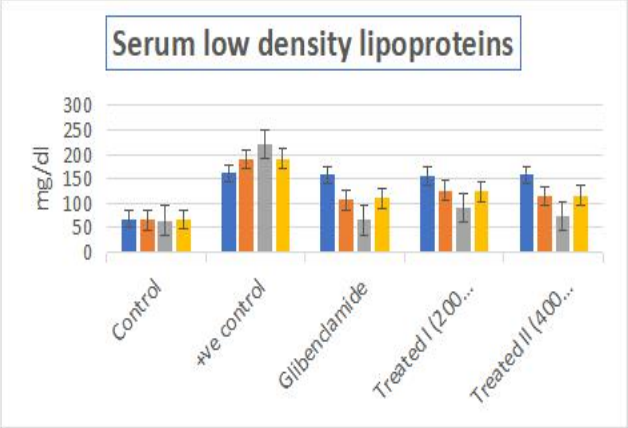

Figure no. 9: Effect of various treatment on serum very- low density lipoproteins (mg/dl)

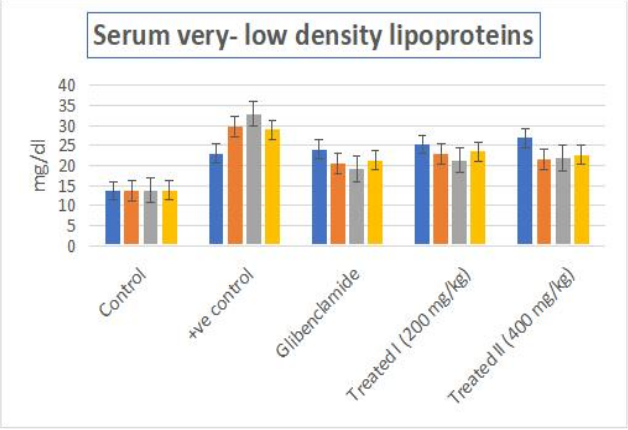

Figure no :8 Effects of treatments on Low Density Lipoprotein Cholesterol

Results have indicated significant (P≤0.01) decrease in mean serum low density lipoproteins (LDL) level in hyperglycemic rats of +ve control, glibenclamide and herbal formulation treated groups I and II compared to control at day 0. Glibenclamide (standard drug) significantly (P≤0.01) raised mean serum LDL level compared to +ve control. Treatment of diabetic rats with graded doses of herbal formulation (200mg//kg, 400mg/kg) significantly (P≤0.01) restored the mean serum LDL level in dose dependent manner.

Statistical analysis has revealed that mean serum very low- density lipoproteins (VLDL) level was significantly (P≤0.01) increased in STZ treated groups compared to control group at 0 day. Glibenclamide tended to reduce the mean serum VLDL level. herbal formulation at 200mg/kg and 400mg/kg reduced the serum VLDL level in the same pattern resulting in nonsignificant (P>0.05) difference in mean serum VLDL level between treated groups I and II. It is also apparent from results that highest dose of herbal formulation (400mg/kg) is more effective in reducing serum VLDL compared to glibenclamide.

Histopathological Examinations

Tissue section of pancreas collected from sacrificed rats and processed for histopathological examinations .

(A) Pancreas of control group showing normal pancreatic cells and fully active islets of Langerhans in pancreatic parenchyma **B)** Pancreas of +ve control showed very small size of islets of Langerhans, destruction of β cells and loss of cellular contents **(C)** Pancreas of the glibenclamide treated group indicated almost normal appearance of pancreas and normal nuclei of cells **(D)** Pancreas of treated group I showed mild degree of necrotic changes in β cells of pancreas **(E & F)** Pancreas of treated group II showed less number of islets of Langerhans and cell swelling in β cells of pancreas

5. DISCUSSION

These phyto constituents having different structure but with same curative potential act in synergistic way for treatment of different diseases. Herbal remedies are reported to delay the development of diabetic complications. Some herbal products have the potential to produce drug-drug interaction or drug-herb interaction. That's why it is essential to standardize the herbal medicines by matching it with the International Standards in order to eliminate the risk of side effects.

Pharmaceutical industries and health authorities are concerned about the quality and safety of the medicinal plant materials and final products because the use of herbal medicines is increasing rapidly[14]. Exact identification of raw material is an important pre-requisite to ensure good quality of herbal medicine which ultimately contributes towards the efficacy and safety of medicine. In quality control testing of herbal medicine, phytochemical and physicochemical studies are of main concern. Before studying any medicinal property of herbal treatment, quality control testing is needed for drug authenticity, because drug efficacy essentially depends upon its chemical and physical properties[15]. It is also important as it helps in a constituent/constituents group characterization that frequently leads towards the structure-activity relationship establishment as well as information about mechanism of action of drug

Qualitative phytochemical screening of plant extract was performed by using aqueous extract. Results have indicated the presence of alkaloids, flavonoids, phenols, glycosides and saponins in herbal extract. These phyto constituents are potent hypoglycaemic agents. Alkaloids are reported to produce anti-hyperglycaemic effect through potentiating insulin secretion from the pancreatic β cells, by modulation of antioxidant enzymes and dropping oxidative damage. An important flavonoid (quercetin) has also been reported to decrease the blood sugar

level, hepatic gluconeogenesis, glycogenolysis and increased glucose uptake resulting in anti-hyperglycemic potential[16].

Possible mechanism of action of anti-hyperglycemic activity of herbal mixture extract in diabetic rats may be due to increased insulin discharge from existing β cells as well as increased transfer of glucose into peripheral tissues. qRT-PCR analysis also confirmed up regulation of Pdx-1 and Ins-1 genes which are directly related with pancreatic regeneration and insulin secretion.

Hyperlipidemia is a common complication of diabetes mellitus. The present results showed significant (P≤0.01) rise in serum cholesterol, triglycerides, LDL and VLDL levels in the diabetic rats while HDL level was decreased.

Treatment of diabetic rats with different doses of herbal formulation extract resulted in gradual decrease in serum cholesterol, triglycerides, VLDL and LDL levels with increase in HDL level at treatment. The lipid-lowering trend of aqueous herbal extract may be due to the presence of flavonoids which are reported to lower the levels of cholesterol and triglycerides and also due to the action of HMG-CoA reductase enzyme that is cholesterol biosynthesis rate-limiting enzyme.

Flavanones (flavonoids) also reported to lower the activity of another absorption and esterification of cholesterol. The results of our study are in accordance with the findings of other researchers who reported that use of different plant extracts potentially restored atherosclerosis that is an important complication of diabetes, by reversing the serum activities of cholesterol, triglycerides and LDL close to normal.

HDL is known as good cholesterol. Various research studies have reported that increase in HDL-cholesterol is linked with decline in coronary disease. Current study proved anti-hyperlipidaemic effects of polyherbal formulation by significantly (P≤0.01) increasing serum HDL and lowering cholesterol and triglycerides levels after 8 weeks treatment of diabetic rats with polyherbal formulation. These results are in agreement with one of the previous findings in which use of aqueous extracts of flower and root of *A. lanata* in diabetic animals improved blood glucose level[17].

According to histopathological evaluation, pancreas of +ve control group (STZ induced diabetic rats) showed small size of islets of langerhans, destruction of β cells and loss of cellular contents indicating necrotic changes. Diabetic rats treated with 400mg/kg body weight of herbal formulation have pancreatic parenchyma with normal appearance however islet of langerhans were less in number and β cells were destroyed indicating psychotic changes. Moreover, pancreas of hyperglycemic rats treated with 400mg/kg herbal formulation showed moderate number of islet of langerhans having active β cells indicating active pancreas[18].

These changes may be due to presence of antioxidant and anti-diabetic potential of phytoconstituents e.g., flavonoid, terpenoids and quercetin and minerals such as Zn in herbal extract that defend and improve the viability key cholesterol-regulating enzyme that plays important role in the and regeneration of damaged β cells and subsequent release of insulin.

6. CONCLUSION

Use of plant extract in diabetic rats significantly reduced hyperglycaemia and improved lipid profile, performance of pancreatic β cells and insulin secretory capacity by upregulating the expression of Ins-1 and Pdx-1 genes that are directly involved in β cells regeneration and insulin release.

Current study has also supported the presence of various phytoconstituents in the formulation which might be responsible for reducing the ROS mediated β cells apoptosis. It is concluded from our research study that herbal formulation is an effective anti-diabetic agent as it can repair free radical damage, prevent pancreatic cell apoptosis and ameliorate insulin secretory capacity.

There is need to obtain more information about the nature of the cellular stress pathways. Role of hyperglycaemia and hyperlipidaemia in the development of diabetes and oxidative stress should be analysed in more detail. With the rapidly increasing incidence of diabetes worldwide, there is a great need to develop safe and effective functional biomaterials with anti-hyperlipidaemic and anti-hyperglycaemic potential.

7.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

8. ACKNOWLEDGEMENT

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