Research Article

ANTIDIABETIC POTENTIAL OF POLY HERBAL FORMULATION ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Aim: The present study was aimed to design the effect of poly herbal formulation against alloxan induced diabetic rats. The formulation was prepared by using three common herbal plants namely *Alternanthera sessilis* Linn., *Amaranthus viridis* Linn., and *Boerhavia diffusa* Linn., and glibenclamide as a standard. Methods: The rats were divided in to five groups each group comprising of five rats. The animals were grouped as: Group I-Normal control, Group II- Alloxan induced (150mg/kg b.wt), Group III- Alloxan + Poly Herbal Formulation (200mg/kg b.wt0, Group IV- Alloxan + Poly Herbal Formulation (400mg/kg b.wt) and Group V-glibenclamide (1mg/kg b.wt) for 30days. After the experimental period of 30 days, the blood and tissue samples were collected for preclinical trials. The parameter studied were fasting blood glucose, serum insulin, glucokinase, glucose -6- phosphatase, total protein, hepatic glycogen, serum cholesterol, triglycerides, serum urea and creatinine, lipid peroxide and superoxide dismutase. Results: Alloxan induced diabetic rats showed significant increase in fasting blood glucose, glucose -6- phosphatase, serum urea and creatinine, serum cholesterol and triglycerides and lipid peroxide and it also decrease in serum insulin, glucokinase, serum protein, hepatic glycogen and superoxide dismutase. Oral administration of Poly herbal formulation restored the levels of biochemical parameters and the level of glucose metabolizing enzymes. Conclusion: From the above findings, suggested that the poly herbal formulation possess antidiabetic activity and it can be used as starting point for the development of herbal based novel drug against diabetes.

Keywords: Diabetes, *Alternanthera sessilis* Linn., *Amaranthus viridis* Linn., *Boerhavia diffusa* Linn., Alloxan and glibenclamide.

INTRODUCTION

Diabetes is one of the oldest known diseases of humankind whose devastating effect is increasing by the day and severity at epidemic level[1]. It is a disease of disordered metabolism of carbohydrate, that also affect protein and fat which is caused by the complete or relative insufficiency of insulin action[2].The prevalence of diabetes is increasing due to life style modifications, aging, urbanization and increasing etiology of obesity and decreased physical activity[3,4]. The early symptoms of diabetes include elevated blood sugar levels (glycosuria), dehydration, weight loss, blurred vision and polyphagia. In conventional medical practice, the present therapies of diabetes mellitus are reported to have side effects. The glucose-lowering drugs include insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, metformin, thiazolidine-diones), α-4glucosidase inhibitors (miglitol, acarbose). The peptide analogs, such as exenatide, liraglutide and DPP-4 inhibitors, increase GLP-1 serum concentration and slow down the gastric emptying. Management of diabetes mellitus with insulin is associated with draw backs such as insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment. Besides the adverse effects of insulin and oral glucose-lowering drugs may include severe hypoglycemia at high doses, lactic acidosis, idiosyncratic liver cell injury, permanent neurological deficit, digestive discomfort, headache, dizziness and even death.

Therefore, since of the side effects associated with the present allopathic drugs for diabetes, need of an hour is to develop effective, safe and cheap drugs for diabetes management. Such effective, safe and cheap drugs could be obtained by using medicinal plants which have been used by humans to prevent or cure diseases including diabetes since the dawn of civilization. These plants based herbal medicines are thought to be effective, safe and affordable to the common population in the underdeveloped and developing countries of the world.

Based on the literature survey, three common plants such as *Alternanthera sessilis* Linn. (Family: Amaranthaceae), *Amaranthus viridis* Linn. (Family: Amaranthaceae), *Boerhavia diffusa* Linn. (Family: Nyctaginaceae) were selected for the preparation of poly herbal formulation and the formulation is used to screen the antidiabetic potentials against alloxan induced diabetic rats.

Amaranthus viridis Linn. is very useful to cure the diabetes, liver disorders, anaemia and inflammation. *Alternanthera sessilis* Linn. has wound healing property and antioxidant activity. *Boerhavia diffusa* Linn. is used in the treatment of diabetes, inflammation, analgesics and liver disorders.

Hence the present study was an attempt to evaluate the antidiabetic potentials of polyherbal formulation on alloxan induced albino rats.

MATERIALS AND METHODS

Collection and authentication of plants

Plant source selected for the present study was (*Alternanthera sessilis* Linn., (Voucher specimen number: GC.Herb.Bot.2294), *Amaranthus viridis* Linn. (Voucher specimen number: IFE − 17424) and *Boerhavia diffusa* Linn. (Voucher specimen number: LIH No. 6562). Leaves of the selected plants were collected from in and around trichy. The plant was identified and authenticated with the specimen deposited at RAPINAT herbarium, department of botany, St. Joseph's college, Trichy.

Preparation of aqueous plant extract

Fresh plant material was shade dried and powdered coarsely using electric blender. 200gm of coarse powder of (*Alternanthera sessilis* Linn., *Amaranthus viridis* Linn., and *Boerhavia diffusa* Linn.) each plant powder was taken and extracted with water. The plant material was mixed with six parts of water and it was boiled and reduced one third. It was filtered and evaporated to dryness. Paste form of the extract obtained was subjected to pre- clinical screening.

Preparation of polyherbal formulation

Aqueous extract of the selected plants (*Alternanthera sessilis* Linn., *Amaranthus viridis* Linn., and *Boerhavia diffusa* Linn.) was prepared individually. Equal volume (1:1:1) of each extract was mixed to make polyherbal formulation.

Experimental animals

Healthy adult wistar strain of Albino rats, two to three months old and weighing 100g-120g were obtained from Biogen, Bangalore. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. Animals were housed in standard polypropylene cages. Animals were fed with standard rat chow pellet obtained from Sai Durga Foods and Feeds, Bangalore, India and water *ad libitum.* All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

Induction of Diabetes

Diabetes mellitus was induced in a batch of normoglycemic albino rats, starved for 16 hours, 150mg/kg body weight of alloxan monohydrates are dissolved in phosphate buffer saline and injected intraperitoneally[5].This dose of alloxan produced persistent hyperglycemia after 4 days revealed by determination of urine sugar levels by BQR method. The diabetes induced rats were chosen and grouped for further studies.

Experimental Design

Wistar strains of albino rats weighing 100gm- 120gm were used as the experimental models. The rats were divided into five groups comprising of six rats each.
Group I: Normal control
Group II: Animals were induced with alloxan in phosphate buffer saline at a dosage of 150mg/kgbw IP
Group III, IV: Animals were induced as in Group II. After 4 days of alloxan induction, diabetic animals were treated with aqueous extract of polyherbal formulation 200, 400mg/kgbw for 30 days orally
Group V: Animals were induced as in Group II. After 4 days of alloxan induction, diabetic animals were treated with glibenclamide 1mg/kgbw for 30 days orally.

After the experimental period, animals were sacrificed by cervical decapitation. Blood was collected, and serum was separated by centrifuging at 3000 rpm for 10 minutes. Pancreas and Liver was dissected out and washed in ice- cold saline. Brain was homogenized in 0.1 M phosphate buffer, pH 7.4 and used for various experiment.

Parameters Studied

Fasting blood glucose[6]**,** Serum insulin , Glucokinase[7]**,** Glucose - 6- phosphatase[8], Total protein [9], Hepatic glycogen[10], Serum cholesterol[11], Triglycerides[12], Serum urea[13],and Creatinine[14], Lipid peroxide[15] and Superoxide dismutase[16].

Statistical analysis

All the results were expressed as mean ± S.E.M The data were statistically analyzed by one-way analysis of variance (ANOVA) and P values <0.05 were considered significant.

RESULTS

Table 1 shows the level of blood glucose, serum insulin and hepatic glycogen of the experimental animals. Alloxan induced animals showed an elevated level of blood glucose and significantly decreased the levels of serum insulin and hepatic glycogen when compared to normal rats. Animals treated with the formulation (Group III & IV) showed marked decrease in blood glucose level with a subsequent increase in the serum insulin and hepatic glycogen levels which was comparable to the glibenclamide group (Group V).

Table 2 represents the levels of the glucose metabolizing enzymes in the experimental animals. The group II animals showed a marked increase in the Glucose -6-phosphatase levels with a decrease in the glucokinase levels. On treatment with the formulation the levels were restored to near normal.

The levels of SOD and LPO in the animal models are depicted in Table 3. The group II animals showed increased level of lipid peroxidation with a decreased level of superoxide dismutase enzyme. On treatment with the formulation the levels were restored to near normal.

The results depicted in Table 4 clearly indicate significant increase in the serum urea and creatinine, triglycerides and cholesterol levels with a marked decrease in serum protein levels in alloxan induced diabetic rats. The formulation treated groups show a marked decrease in the urea, creatinine, triglycerides and cholesterol levels and an increase in serum protein levels when compared with Group II animals.

DISCUSSION

Diabetes mellitus is a disorder of carbohydrate metabolism caused by the total (or relative) absence of insulin, which manifests clinically as an elevated blood glucose. Alloxan is an oxidation product of uric acid, 2, 4, 5, 6-pyrimidinetetrone; administration to experimental animals destroys beta cells in the pancreas, can cause diabetes mellitus. It may produce the hypoglycaemia resulting from insulin liberation, followed by hyperglycaemia resulting from destruction of the islets of Langerhans (alloxan diabetes).

Alloxan is known to produce diabetes by damaging β-Cells in pancreas in experimental animals. Many medicinal plants have been traditionally used in India and other parts of the world, since long time for their sugar lowering effect. Herbal products are

considered to be least toxic and free from side effects when compared with their synthetic counterparts[17]. The possible mechanism of action of the poly herbal formulation to reduce the blood glucose level might be increasing the pancreatic secretion of insulin from β-cells of islets and are more effective for controlling diabetes by various mechanisms which may result in enhanced transport of blood glucose to peripheral tissue, improvement of carbohydrate metabolizing enzymes towards the reestablishment of normal blood glucose level.

Alloxan, a β-cytotoxin which causes a massive destruction of β-cells of the islets of Langerhans resulting in reduced synthesis and release of insulin, leading to hyperglycemia. The primary actions of insulin on metabolism includes control of cellular intake of certain substances, most prominently glucose in muscle and adipose tissue, increase of DNA replication and protein synthesis via control of amino acid uptake and modification of the activity of numerous enzymes. In diabetes mellitus deranged glucagon-mediated regulation of cyclic AMP formation and insulin deficiency leads to increased glucose level in blood [18]. In the present study, oral administration of polyherbal formulation at a dose levels of 200 & 400mg/kg bw. for 30 days enhances insulin production. This may be due to the regenerating effect of pancreatic β-Cells. The polyherbal formulation increased insulin levels in a dose dependent manner and was comparable with that of standard drug.

In diabetes, the glycogen content of the skeletal muscles and liver, markedly depleted and the reduced level of hepatic glycogen are due to inadequate insulin secretion. Insulin deficiency inactivates glycogen synthetase system[19].

Liver is the major site of synthesis and storage of glycogen. Administration of alloxan causes tissue necrosis leading to a decrease in glycogen content. The depletion may also be because of enhanced glycogenolysis and inhibition of glycogen synthase, due to insulin deficiency[20]. Significant increase in the liver glycogen by administration of herbal formulation might be attributable to the regeneration of pancreatic beta cells and stimulation of insulin release from the β-cells of islets that stimulates the activity glycogen synthase and inhibits the phosphorylase enzyme.

Liver plays an important role in the maintenance of blood glucose level by regulating its metabolism. Hexokinase, which brings about the first phosphorylation step of glucose metabolism, is significantly reduced in diabetes and this might be the reason for the diminished consumption of glucose in the system and increased blood sugar level[21].Oral administration of the herbal formulation increased the activity of the enzyme glucokinase due to the activation of mRNA coding for hexokinase synthesis.

The inability of the tissues to utilize the blood glucose induces it to turn on the gluconeogenic pathway, to meet the energy demands of the cell. Glucose-6-phosphatase is present only in hepatic tissue and is essential in regulating the gluconeogenic pathway. Due to insulin deficiency, tissues are incapable to utilize peripheral glucose, hence increase the synthesis of glucose-6-phosphatase to enhance gluconeogenesis[22].On administration of the herbal formulation dose dependently decrease the glucose-6-phosphatase activity. It is evident that the herbal formulation decreases the activity of glucose-6- phosphatase by enhancing the utilization of glucose by tissue thereby inhibiting gluconeogenesis.

Antioxidant capacity is reduced to a significant extent in the alloxan induced diabetic rats, due to the higher requirement of antioxidants in order to regulate the reactive oxygen species

(ROS) homeostasis. Nevertheless, enhanced antioxidant capacity in conjunction with reduced lipid peroxidation could be attained by regular ingestion of rich source of antioxidant compounds. ROS can be primarily eliminated by essential free radical scavenger enzyme, superoxide radical. As it is obvious from the Table 3. that activities of antioxidant related enzyme were deteriorated by the administration of alloxan. When the activity of the important antioxidant enzyme was diminished, the superoxide anion and hydrogen peroxide radical (H_2O_2) are available in excess, prompting the production of ROS and dissemination of lipid peroxidation. The level of SOD diminished in diabetic rats[23].Supplementation of PHF in alloxan induced diabetic rats significantly boosted enzymatic antioxidant defense system including superoxide dismutases (SODs) by administration of the Polyherbal extract can decompose superoxide and hydrogen peroxide in the cells, and hence a Polyherbal boosts the main defense against oxidative injuries. Besides, the role of SOD in blocking pathways of hyperglycemic damage is well established.

Based on the findings of the present study, it may be concluded that an increase in oxidative stress occurs in diabetic rats (group II) as an increased level of LPO and decreased level of antioxidant enzyme (SOD). The herbal formulation demonstrated that the pharmacological activity in reversing the altered parameters due to diabetes mellitus. Combination of three herbal plants in high doses (400mg/kg bw) appeared to be more effective as antidiabetic and antioxidant agents. The herbal formulation reinforces the constitutive cellular defense system by mimicking the endogenous LPO and enhancing the level of antioxidant tissue defense, SOD.

The blood urea and creatinine are considered as significant markers of renal dysfunction. Alloxan causes renal damage due to abnormal glucose regulation including elevated glucose and glycosylated protein levels, haemodynamic changes within the kidney and increased oxidative stress in diabetic rats[24]. The formulation refurbished the serum urea and creatinine levels symbolizing the renal protection and also prevents protein and nucleic acid degradation.

Increased catabolism of protein in diabetes mellitus can be attribute to the increased gluconeogenesis in the tissues to meet the energy demands. Also, with the damage caused to the liver by alloxan decreases the rate of protein synthesis. Significant reduction in total protein was observed in diabetic (Group II) which was due to decreased protein synthesis and increased protein catabolism.

Insulin deficiency leads to various metabolic aberrations in the animals such as decreased protein content. Insulin deficiency causes excessive catabolism of protein and the amino acid released were used for gluconeogenesis[25].

The formulation with its regenerative potential caused a profound increase in insulin secretion, thereby the test drug enhanced the protein sparing action of glucose which helped to maintain serum protein levels in treated (Group III and Group IV) animals. Table: 4 showed the dose dependent resumption in serum protein level. The formulation was found to have insulinogenic activity which enhanced the uptake of glucose by cell and there by prevented proteolysis for energy needs.

In the present study diabetic (Group II) rats showed elevation in the levels of cholesterol and triglycerides (Table: 4) due to the lack or insufficiency of insulin in diabetic rats. After the treatment with formulation there was a significant decrease in (Group III and IV) treated animals when compared to untreated disease

control animals (Group II). Serum lipids are elevated in diabetes due to lipolytic hormonal action. Insulin deficiency in the diabetic state leads hypertriglyceridemia and hyper cholesterolemia[26].

In the pathogenesis of diabetes, lipid plays a significant factor. Increased level of cholesterol present in plasma represent a risk factor for coronary artery disease[27].

Increased level of total cholesterol and triglycerides was observed in alloxan induced diabetic rats. Hypercholesterolemia in the rats received alloxan is caused by increased Intestinal absorption and increased cholesterol biosynthesis[28].Treatment with poly herbal formulation reduced total cholesterol and triglycerides level to a significant extent in dose dependent manner. It is assumed that PHF may exert its hypercholesterolemic effect either due to decreased intestinal absorption or decreased cholesterol biosynthesis. The lipoprotein in the diabetic rats are oxidized and may be cytotoxic, which can be reversed by the administration of antioxidant via PHF. The result clearly demonstrated that the PHF recovered the imbalanced lipid profile of alloxan induced diabetic rats in dose dependent manner.

Table 1: Levels of fasting glucose, insulin and hepatic glycogen in experimental models

Groups	I	II	III	IV	V
Fasting Glucose mg/dl	82.48 ± 0.95	303.23 ± 1.61*	220.72 ± 8.06	114.93 ± 0.44**	100.02 ± 0.42***
Insulin IU/ml	5.27 ± 0.20	3.20 ± 0.17*	4.23 ± 0.20	4.53 ± 0.18**	5.10 ± 0.12***
Hepatic Glycogen mg/g of tissue	2.82 ± 0.03	0.44 ± 0.02*	1.34 ± 0.17	2.51 ± 0.03**	2.59 ± 0.01***

mg/dl – milligram per deciliter; IU/ml – International unit per milliliter ; mg/g –milligram per gram
Values are mean ± SEM (n=6). *p<0.05 statistically significant when compared with normal control, **p<0.05 statistically significant when compared with alloxan group, ***p<0.05 statistically significant when compared with alloxan group.

Table 2: Effect of polyherbal formulation on glucose metabolizing enzymes in the alloxan induced diabetic rat

Groups	I	II	III	IV	V
Glucokinase (µg of glucose utilized/min/g tissue)	731.94 ±6.05	433.33 ±7.35*	502.77 ±7.35	576.39±25.27**	655.55±16.90***
Glucose-6- phosphatase (µg of Pi liberated/ min/g tissue)	13.31 ± 0.64	38.19 ± 2.00*	30.09 ± 2.32	21.41 ± 0.58**	19.33 ± 0.70***

µg of glucose utilized/min/g tissue – Microgram of glucose utilized per min per gram tissue
µg of Pi liberated/ min/g tissue – Microgram of inorganic phosphate liberated per min per gram tissue
Values are mean ± SEM (n=6). *p<0.05 statistically significant when compared with normal control, **p<0.05 statistically significant when compared with alloxan group, ***p<0.05 statistically significant when compared with alloxan group.

Table 3: Levels of superoxide dismutase and lipid peroxidation in the animal models

Groups	I	II	III	IV	V
SOD (µg of epinephrine oxidized/ g tissue)	343.00 ± 3.12	146.40 ±1.93*	236.20 ± 5.14	276.20 ± 3.82**	297.80 ± 1.74***
LPO (nM of MDA/ g tissue)	29.26 ± 0.54	236.72 ± 9.46*	118.55±12.35	61.56 ± 2.02**	51.00 ± 2.02***

nM of MDA formed/g of tissue – Nanomole of malondialdehyde formed per gram of tissue,
µg of epinephrine oxidised/ g tissue – Microgram of epinephrine oxidised per gram tissue.
Values are mean ± SEM (n=6). *p<0.05 statistically significant when compared with normal control, **p<0.05 statistically significant when compared with alloxan group, ***p<0.05 statistically significant when compared with alloxan group.

Table 4: Levels of urea, creatinine, serum protein, triglycerides and serum cholesterol in normal, diabetes induced, and formulation treated diabetic animals

Groups	I	II	III	IV	V
Urea (mg/dl)	33.33 ± 3.85	86.67 ±3.85*	62.22 ± 2.22	51.11 ± 2.22**	40.00 ±3.85***
Creatinine(mg/dl)	0.75 ± 0.06	2.36 ± 0.10*	1.38 ± 0.07	1.02 ± 0.01**	0.78 ± 0.02***
Serum protein (g/dl)	7.00 ± 0.23	2.53 ± 0.29*	4.27 ± 0.18	5.87 ± 0.18**	5.87 ± 0.18***
Triglycerides(mg/dl)	71.11 ± 2.94	140.00 ±1.9*	125.56 ±.94	110.00 ± 1.9**	86.67±1.92***
Serum cholesterol (mg/dl)	157.78±4.44	286.67±7.70*	244.44±9.69	206.67±7.70**	180 ±3.85***

mg/dl – milligram per deciliter; g/dl – gram per decilitre
Values are mean ± SEM (n=6). *p<0.05 statistically significant when compared with normal control, **p<0.05 statistically significant when compared with alloxan group, ***p<0.05 statistically significant when compared with alloxan group.

CONCLUSION

From the above findings revealed that the poly herbal formulation comprising of *Amaranthus viridis* Linn., *Alternanthera sessilis* Linn. and *Boerhavia diffusa* has potent anti-hyperglycemic activity in alloxan induced diabetes as evident from its capacity to enhance insulin synthesis and regulates various biochemical parameters. The effect was dose dependent and more pronounced activity was observed in higher dose. Further in-depth studies must be carried out to isolate the active constituents present in tested formulation and to explore the molecular mechanism of antidiabetic potential.

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