PROPHYLACTIC ROLE OF A POLY HERBAL FORMULATION ON ALLOXAN INDUCED DIABETES IN EXPERIMENTAL MODELS

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Received: January 6, 2013; Accepted: March 2, 2013

Abstract: Diabetes is an epidemic disorder, including chronic complications characterized by damage, dysfunction and eventual failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind health in all parts of the world. The traditional medicine proved to possess good therapeutic value showing a bright future in the treatment of diabetes mellitus. The present study was designed to evaluate the antidiabetic potential of a poly herbal formulation comprising of Biophytum sensitivum Linn., Bambusa arundinacea Retz., Artocarpus heterophyllus Lam., Trigonella foenum-graecum Linn. Albinorats of either sex were used as experimental models. The rats were divided into six groups each comprising of six rats each. The groups were group I- normal control, group II- alloxan induced disease control (150mg/kg b.wt), group III- alloxan + formulation (500mg/kg b.wt), group IV- alloxan + glibenclamide (200mg/kg b.wt) and group VI- formulation treated (750mg/kg b.wt) respectively. After the experimental period of 45 days, the blood and tissues were collected and pre-clinical trials were carried out. The parameters studied were plasma glucose, hepatic glycogen, serum insulin, glycosylated hemoglobin, glucose-6-phosphatase, glucokinase, and serum marker enzymes (AST, ALT and ALP). Alloxan induced disease control group showed significant increase in plasma glucose, glycosylated hemoglobin, glucose-6-phosphatase, and serum marker enzymes (AST, ALT and ALP). Alloxan induced disease control group showed significant increase in plasma glucose, glycosylated hemoglobin, glucose-6-phosphatase, and serum marker enzymes (AST, ALT and ALP). It also showed significant decrease in hepatic glycogen, glucokinase, and serum insulin levels. Oral administration of the formulation restored the level of biochemical parameters and serum marker enzyme levels. From the present observation, it is evident that the formulation could be an effective antidiabetic drug without any toxicity.

Key words: Poly herbal formulation, Alloxan, Diabetes

INTRODUCTION

Diabetes is an epidemic disorder, including chronic complications characterized by damage, dysfunction and eventual failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels [1]. The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind health in all parts of the world. Asia-Pacific region is of prime
importance to the epidemiology of diabetes. The region combines a high proportion of the world’s population with rapidly rising diabetes prevalence rates. In India the figures are predicted to rise from an estimated 15 million in 1995 to 57 million in 2025 [2].

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called “alloxan diabetes”) in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin-producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The toxic action of alloxan is initiated by free radicals formed in this redox reaction which damages the β cells [3].

The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind in all parts of the world. Traditional medicine has proved to possess good therapeutic value showing a bright future in the treatment of diabetes mellitus [4]. *Biophytum sensitivum* Linn. has been proven to stimulate the weight of spleen and thymus thus increasing the production of immune cells. It enhances the antibody production by increasing the antibody forming cells [5]. The sprouts of *Bambusa arundinacea* Retz. are beneficial in dressing the wounds and in ulcers, dyspepsia, nausea, intestinal worms and flatulence. Roots are diuretic, tonic, laxative and cooling and useful in relief from kapha, pitta, leprosy, skin diseases, burning sensation and ringworm [6]. *Artocarpus heterophyllus* Lam. is used for curing inflammation, constipation, wound healing and skin diseases. Bark and leaves are used for the remedy of chest pain and vomiting. Roots are used in the treatment of respiratory ailments which include difficult and painful breathing [7]. The leaves of *Trigonella foenum-graecum* Linn. are aromatic, cooling and mild laxative. The seeds exercise soothing effect on the skin. It relieves irritation of the skin and alleviates swelling and pain. Seeds have hypolipidemic, hypoglycaemic, gastroprotective, antioxidant and immunomodulatory properties. It has been traditionally used for diabetes and also to stimulate lactation [8]. The present study was hence designed to evaluate the therapeutic value of the formulation of these plants in the treatment of alloxan induced diabetes.

**MATERIALS AND METHODS**

**Identification and authentication:** Plant sources selected for the present study were *Biophytum sensitivum* Linn (leaves), *Bambusa arundinacea* Retz (leaves), *Artocarpus heterophyllus* Lam (leaves), *Trigonella foenum graecum* Linn (seeds). Plants were collected from Trichy, identified with the help of flora of Presidency of Madras and authenticated with the specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph’s college, Trichy.

**Preparation of the formulation:** Aqueous extract of each plant was prepared separately as follows. The plant materials were shade dried and coarsely powdered with electrical blender. 200gm of the plant powder was mixed with 1200 ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract was obtained. The aqueous extracts of *B. sensitivum* Linn, *B. arundinacea* Retz, *A. heterophyllus* Lam and *T. foenum graecum* Linn were mixed in the ratio of 1:2:2:4 respectively.

**Experimental models:** Thirty six albino rats of Wistar strain, each weighing 150-180g were selected and used for the present study. The animals were housed in clean polypropylene cages and were fed with standard pellet diet and water *ad libitum*. The animals were exposed to alternate cycle of 12h of darkness and light each and maintained in controlled temperature (22 ± 2° C).

**Experimental design:** The animals were divided into 6 groups comprising of 6 rats each. Group I
served as normal control. Group II disease control received intraperitoneal injection of alloxan (150mg/kgbw) as a single dose. Alloxan at the above mentioned dose was administered to Groups III, IV and V. Group III and IV were treated with the formulation at a dose level of 500mg, 750mg/kg body weight and group V was treated with glibenclamide at a dose of 200mg/kg body weight for 45 days. Group VI received 750mg/kg body weight of the formulation alone for 45 days. 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. Liver was dissected out and washed in ice-cold saline. The parameters studied were plasma glucose [9], glycosylated hemoglobin [10], serum marker enzymes (AST, ALT and ALP) [11], glucose-6-phosphatase [11], glucokinase [12], hepatic glycogen [13] and serum insulin  [14].

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

RESULTS

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

Table 2 indicates the levels of the glucose metabolizing enzymes in the experimental animal models. 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.
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 hepatic marker enzymes AST, ALT and ALP in the animal models are depicted in Table 3. The disease control group shows an elevation in the levels of these enzymes. The enzyme levels were restored to near normal in the formulation treated groups.

**DISCUSSION**

Diabetes is characterized by abnormal metabolism of blood sugar and defective insulin. Alloxan, a beta-cytotoxin causes a massive destruction of β-cells of the islets of langerhans resulting in reduced synthesis and release of insulin, leading to hyperglycemia [15]. 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. The decrease in the uptake of glucose may also be a reason for the increased blood glucose level. The possible mechanism of action of the formulation to reduce the glucose level might be the potentiation of pancreatic secretion of insulin from β-cells of islets that result in enhanced transport of blood glucose to peripheral tissue.

The excess of glucose present in the blood during diabetes react with hemoglobin and form glycosylated hemoglobin. Glycosylated hemoglobin was found to be increased in diabetic mellitus and the amount of increase is directly proportional to that of fasting blood glucose level [16]. The various proteins including hemoglobin, albumin, collagen and crystalline proteins undergo nonenzymatic glycation in diabetes [17]. The hemoglobin level was decreased in diabetic rats that may be due to increased formation of glycosylated hemoglobin.

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 glucogenolysis and inhibition of glycogen synthase, due to insulin deficiency [18]. Significant increase in the liver glycogen by administration of herbal formulation might be attributable to the stimulation of insulin release from β-cells that stimulates the activity of a multifunctional enzyme glycogen synthase.

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, hepatic tissues are incapable to utilize peripheral glucose and hence increase the synthesis of glucose-6-phosphatase to enhance gluconeogenesis [19].

On administration of the herbal formulation dose dependently decrease in 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 the hepatic tissue thereby inhibiting gluconeogenesis.

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 [20]. Oral administration of the formulation increased the activity of the enzyme glucokinase possibly due to the activation of mRNA coding for hexokinase synthesis.

The marker enzymes are located in the cytoplasm of hepatocytes under normal conditions. Hence only low levels are found in the circulation. These enzymes are leaked out into the bloodstream by the adverse effect of alloxan.
Alloxan induction causes extensive damage of the liver which results in an increase in serum marker enzymes such as AST, ALT and ALP in diabetic animals [21]. Administration of the formulation to alloxan induced diabetic rats reduced the levels of serum marker enzymes AST, ALT and ALP indicating that the formulation protects the liver from the adverse effects of alloxan and repairs the damage caused. 

CONCLUSION

The efficacy of any drug is essentially dependent on its ability to reduce the harmful effects of the toxin ingested. The present study shows the antidiabetic efficacy of the plants selected which is evident from the restoration of the biochemical picture of the animals.

REFERENCES