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RESEARCH ARTICLE

ANTIDIABETIC ACTIVITY OF A HERBAL FORMULATION IN ALLOXAN INDUCED TOXICITY IN ALBINO RATS

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Abstract

Diabetes is a chronic complication characterized by damage, dysfunction and ultimate failure of eyes, kidneys, nerves, heart and blood vessels. The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind in all parts of the world. Traditional medicine has been used to treat various ailments from the vedic era and possess good therapeutic value. The use of plants in traditional medicines has been mentioned in the Charaka Samhita.. The present study was designed to evaluate the antidiabetic potential of a poly herbal formulation comprising of *Cinnamomum zeylanicum* Blume, *Andrographis paniculata* Nees, *Cuminum cyminum* L in the ratio 1:2:1. Albino rats 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 (120mg/kg b.wt), Group III- Alloxan+ Formulation (500mg/kg b.wt), Group IV- Alloxan + Formulation (750mg/kg b.wt), Group V- Formulation treated (750mg/kg b.wt) and Group VI- Alloxan + Glibenclamide (100mg/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, serum and tissue proteins, serum urea and creatinine. Alloxan induced disease control group showed significant increase in plasma glucose, Glycosylated hemoglobin, glucose-6-phosphatase, serum urea and creatinine. It also showed significant decrease in serum insulin, hepatic glycogen, glucokinase, serum and tissue protein. Oral administration of the formulation restored the level of biochemical parameters and glucose metabolising enzymes. From the present observation, it is evident that the formulation could be an effective antidiabetic drug in alloxan induced diabetes.

INTRODUCTION

Diabetes is a condition of hyperglycaemia which includes chronic complications categorized by damage, dysfunction and ultimate failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Cohen et al., 2007). The rapidly increasing incidence of diabetes mellitus is becoming a serious threat to mankind 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 15 million in 1995 to 57 million in 2025(Cockram, 2000).

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is 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 beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction (Lenzen, 2008).

The present study was designed to analyse the anti diabetic effect of a herbal formulation comprising of *Cinnamomum zeylanicum* Blume, *Andrographis paniculata* Nees, *Cuminum cyminum* L in the ratio 1:2:1.

Cinnamomum zeylanicum Blume possess various medicinal properties. It has been recorded that the volatile oil obtained from the bark, leaves and root vary in its chemical constituents and hence each part of the plant may possess different therapeutic effect (Shen et al., 2002). The primary constituents of the plant are cinnamaldehyde in the bark, eugenol in the leaves and camphor in the roots (Gruenwald, 2010). The plant possess antimicrobial and anti parasitic activity, lowers blood glucose and blood pressure, decreases cholesterol levels in the serum, anti inflammatory, antinociceptive and anti gastric ulcer possess antioxidant & hepatoprotective properties, prevents alzheimer's disease (Priyanga Ranasinghe,2013).

Andrographis paniculata Nees is called the "King of Bitters". Andrographolide, neoandrographolide and kalmeghnin are the active principles of the plant (Niranjan, 2010). The plant has been traditionally used in the treatment of liver diseases which has been mentioned in Charaka Samhita (Dhiman Anju,2012). It is used in fevers and to remove toxins from the body. It is also used to treat GI and upper respiratory tract infections and in herpes. It also reveals antibacterial, antimalarial, filaricidal, antridiarrhoeal properties (Niranjan, 2010).

The main phytochemical constituents of *Cuminum cyminum* L are Cuminaldehyde, Limonene, eugenol, α- and β- pinenes (Johri, 2011; Dorman and Deans,2000). Cumin seeds are being used in the treatment of diarrhoea, dyspepsia, flatulence, morning sickness and colic. It promotes the assimilation of other herbs (Deepak, 2013). It also exhibits antimicrobial (Johri, 2011; Dorman and Deans, 2000), anti epileptic (Janhmadi et al., 2006) and anti tumorogenic (Gagandeep et al., 2003) effects. Hence the present study was designed to evaluate the anti diabetic effect of these plants as a formulation.

MATERIALS AND METHODS

Identification and authentication:

Plant sources selected for the present study were *Cinnamonum zeylanicum* Blume (bark), *Andrographis paniculata* Nees (Aerial parts),*Cuminum cyminum* L (seeds). Plants were collected and purchased 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. 200gmof 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 *Cinnamonum zeylanicum* Blume (bark), *Andrographis paniculata* Nees (Aerial parts),*Cuminum cyminum* L (seeds) were mixed in the ratio of 1:2:1 respectively and the formulation was used for the study.

Experimental models:

Albino rats of Wistar strain, each weighing 150-200g were selected and used for the present study. The animals were acclimatized to the laboratory conditions for 10 days prior to the commencement of the experiment. 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 an ambient temperature (22 ± 2° C), with 65± 5% humidity.

Experimental design:

Animals were divided into six groups of six rats each. Group I served as Normal control, Group II- Alloxan induced disease control (120mg/kg b.wt- IP), Group III- Alloxan+ Formulation (500mg/kg b.wt), Group IV- Alloxan + Formulation (750mg/kg b.wt), Group V- Formulation treated (750mg/kg b.wt) and Group VI- Alloxan + Glibenclamide (100mg/kg b.wt) respectively for a period of 45 days.

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 (Folin and Wu, 1919), glycosylated haemoglobin (Nayak and Pattabiraman,1981), glucose-6-phosphatase (King,1965), glucokinase (Brandstrup et al.,1957), hepatic glycogen

(Morales et al,1973), serum insulin (Morgan,1963), serum and tissue proteins (Lowry et al., 1951), serum urea (Natelson et al.,1951) and creatinine (Bonsnes and Taussky, 1945).

Statistical Analysis

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

RESULTS

The results depicted in **Table 1** clearly indicate significant increase in the blood glucose, urea and creatinine levels with a marked decrease in serum and tissue protein levels in alloxan induced diabetic rats. The formulation treated groups show a marked decrease in the blood glucose, urea and creatinine levels and an increase in serum and tissue protein levels when compared with Group II animals.

The present study showed a significant decrease in insulin and hepatic glycogen with an elevation in the glycosylated haemoglobin level in alloxan induced diabetic rats (Group II) when compared with normal control rats (Group I) as shown in **Table 2.** The formulation treated groups showed an increase in the insulin and hepatic glycogen levels and simultaneously decreased the level of glycosylated haemoglobin. The effect of test drug was also comparable to that of standard drug, glibenclamide (Group VI).

Table 3 depicts a significant decrease in glucokinase and an increase in the glucose -6-phosphatase activity in alloxan induced diabetic rats when compared to normal control group. The formulation treated groups (III and IV) showed a profound (P<0.05) increase in glucokinase activity and inhibition of glucose 6 phosphatase.

Table 1

Estimation of Serum Glucose, Urea and Creatinine, Serum and Tissue Protein in diabetes induced and formulation treated animals

Group	S.Glucose (mg/dl)	S.Urea (mg/dl)	S.Creatinine (mg/dl)	S.Protein (mg/dl)	T.Protein (mg/g)
Gp I	118 ± 0.91	28.98± 1.67	1.25 ± 0.09	6.8 ± 1.2	83.67 ± 0.96
Gp II	340 ± 1.02*	68.61 ± 0.25*	8.75 ± 0.95 *	2.62 ± 0.96*	41.32 ± 1.03*
Gp III	156.2 ± 0.56	38.63 ± 0.67	3.61 ± 0.61	5.41 ± 0.11	64.67 ± 0.62
Gp IV	109.62 ± 0.44**	24.23 ± 0.42**	1.10 ± 0.36**	5.98 ± 0.98**	82.33 ± 0.78**
Gp V	111 ± 0.73	28.09 ± 0.62	0.98 ± 1.10	6.01 ± 0.63**	76.14± 0.49
Gp VI	119 ± 0.72**	23.45 ± 0.55**	1.06 ± 0.76 **	5.93 ± 0.52	83.31 ± 0.61**

Values are mean ± S.E.M. (n=6)

*p<0.05 Statistically significant when compared with normal control

** p<0.05 Statistically significant when compared with alloxan treated group

Table 2
Estimation of hepatic glycogen, serum insulin, glycosylated haemoglobin in diabetes induced and formulation treated animals

Group	Hepatic Glycogen (mg/g)	Serum Insulin (µIU/ml)	Glycosylated Hemoglobin (%)
Gp I	38.87 ± 0.33	38.6 ± 4.5	2.44 ± 0.29
Gp II	18.54 ± 0.81*	8.2 ± 1.3*	4.68 ± 0.68*
Gp III	26.66 ± 0.99	30.6 ± 2.1	3.01 ± 0.19
Gp IV	34.76 ± 0.63**	34.5 ± 1.9**	2.52 ± 0.66**
Gp V	31.89 ± 0.62	36.4 ± 0.88**	2.38 ± 1.4**
Gp VI	36.45 ± 0.98**	30.6 ± 0.68	2.68 ± 0.76**

Values are mean ± S.E.M. (n=6)
*p<0.05 Statistically significant when compared with normal control
** p<0.05 Statistically significant when compared with alloxan treated group

Table 3
Assay of the activity of glucose-6-phosphatase, glucokinase in diabetes induced and formulation treated animals

Group	Glucose -6 phosphatase (mg/g)	Glucokinase (µIU/ml)
Gp I	0.150 ± 0.021	207.5 ± 6.4
Gp II	0.252 ± 0.028*	115.4 ± 8.9*
Gp III	0.201 ± 0.036	163 ± 4.2
Gp IV	0.199 ± 0.006**	217.2 ± 3.9**
Gp V	0.172 ± 0.52	198 ± 0.98
Gp VI	0.163 ± 0.36**	186.5 ± 0.39**

Values are mean ± S.E.M. (n=6)
*p<0.05 Statistically significant when compared with normal control
** p<0.05 Statistically significant when compared with alloxan treated group

DISCUSSION

Plants ease hyperglycemia by promoting regeneration of beta cells or by protecting the cells in pancreas from destruction. The plants restrict glucose load and promotes unrestricted endogenous insulin action (Jadhav et al.,2009). Alloxan is widely used to induce diabetes in experimental animals. The cytotoxic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Highly reactive hydroxyl radicals are also formed by Fenton reaction. The actions of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of beta cells and thus increase the blood sugar level (Ankur and Shahjad, 2012).

Alloxan is taken up by the pancreatic beta cells, a process which determines alloxan diabetogenecity. Beta cells utilise various reducing agents like GSH, cysteine, ascorbate and protein bound sulphydryl groups for the reduction processes. Alloxan binds to two sugar binding sulphhydryl groups in the glucokinase thus inactivating the enzyme.

Alloxan is hydrophilic and has a similar shape as that of glucose. This promotes its selective uptake in the beta cells and also permits it to be transported through the GLUT transporters in the membranes (Ankur and Shahjad, 2012). The possible mechanism of the antidiabetic potential of the plant extract may be due to the regeneration of β cells of pancreatic secretion of insulin or enhanced transport of blood glucose to peripheral tissue.

Inhibition of Glucokinase reduces glycolysis and ATP synthesis. This further suppresses glucose induced insulin production (Lenzen and Panten 1988; Tiedge etal., 2000).

Glucose-6-phosphatase is an important regulatory enzyme in gluconeogenesis. In diabetic animals the enzyme levels were observed to increase. The increased activity of glucose 6-phosphatase in liver of the alloxan induced diabetic rats may be due to insulin insufficiency.

Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase (Murray et al., 2000).

In the formulation treated rats glucose-6-phosphatase was significantly reduced in liver which may be due to increased insulin secretion, which is responsible for the repression of the gluconeogenic key enzymes.

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 (Vasabthakumari and Shyamaladevi, 1998). 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 and tissue protein levels in Group III and Group IV animals. Insulin stimulates the uptake of amino acid and plays a major role in activating the synthesis of proteins.

In diabetes, the glycogen content of the skeletal muscles and liver, markedly depleted and the reduced level of hepatic glycogen is mainly due to inadequate insulin secretion. Insulin deficiency inactivates glycogen synthetase system (Chakrabarthi et al., 2003).

Regeneration of the necrosed hepatic tissue reversed the functional status of the cells, increasing the expression of glycogen synthase and increasing glycogen synthesis and storage in the liver (Sumana & Suryawanshi, 2001). Hence the increased levels of hepatic glycogen in the formulation treated groups indicate the regeneration of the necrosed hepatic cells, restoring the normal function in the hepatocytes.

The blood urea and creatinine are considered as significant markers of renal dysfunction. Diabetes mellitus also causes renal damage due to abnormal glucose regulation including elevated glucose and glycosylated protein levels, haemodynamic changes within the kidney and increased oxidative stress (Aurell and Bjorck, 1992). The formulation refurbished the serum urea and creatinine levels symbolizing the restoration of normal biochemical picture in the test animals.

CONCLUSION

The results indicate that the formulation comprising of Cinnamomum *zeylanicum* Blume, *Andrographis paniculata* Nees, *Cuminum cyminum* L possess hypoglycaemic effect in alloxan induced diabetes as evident from its capacity to enhance insulin synthesis, regulation of the biochemical indices and regeneration of the necrosed hepatic cells.

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