Hypoglycaemic and Antioxidant Activity of SPHAG - a Poly Herbal Formulation in Alloxan Induced Wistar Albino Rats

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Abstract

Management of Diabetes mellitus is one of the greatest challenges among the developing and developed countries. The use of herbal medicine is steadily growing in the management of various diseases all over the world. The present study has been taken up on a poly herbal formulation -SPHAG, a combination of aqueous extracts of plants *Solanum nigrum*, *Premna corymbosa*, *Holarrhena pubescens*, *Alstonia scholaris* and *Gymnema sylvestre* for its hypoglycaemic and antioxidant activity in alloxan induced diabetic Wistar albino rats. The four groups containing 6 animals in each group, like Healthy Control, Disease Control, SPHAG lower dose(250 mg/kg.b.wt.) and SPHAG higher dose (500 mg/kg.b.wt.) were maintained. The oral administration of SPHAG had showed significant reduction in the glucose level and HbA1C level when compared with Disease Control. Biochemical parameters comprising of liver function and renal function tests had shown improved health status in SPHAG treated groups over Disease Control group. The antioxidant enzymes superoxide dismutase, Glutathione peroxidase levels in blood and tissues including liver, kidney and heart were found to be decreased in the Disease control group. SPHAG treated animals showed significant improvement on the antioxidant enzyme levels and the efficacy is found to be dose dependent. Thus, the present study has demonstrated hypoglycaemic and antioxidant potential of SPHAG in the experimental animals. The synergistic contribution of major phyto-constituents of SPHAG i.e. flavonoids and phenols are expected for its biopotency and efficacy.

Key words: hypoglycaemic activity, antioxidant potential, superoxide dismutase, peroxidise

Introduction

Diabetes mellitus is recognized as one of the leading cause of morbidity and mortality in the world. Diabetes is a condition characterized by hyperglycaemia resulting from the body's inability to use blood glucose for energy (1-3). It is one of the major complications affecting both quality and length of life. It is characterized by altered carbohydrate, lipid and protein metabolism. Diabetes is caused by insufficient production of the hormone insulin by the pancreas or insensitivity of cells to the effects of insulin (4-5). India has the highest prevalence of diabetes in the world, accounting for almost one sixth of the world's diabetic patients. Currently only 10% of patients in India are receiving appropriate treatment (6-8).

The reasons for this could include the variability of healthcare available in different areas and cost of the medicine. Thus there is an urgent need to develop a cost-effective protocol for diabetes care aimed at improvised treatment with cost-effective method. The use of plants as a source of medicine will be one of the promising way of management of Diabetes in the developing countries like India (9-11). The present study has been taken up to evaluate the hypoglycaemic and antioxidant effect of the polyherbal formulation SPHAG. SPHAG is the plant product developed by the combination of aqueous extracts of plants *Solanum nigrum, Premna corymbosa, Holarrhena pubescens, Alstonia scholaris and Gymnema sylvestre.*

Materials and Methods

Plant Collection and Identification

The plant materials *Solanum nigrum* Leaf, *Premna corymbosa* Leaf, *Holarrhena pubescens* Bark, *Alstonia scholaris* Leaf and *Gymnema sylvestre* Leaf were purchased from the local drug supplier of the institute and these plants were authenticated in the Pharmacy Division of National Research Institute for Panchakarma (NRIP), Cheruthuruthy.

Plant product SPHAG

SPHAG is a polyherbal formulation made out of the combination of aqueous extracts of plants *Solanum nigrum* Leaf, *Premna corymbosa* Leaf, *Holarrhena pubescens* bark, *Alstonia scholaris* Leaf and *Gymnema sylvestre* Leaf. The aqueous extract was prepared as per the Ayurvedic Pharmacopeia of India and

the product was developed by combination of equal quantity of extracts (1:1:1:1:1). The product was stored in 4 -8^0 C for the purpose of animal experiment.

Chemicals and Reagents

Chemicals of Analytical Grade from SRL India and Biochemical kits from Transasia Ltd., Mumbai, Randax Ltd., UK and Bayer India Ltd., Mumbai were used.

Phytochemical Analysis

Phytochemical analysis was carried out as per the standard protocols like Salkowski test, Dragendorff's test, Keller Kilani test and Ellagic acid test protocols (12-15).

Animal Experimentation

Wistar albino rats were procured from the Small Animal Breeding Station, Government Veterinary College, Thrissur, Kerala. Animals were acclimatized to the standard laboratory condition before starting the experiment. The animal studies were carried out in the National Research Institute for Panchakarma, Cheruthuruthy as per CPCSEA guidelines and with the approval of Institutional Animal Ethical Committee.

Six to seven months old Wistar albino rats of both sexes weighing 150-200 gm were used for the experiment. The animals were fed with standard laboratory pellet chow (Amrit, Bangalore) and given water *ad libitum*. All rats were clinically healthy. The animals were randomly divided into four groups of six animals each as per the standard protocol (16-18).

Diabetes mellitus was induced by injecting 4% alloxan monohydrate 150 mg/kg.b.wt., i.p. (intra-peritoneal) in normal saline. All the rats were fed with the same normal diet. 12 hrs fasting blood glucose levels was analyzed on the 4^{th} day after injecting alloxan, and the rats with blood glucose level > 150 mg/dl were selected for the present study. The experimental animals were grouped as follows.

Group I : Healthy Control Group (HC)

These animals were not treated with alloxan. They received standard food and water throughout the experimental period i.e. 15 days.

Group II: Disease Control Group (DC)

These animals were treated with alloxan and their blood glucose levels were > 150 mg/dl. They received standard food and water throughout the experimental period

Group III: Experimental Group: Test Drug-Lower Dose (LD)

These animals were treated with alloxan and their blood glucose levels were > 150 mg/dl. They received standard food and water throughout out the experiment along with daily single dose of SPHAG extract 250 mg/kg. b.wt..

Group IV: Experimental Group- Test Drug-Higher Dose (HD)

These animals were treated with alloxan and their blood glucose levels were > 150 mg/dl. They received standard food and water throughout out the experiment along with daily single dose of SPHAG extract 500 mg/kg. b.wt.

On the 16^{th} day, blood samples were collected from retro orbital puncture under keeping the animals for 12 hrs fasting. At the end of the experiment, the animals were sacrificed by cervical dislocation under diethyl ether anaesthesia and the tissue samples of liver, kidney and heart were collected for evaluation of antioxidant enzymes in tissue level among all the experimental groups.

Biochemical and Antioxidant Assays

Biochemical parameters including glucose level, Albumin, globulin, total protein status, lipid profile, liver function parameters and renal function parameters were evaluated (19-20). Antioxidant enzymes superoxide dismutase and glutathione peroxidase levels were also estimated as per the standard clinical laboratory protocols (21-23).

Superoxide Dismutase

The role of superoxide dismutase (SOD) was to accelerate the dismutation of the toxic superoxide radical ($O2^{\cdot}$), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. The method employed xanthine and xanthine oxidase to generate superoxide radicals, which reacted with 2-(4-idophenyl)-superxoixde dismutase 3-(4-nitrohpenol)-5- phenyltetrazolium chloride to form a red formazan dye. The superoxide dismutase activity was then measured by the degree of inhibition of the reaction.

Glutathione Peroxidase

Glutathione peroxidase (GPx) catalyses the oxidation of Glutathione by Cumene Hydro peroxides. In the presence of Glutathione reductase and NADPH the oxidized Glutathione (GSSG) was immediately

converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

Statistical Analysis

The data were expressed as mean ± SEM and statistically analyzed by one way ANOVA.

Results

The present study has demonstrated the hypoglycaemic and antioxidant potential of SPHAG –a polyherbal formulation in the alloxan induced diabetic Wistar albino rats. The phytochemical analysis on the SPHAG has shown that the presence of flavanoids, phenols, alkaloids and other components at various levels (Table 1).

During the treatment with alloxan for inducing experimental diabetes condition, it was observed that the rise in blood glucose level reached its maximum peak to 286 mg % on the third day after the injection and found to be stable throughout the study period. Treatment with SPHAG on two different doses 250 mg/kg.b.wt and 500 mg/kg/b.wt caused significant reduction in blood glucose level while comparing with Disease control group. The maximum reduction was achieved at higher dose and blood glucose reached nearly 155 mg %.

The HbA1C level was found to be 9.73 in disease control, and it has been reduced to 6.86 and 5.71 in lower and higher dose of SPHAG treated groups respectively. The other biochemical parameters like renal function tests including urea and creatinine, liver function tests including SGOT, SGPT, and lipid profile parameters had shown that two to three fold elevated level in disease control group (Table 2). There was significant reduction of these levels in the SPHAG treated groups. Thus the overall study demonstrated that the polyherbal formulation SPHAG is having significant hypoglycaemic activity in alloxan induced diabetic rats.

The antioxidant enzymes such as superoxide dismutase and glutathione peroxidase levels in blood and tissue samples (liver, kidney and heart) were estimated in all the experimental animals. Alloxan induced Disease control group exhibited the significant reduction of antioxidant enzymes when compared with healthy control group. The SPHAG treated diabetic rats exhibited improved status of antioxidant levels in blood and it was comparable to healthy control group (Table 3). The antioxidant enzymes in liver, kidney and heart tissues have also shown the elevated levels of SOD and Gpx in SPHAG treated animals over the Disease control group (Figure 1-2).

Table 1. Phytochemical Analysis of the SPHAG

Phytochemical Analysis	SPHAG
Alkaloids	++
Flavanoids	+++
Saponins	++
Carbohydrates	++
Proteins	++
Phenols	+++
Tannins	+
Glycosides	-
Steroids	++

+++ Strongly Present ++ Moderate Present + Present - Nil

Table 2. Effect of SPHAG on biochemical parameters in alloxan induced diabetic rats.

Parameters	Experimental Groups			
	Group I: Healthy Control	Group II: Disease Control	Group III: SPHAG- Lower Dose (250 mg/kg.b.wt.)	Group IV: SPHAG- Higher Dose (500 mg/kg.b.wt.)
Glucose	98.50 ± 13.80	285.46 ± 34.60*	185.50 ± 26.90*	153.50 ± 21.00*
HBA1c	4.93 ± 0.57	9.73 ± 1.25	6.86 ± 1.60	5.71 ± 1.06
Mean Blood Glucose (MBG)	77.50 ± 15.7	193.26 ± 19.80*	158.65 ± 21.30*	114.80 ± 23.85*
Protein	6.7 ± 0.3	6.16 ± 0.85	6.6 ± 0.55	7.1 ± 1.30
Albumin/Globulin ratio	2.6 ± 0.57	1.5 ± 0.63	1.8 ± 0.30	2.2 ± 0.51
Urea	43.66 ± 5.07	77.0 ± 7.21**	69.50 ± 6.80*	53.16 ± 8.30*
Creatinine	0.9 ± 0.05	1.6 ± 0.28*	1.20 ± 0.20**	1.1 ± 0.10*
SGOT	79.9 ± 14.73	164.0 ± 38.54*	110.0 ± 14.50*	93.25 ± 13.7*7
SGPT	52.65 ± 11.80	68.20 ± 15.64*	46.30 ± 10.70*	50.48 ± 13.77*
Cholesterol	79.90 ± 30.50	166.50 ± 26.80*	88.60 ± 13.52*	72.30 ± 17.50*
Triglycerides	67.00 ± 12.50	215.30 ± 63.70*	169.67 ± 45.20	112.40 ± 36.40*

Values are expressed as Mean ± S.D; n=6 animals in each group. p <0.05, p<0.01 when compared to disease control.

Table 3 : Estimation of Antioxidant enzyme levels in the SPHAG treated animals.

Experimental Groups	Antioxidant enzymes level in blood	
	SOD (U/mg protein)	GPx (U/mg protein)
Group I: Healthy Control	9.67 ± 0.53	4093.90 ± 669.50
Group II: Disease Control	5.63 ± 0.70*	3357.65 ± 477.69*
Group III: SPHAG- Lower Dose (250 mg/kg.b.wt.)	6.48 ± 0.37*	4172.60 ± 627.69**
Group IV: SPHAG- Higher Dose (500 mg/kg.b.wt.)	7.10 ± 0.43*	4664.25 ± 714.91**

Values are expressed as Mean ± S.D., n=6 animals in each group. p <0.05, p<0.01 when compared to Disease control.

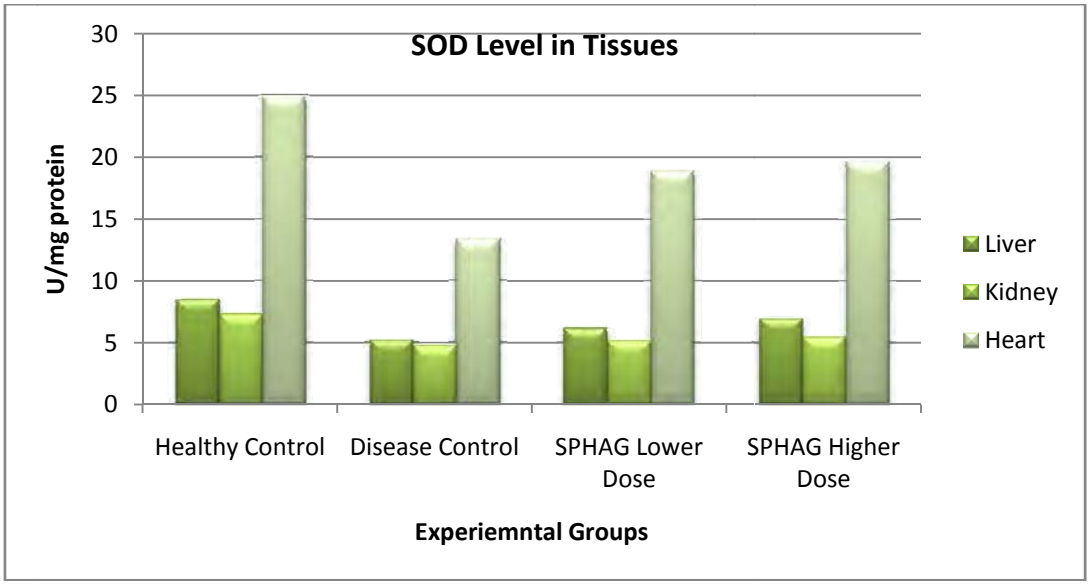

Figure. 1. Effect of SPHAG on Superoxide dismutase level in different tissues of alloxan induced diabetic rats

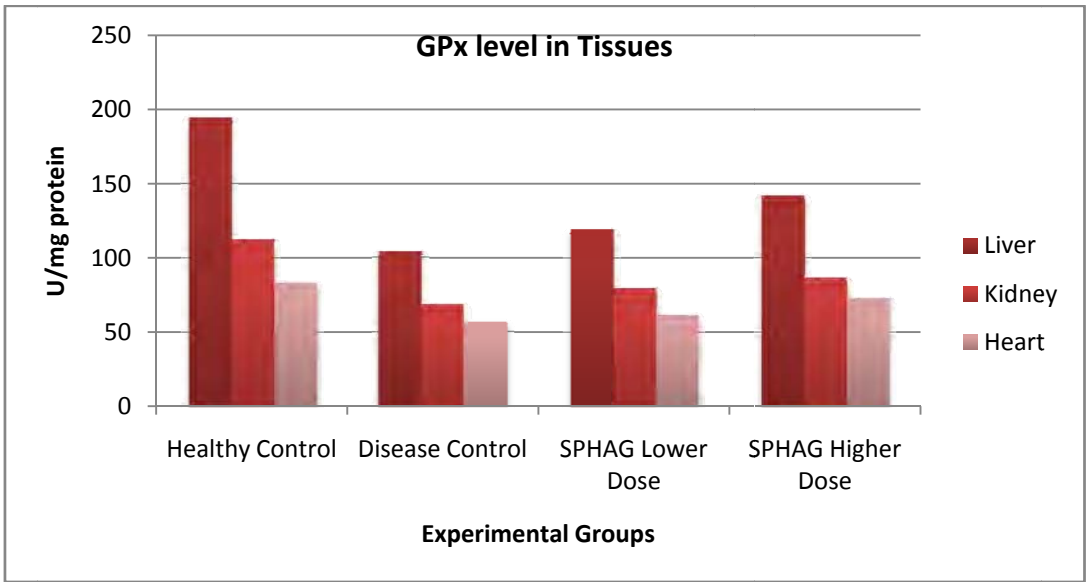

Figure. 2. Effect of SPHAG on Glutathione peroxidase level in different tissues of alloxan induced diabetic rats.

Discussion:

Diabetes mellitus is a clinical condition caused by indented or acquired deficiency of insulin by the pancreas or by ineffectiveness of insulin produced. Such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's system (24). The depletion of defensive body chemicals called antioxidants may increase the risk complication from the most common form of diabetes mellitus. Some complications of diabetes are associated with increased activity of free radicals induced lipid peroxidation and accumulation of lipid peroxidation products (25-26).

Apart from the currently available therapeutic options in modern medicine, many herbal medicines are also recommended for the treatment of diabetes. As every country have their own indigenous plant based medicines for the treatment and prevention of diabetes mellitus (27-30), the present study has been taken up with a novel formulation developed (SPHAG) comprising of some selected medicinal plants that are traditionally used in India for various ailments.

In principle, the study has shown that SPHAG has the significant hypoglycaemic activity at the 250 and 500 mg/kg.b.wt dosages, and the efficacy of the drug is found to be dose dependent. Even though, the administration of SPHAG did not bring the glucose level to the normal range at the selected doses, there was significant decrease in glucose and HbA1C level in the SPHAG treated groups when compared with Disease control group.

The other biochemical parameters like lipid profile, liver function and renal function tests were shown that the test drug has decreased their elevated levels in the treatment groups. It proves the safety of the SPHAG at the prescribed dosages.

So, the present study concluded that SPHAG has potential hypoglycaemic activity and antioxidant activity in the alloxan induced diabetic animal model system. It is understood that the strong presence of phyto-constituents such as flavanoids and phenols may be contributing individually and synergistically for the efficacy of the SPHAG. Further research is very much required on identification of functional compounds and their synergic action with respect to hypoglycaemic and antioxidant functions.

References:

[1] King H, Rewers M. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults: WHO Ad Hoc Diabetes Reporting Group. Diabetes Care 1993;16: 157-177.

[2] Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabet Met. 1997; 14 (Suppl.5); S1-S85.

[3] International Diabetes Federation: Diabetes Atlas 2000. Brussels, International Diabetes Federation, 2000.

[4] Morgan CL, Currie CJ, Peters JR: Relationship between diabetes and mortality: A population study using record linkage. Diabetes Care 2000; 23: 1103-1107.

[5] Ramachandran A, Snehalatha C, Latha E, Manoharan M, Vijay V: Impacts of urbanization on the lifestyle and on the prevalence on diabetes in native Asian Indian Population. Diabetes Res Clin Pract. 1999; 44: 207-213.

[6] Ramachandran A. Snehalatha C, Kapur A, Vijay V, Mohan V, Das AK, Rao PV, Yajnik CS, Prasanna Kumar KM, Nair JD. Diabetes Epidemiology study group in India: High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. Diabetologia 2001; 44: 1094-1101.

[7] Mohan V, Pradeepa R. Epidemiology of diabetes in different regions of India. Health Administrator 2009; 22: 1-18.

[8] Raheja BS, Kapur A, Bhoraskar A et al. DiabCare Asia-India Study: Diabetes care in India-Current status. J Assoc Physicians India 2001; 49: 717-722.

[9] Jayaprasad B, Thamayandhi D, Sharavanan PS. Traditionally suing antidiabetic medicinal plants in Tamil Nadu. International Journal of Res in Pharmaceutical and Biosciences. 2012; 2 (1): 1-8.

[10] Anitha VR, Mohan T, Athiperumalsami, Sutha S. Ethnobotanical plants used by Kanilkars of Tirunelweli district, Tamil Nadu to treat skin diseases. Ethno botanical leaflets. 2008; 12:171-180.

[11] Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. J. Ethnopharmacol. 2002; 81: 81-100.

[12] Maluventhan Viji, Sangu Murugesan. Phytochemical analysis and antibacterial activity of medicinal plant Cardiospermum halicacabum Linn. Journal of Phytology. 2010; 2(1):68-77.

[13] Hassan SW, Umar RA, Lawal M, Bilbis LS, Muhammad BY and Dabai YU. Evaluation of antibacterial activity and phytochemical analysis of root extracts of Boscia angustifolia. African journal of Biotechnology. 2006; 5(18):1602-1607.

[14] EL- Olemyl MM, AL-Muhtadi FJ, Afifi AA. Experimental phytochemistry. A Laboratory manual college of Phramcy, King Saud University. King Saud University Press. 1994; 1-34.

[15] Trease GE, Evans WC. A textbook of Pharmacognosy. 11th Edition. Bailliere Tindall Publication. London. 1978; 530.

[16] Nataraja Thamizh Selvam, Nimmi R, Usha Rani K, Saraswathy VN, Venugopalan TN, Sanjayakumar YR, Jaya N. Evaluation of Antioxidant activityof methanolic extract of Pandanus odoratissimus in alloxan induced Wistar albino Diabetic rats. The Antiseptic. 2008; 5: 250-253

[17] 17. Herbert OC, Mbagwu, Clement Jackson, Idonegesit Jackson, Godwin Ekpe, Udeme Eyaeko. Grace Evaluation of the hypoglycemic effect of aqueous extract of Phyllanthus amarus in alloxan-induced diabetic albino rats. Int J Pharm Biomed Res. 2011; 2(3):158-160.

[18] Vadivelan R, Dipanjan M, Umasankar P, Dhanapal SP, Sathishkumar N, Antony S, Elango K. Hypoglycemic, antioxidant and hypolipidemic activity of Asparagus racemosus on streptozotocin-induced diabetic in rats. Advances in Applied Science Research. 2011; 2(3):179-185.

[19] Compagnone D, Federeci G, Massoud R, Santoro L, Anichini M. Analysis for transaminases in serum with an amperometric glutamate. Clinical Chemistry, 1992; 38:2306-2310.

[20] Chandan BK, Sharma AK, Anand KK. Boerhavia diffusa: A study of hepatoprotective activity. Journal of Ethnopharmacology. 1991; 31: 299-307.

[21] Kono Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 1978; 186: 189-195

[22] Kaushik P, Kaushik D, Sukbhir LK. In vivo antioxidant activity of plant Abutilon indicum. J Pharm Edu Res. 2011; 2(1): 50-53.

[23] Habbu P, Shastri, R, Mahadevan KM, Joshi H, Das S. Hepatoprotective and antioxidant effects of Argyreia speciosa in rats. Afr J Tradit Complement Altern Med. 2008; 5(2): 158-64.

[24] Ramachandran A, Snehalatha C, Viswanathan V. Burden of type 2 diabetes and its complications- the Indian Scenario. Curr. Sci. 2002; 83: 1471-1476.

[25] Glugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabet. Care. 1996; 19: 257-267.

[26] Lipinski B. Pathophysiology of oxidative stress in diabetes mellitus. J. Diabet. Complications. 2001; 15: 203-210.

[27] Raman BV, Krishna ANV, Rao BN, Saradhi MP. Plants with Antidiabetic activities and their medicinal values. 2013; 3 (3): 11-15

[28] Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S, Ravikumar A. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. Evid Based Complement Alternat Med. 2011; 5 (1): 147-156.

[29] Kesari AN, Gupta RK, Watal G. Hypoglycemic effects of Murraya koenigii on normal and alloxan-diabetic rabbits. J Ethnopharmacol 2005; 97 (2): 247-251.

[30] Aritajat S, Wutterapol S, Saenphet K. Anti-diabetic effect of Thunbergia laurifolia Linn. Aqueous extract. Southeast Asian J Trop Med Public Health 2004; 35(2):53-58.