Antidiabetic, Anticholesterolemic and Antioxidant Activity of A Herbal Formulation

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Summary

Antidiabetic, anticholesterolemic and antioxidant activities of HF (100 mg/kg and 300mg/kg, p.o., per day for 4 weeks) have been evaluated in Wistar rats. Alloxan monohydrate (150 mg/kg, i.p) was used to induce hyperglycemia, hypercholesterolemia and raised serum lipid peroxide levels. Blood glucose and serum cholesterol were determined using standard diagnostic kits. Antioxidant activity was measured by thiobarbituric (TBA) method. HF (300 mg/kg., per day for 4 weeks) significantly ($p<0.05$) decreased blood glucose, serum cholesterol level and exhibited antioxidant activity in alloxan induced diabetic rats. Thus, the findings of the present study support the folklore use of this herbal formulation for its antidiabetic, anticholesterolemic and antioxidant activities.

Keywords: Herbal formulation, alloxan, antidiabetic, anticholesterolemic, antioxidant

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Introduction

In recent years, developed nations have witnessed an explosive increase in the prevalence of diabetes mellitus (DM) predominantly related to lifestyle changes and obesity. There is an estimated 143 million people worldwide suffering from the disease and this is almost five times the estimate ten years ago. It has been predicted that the number may probably double by the year 2030. The human population worldwide appears to be in the midst of an epidemic of diabetes [1]. Diabetes Mellitus is a group of metabolic disorders characterized by hyperglycemia, hyperlipidemia, polyphagia, polydypsia, ketosis, nephropathy, neuropathy and cardiovascular disorders with absolute or relative deficiencies in insulin secretion and/or insulin action [2]. There is an increasing demand by patients to use the natural products with antidiabetic activity due to the side effects associated with the use of insulin [3]. Plant drugs and herbal formulations are frequently considered to be less toxic and more free from side effects than synthetic ones [4]. Based on the WHO recommendations, a large number of agents of plant origin used in traditional medicine have been attributed to its antihyperglycemic effects [5]. Some herbal formulation like Diasulin, DHF-39 and *Ocimum sanctum* (Linn) seed oil have the potential to reduce blood glucose, blood cholesterol and free radicals in experimental animal models [6-8]. Most of the plants containing substances like glycosides, alkaloids, terpenoids, and flavonoids are frequently implicated as having antidiabetic effects [9]. The folklore HF consists of *Glycyrrhiza glabra, Nelumbo nucifera, Zingiber officinale, Eclipta alba, Hibiscus rosasinensis, Hemidesmus indicus, Rosa damascena, Quercus infectoria* and *Terminalia chebula.* There is no data available in the literature regarding the antidiabetic effects of this folklore formulation. Therefore, the objective of the present study was to explore the potential of the folklore formulation for its antidiabetic, anticholesterolemic and antioxidant activities in rats.

Materials and Methods

Extract

Each 1 gm of folklore HF consists of *Glycyrrhiza glabra*-0.08 g, *Nelumbo nucifera*-0.08 g, *Zingiber officinale*-0.08 g, *Eclipta alba*-0.08 g, *Hibiscus rosasinensis*-0.08 g, *Hemidesmus indicus*-0.08 g, *Rosa damascena*-0.08 g, *Quercus infectoria*- 0.04 g and *Terminalia chebula*-0.08 g. The aerial parts of each of the above plants were sun dried and blended to coarse particles. 0.5 kg of the blended aerial parts of the plant was extracted with 1:1 of acetone- ethanol for 72 h. The extract on removal of the solvent was allowed to cool, dried and powdered. All the dried extracts were blended to produce a HF. Appropriate concentration of the extracts was made in distilled water.

Drugs

Alloxan monohydrate (Sigma, Mumbai), TBA (Sigma, Mumbai), metformin (USV Limited, Daman) and HF (100mg/kg and 300mg/kg, p.o., per day) were used in the study.

2.3 Animals

Albino rats (150-200 g) of Wistar strain were obtained from Serum Institute, Pune. Animals were housed into groups of five at an ambient temperature of 25 ± 1ºC with 12 h light: 12 h dark cycle. Animals had free access to standard laboratory pellet and water.

They were deprived of food but not water 4 h before the experiment. The Institutional Animal Ethical Committee approved the protocol of this study.

Induction of Hyperglycemia, Hypercholesterolemia and Oxidative Stress in Rats

Rats were fasted for 24 h before the induction of diabetes by single injection of freshly prepared alloxan monohydrate (150 mg/kg, i.p.). Parameters studied were:

a) Blood glucose level on 2nd day, 1st week, 2nd week and 4th week

b) Total cholesterol level at the end of 4th week

c) Antioxidant activity at the end of 4th week

Experiment Design

Rats were housed into seven groups of 5 animals each. Rats of group I received standard food and water. Rats of group II received HF (100mg/kg, p.o, per day, for 4 weeks), Rats of group III received HF (300mg/kg, p.o, per day, for 4 weeks), Rats of group IV received freshly prepared Alloxan monohydrate (150mg/kg, i.p.). Rats of group V received Alloxan monohydrate (150mg/kg, i.p.) and HF (100mg/kg, p.o, per day, for 4 weeks). Rats of group VI received Alloxan monohydrate (150mg/kg, i.p) and HF (300mg/kg, p.o, per day, for 4 weeks). Rats of group VII received Alloxan monohydrate (150 mg/kg, i.p) and standard drug metformin (500mg/kg, p.o, per day, for 4 weeks).

The animals in all groups had free access to standard diet and water. HF treatment was started after 2 days of alloxan treatment following confirmation of diabetic state in rats and it was continued for a total duration of 4 weeks.

Blood sample Collection

Blood sample was withdrawn by puncture of retro-orbital plexus in rats [10].

Biochemical parameters

Blood glucose was estimated enzymatically by GOD/POD method using kits from Autospan, Mumbai. Serum cholesterol was measured by CHOD-PAP methods using kits from Agappe Diagnostics. Lipid peroxides were measured in serum as thiobarbituric acid reactive substances (TBARS) as per Satoh [11].

Statistical Analysis

All data are expressed as mean $\pm$ SEM. Statistical analysis was done by using one way analysis of variance (ANOVA) followed by Dunnett's test. $P<0.05$ was considered significant.

Results

Effect of herbal formulation on blood glucose level (BGL) and serum cholesterol

The BGL on 1st week and serum cholesterol level in vehicle treated group was 65.04$\pm$ 3.6 mg/dl and 138.8 $\pm$ 3.8 mg/dl respectively. Animals treated with HF (100 mg/kg and 300 mg/kg) alone did not show any significant change in BGL and serum cholesterol as compared to vehicle treated group. There was a significant reduction ($p<0.05$) of BGL and serum cholesterol levels in HF (300mg/kg, p.o, per day, for 4 weeks) treated alloxan-induced diabetic rats as compared to alloxan-induced diabetic rats [Table 1].

Estimation of anti-lipid peroxidation activity of herbal formulation

The antioxidant activity in vehicle treated animals was found to be 2.95 ± 0.35 nM of MDA. Animals treated with HF (100 mg/kg and 300 mg/kg) alone did not show any significant change in antioxidant activity as compared to vehicle treated group. HF (100 mg/kg and 300mg/kg, p.o, per day, for 4 weeks) treatment in alloxan-induced diabetic rats caused a significant ($p<0.05$) reduction in lipid peroxidation compared to alloxan-induced diabetic rats at the end of treatment schedule [Table 2].

Table 1: Effect of HF (100 mg/kg and 300mg/kg, p.o, per day for 4 weeks) on blood glucose and serum cholesterol levels in alloxan induced diabetic rats.

Group	Treatment	Blood glucose level (mg/dl)			Serum Cholesterol (mg/dl)
		1st Week	2nd Week	4th Week	
I	Vehicle	65.04 ± 3.6	52.57 ± 0.65	51.85 ± 1.34	138.8 ± 3.8
II	HF (100 mg/kg)	58.13 ± 2.3	68.04 ± 2.17	65.97 ± 2.21	133.2 ± 11.6
III	HF (300mg/kg)	52.21 ± 1.8	43.92 ± 9.01	42.12 ± 1.36	114.7 ± 3.4
IV	Alloxan (150mg/kg)	124.7 ± 7.91*	96.85 ± 4.70*	94.15 ± 7.81*	189.7 ±20.2*
V	Alloxan (150mg/kg) + HF (100 mg/kg)	104.5 ± 1.0	100.8 ± 2.2	81.15 ± 1.84	153.5 ± 5.70
VI	Alloxan (150 mg/kg) + HF (300mg/kg)	67.55 ± 9.46#	40.62 ± 4.53#	39.15 ± 1.47#	58.28 ±7.40#
VII	Alloxan (150 mg/kg) + Metformin(500mg/kg)	72.52 ± 9.65#	40.62 ±1.57#	45.9 ± 1.47#	56.41 ±6.50#
	F- Value (6,28)	18.52	34.89	14.55	24.00

n=5, the observations are mean ± SEM. *$p<0.05$, as compared to normal control group and #$p<0.05$ as compared to Alloxan group. (ANOVA followed by Dunnett's test).

Table 2: Effect of HF (100 mg/kg and 300mg/kg, p.o, per day for 4 weeks) on antioxidant level in alloxan induced diabetic rats by TBA method.

Group	Treatment	Antioxidant activity (nM of MDA)
I	Vehicle	2.95 ± 0.35
II	HF (100mg/kg)	4.40 ± 1.08
III	HF (300mg/kg)	4.95 ± 0.35
IV	Alloxan (150mg/kg)	13.77 ± 0.28*
V	Alloxan (150mg/kg) + HF (100mg/kg)	3.80 ± 0.63#
VI	Alloxan (150mg/kg) + HF (300mg/kg)	3.76 ± 0.99#
VII	Alloxan (150mg/kg) + Metformin (500mg/kg)	5.43 ± 0.39#
	F- Value (6,28)	31.80

n=5, the observations are mean ± SEM. *$p<0.05$, as compared to normal group and #$p<0.05$, as compared to Alloxan group. (ANOVA followed by Dunnett's test).

Discussion

Several herbal and herbomineral preparations are available in the 'folklore' and indigenous systems of medicine for the treatment of diabetes mellitus. In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than individual plant [12]. Diabetes mellitus is possibly the world's fastest growing metabolic disorder and knowledge on the heterogenecity of this disorder is advanced [13]. The number of functional intact β-cells in the islet organ is of decisive importance in the development course and outcome of diabetes mellitus. Alloxan, a beta cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which decrease utilization of glucose by the tissues [14]. The preliminary phytochemical screening of HF showed the presence of alkaloids, saponins, steroids, flavonoids and tannins [15].

In our study, we have observed that HF (300mg/kg, p.o., per day for 4 weeks) significantly decreased blood glucose in alloxan diabetic rats. During diabetes, enhanced activity of lipases increases lipolysis and releases more free fatty acids into the circulation. Increased fatty acid concentration also increases the β-oxidation of fatty acids, producing more acetyl CoA and cholesterol during diabetes. In normal condition, insulin increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes hypercholesterolemia. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats [16]. Accumulation of triglycerides is one of the risk factor in Coronary Heart Disease (CHD). There was a significant rise in blood glucose level and serum cholesterol level in alloxan treated groups. HF (300mg/kg, p.o., per day for 4 weeks) significantly reduced serum cholesterol in alloxan-induced diabetic rats and thus may prevent the progression of CHD. In aerobic life, lipids containing polyunsaturated fatty acids, proteins, nucleic acids and carbohydrates can be oxidized by free radical mediated reaction. When oxygen is supplied in excess or its reduction is sufficient, reactive oxygen species (ROS) are generated [17]. Hypercholesterolemia can increase production of ROS, which may result in lipid peroxidation leading to increased formation of malondialdehyde (MDA). Serum lipid peroxides were measured as the thiobarbituric acid reactive substances (TBARS). An increase in TBARS levels indicates decrease in antioxidant status. Decrease in rise of TBARS after administration of HF (100 mg/kg and 300 mg/kg, p.o., per day for 4 weeks) suggests that it can decrease lipid peroxidation and improve its antioxidant status. It can thus be concluded that the folklore HF has antidiabetic, anticholesterolemic and antioxidant activity in alloxan induced diabetic rats.

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