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Comparative evaluation of antidiabetic activity of some marketed polyherbal formulations in alloxan induced diabetic rats

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Abstract

A comparison was made between the antidiabetic activities of five different marketed polyherbal formulations in alloxan induced diabetic rats. Serum glucose and other biochemical parameters i.e. total protein, triglyceride and cholesterol were determined at the dose 800 mg/kg body weight, p.o. for 30 days. There is a significant increase in serum glucose ($p<0.001$) and improvement in the other biochemical parameters with treatment of polyherbal formulations which altered in diabetic rats as compared to the standard drug.

Key words : Polyherbal formulations, antidiabetic

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia, hypertriglyceridaemia and hypercholesterolaemia, resulting from defects in insulin secretion or action or both [1.] Two groups of oral hypoglycaemic drugs, sulphonylureas and biguanides, have been used in the treatment of DM. They act by lowering blood glucose levels thereby delaying or preventing the onset of diabetic complications[2]. In traditional practice medicinal plants are used in many countries to control diabetes mellitus. The hypoglycaemic action of these medicinal plants are being studied.[3] Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones[4] .

Five commonly used polyherbal formulations were procured from local market of Wardha, Maharashtra, India to evaluate and compare their antidiabetic activity. The effects produced by these formulations on blood glucose and other biochemical

parameters were evaluated and compared with standard combination of glibenclamide and metformin

Material and methods

Selection and procurement of marketed antidiabetic polyherbal formulations.

Five brands of formulations (solid dosage form) were selected and procured from the local market. The formulations selected were, on the basis of matching the contents maximally, and are consumed by most of the diabetic patients.

BRANDS	BATCH NO.
DW	7-07
HP	Hy-260 H
KR	177-K 406
DB	A035005
GL	9/02

Procurement of animals.

The work was got approved from the Institutional Animal Ethical Committee (Registration No. 535/02/a/CPCSEA/Jan 2002), of Institute of Pharmaceutical Education and Research, Wardha. The adult, healthy albino rats of either sex, weighing in between 150-200g bred in the animal facility of the Institution, were employed for the study. Animals were maintained throughout the study at 24-28 °C, were fed a standard laboratory diet and water *ad libitum* and maintained in specious polypropylene cages and well ventilated animal house with 12 hrs. dark and light cycle.

Procurement of diagnostic kits

Diagnostic kits used for the estimation of Glucose, Cholesterol, Triglyceride and Total Protein were obtained from Merk Limited, Mumbai.

Procurement of diabetic inducer

Alloxan monohydrate was used as the Diabetes inducer in animals and was procured from the Loba Chemicals.

Diabetes induction

Alloxan monohydrate was used for induction of diabetes in rats. The solution was made in normal saline and administered with the single dose of 120 mg/kg body weight, i.p[5].

Dose preparation of the formulation

The weighed amount of powdered formulations was dispersed in 5 % tween 80 and was administered with the dose of 800 mg/kg body weight, p.o[6].

Toxicity evaluation in mice.[7]

The formulations were tested for the acute toxicity (if any) in mice. To determine the acute toxicity of a single oral administration, different doses of the formulation (0.5, 1.0, 1.5 and 2 g/kg BW) were administered to different groups of the mice (three mice were used for each group, control mice received Tween 80). Mortality and general behavior of the animals were observed continuously for the initial four hrs. and intermittently for the next six hrs. and then again at 24 hrs and 48hrs following dose administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsions.

Antidiabetic activity evaluation

Animal groups-

Rats were made diabetic with the single dose of alloxan monohydrate (120 mg/kg body weight, i.p.). After 72 hrs, the animals with fasting blood glucose level >140 mg/dl were considered diabetic. The diabetic rats were randomly divided into eight groups containing six rats in each group. One group of six rats was kept without alloxan monohydrate administration as normal control. The groups were made as follows;

Group I : **Normal,** receive normal rat fed and water, *ad libitum*
Group II : **Diabetic control,** receive 5 % tween 80, with normal rat fed and water, *ad libitum*
Group III : **Diabetic standard,** treated with Glibenclamide + Metformin (5mg/kg + 500 mg/kg, p.o.) in combination.
Group IV : **Diabetic test,** treated with the brand DW (800 mg/kg, p.o.)
Group V : **Diabetic test,** treated with the brand HP (800 mg/kg, p.o.)
Group VI : **Diabetic test,** treated with the brand KR (800 mg/kg, p.o.)
Group VI : **Diabetic test,** treated with the brand DB (800 mg/kg, p.o.)
Group VI : **Diabetic test,** treated with the brand GL (800 mg/kg, p.o.)

Study protocol

The standard and test formulations were administered for 30 days once in a day. After the completion of protocol, the rats were fasted overnight, prior to the blood collection. The blood samples were collected from retro orbital sinus in micro-centrifuge tube. It was kept for 30 min. and centrifuged at 3000 rpm, for 20 min. The serum thus obtained was separated and used immediately for the further estimation. [8]

Statistical analysis

The data obtained in present investigation was subjected to statistical analysis. All results are expressed as Mean ± S.E.M. The data was analyzed using Analysis of variance (ANOVA) and the group means were compared by Dunnet test. Values were considered statistically significant when $p<0.01$.Graph Pad Instat was used for the analysis of data.

Results

Acute toxicity studies

In acute toxicity study no toxic symptoms were observed for all formulations up to dose 2g/kg body weight. All animals behaved normally. No neurological or behavioral effects could be noted. No mortality was found up to 14 days study.

Effect on blood glucose and body weight

Alloxan (120 mg/kg) administration resulted in significant elevation of glucose level and reduction in body weight. Administration of various formulations at dose 800mg/kg administered for thirty days were able to correct this aberration significantly ($p<0.01$). The results of all the formulations tested are presented in Table 1.

Table 1. Effects of polyherbal formulations on body weight and serum glucose in Alloxan- induced diabetic rats

Group(S)	Change in body weight (gm)	Serum Glucose (mg/ dl)	
		Initial	Final
I. Normal	+21.34	77.66 ± 4.91	79.58 ± 3.64
II. Diabetic Control	-31.67	238.56 ± 8.71**	266.48 ± 6.66**
III. Diabetic + Glibenclamide and Metformin	+27.34	231.70 ± 9.55	113.59 ± 4.73**
IV. Diabetic + DW	+14.33	228.06 ± 5.56	139.02 ± 3.63**
V. Diabetic + HP	+11.17	244.58 ± 4.65	123.75 ± 8.35**
VI. Diabetic + KR	+13.66	241.30 ± 8.07	137.52 ± 3.97**
VII. Diabetic + DB	+9.17	242.11± 6.82	130.95± 5.25**
VIII Diabetic + GL	+8.34	241.21± 11.07	151.6± 4.86**

Values are expressed as Mean ± S.E.M. (n= 6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

**$P<0.01$ Results of Gr. I was compared with Gr. II and Gr. II results with Gr. III to VIII

Effect on protein

There was a marked decrease in the plasma protein content of untreated diabetic group ($p<0.01$) when compared with that of the control group. All polyherbal formulations were able to correct this metabolic disturbance significantly as given in Table 2.

Table 2. Effects of polyherbal formulations on some biochemical parameters in Alloxan- induced diabetic rats

Group(S)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Total Protein (g/dl)
I. Normal	111.76± 5.25	80.36± 2.92	7.46± 0.68
II. Diabetic Control	144.31± 5.27	104.73± 4.75	4.04± 0.12
III.Diabetic + Glibenclamide and Metformin	109.08± 6.16	83.86± 3.25	6.86± 0.68
IV. Diabetic + DW	115.06± 3.30	87.7± 4.74	6.06± 0.43
V. Diabetic + HP	116.81± 1.68	86.36± 4.78	6.08± 0.65
VI. Diabetic + KR	116.76± 4.29	88.6± 4.88	5.62± 0.57
VII. Diabetic + DB	118.43± 6.57	89.7± 4.58	6.53± 0.35
VIII Diabetic + GL	119.31± 3.14	89.43± 5.0	5.55± 0.57

Values are expressed as Mean ± S.E.M. (n= 6). Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

***P*<0.01 Result of Gr. I was compared with Gr. II and Gr. II results with Gr. III to VIII.

Effect on lipids

Total cholesterol and triglyceride levels were found to be significantly ($p<0.01$) increased in the vehicle treated diabetic group in comparison with the control group. Treatment with all formulations for a thirty days significantly attenuated ($p<0.01$) the elevated total cholesterol and triglyceride levels in comparison with the vehicle treated diabetic rats given in Table 2.

Discussion and conclusion

Alloxan, a beta cytotoxin, induces diabetes in a wide variety of animal species by damaging the insulin secreting pancreatic beta cell resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the

tissues[9]. Insulin deficiency leads to various metabolic aberrations the animals namely increase blood glucose[10], decreased protein content[11] and increased levels of cholesterol and triglyceride[12].

The present study was conducted to evaluate the antidiabetic activity of some marketed polyherbal formulations. The experimental groups were treated with standard (Glibenclamide + Metformin) and test formulations. The experiment was conducted in accordance with parallel design i.e. each group received single formulation, single time. After completion of the study protocol, it was found that with test and standard treatment, the serum level of glucose, cholesterol, triglycerides and protein inproved significantly ($p<0.01$) as compared to diabetic control.

The significant antidiabetic activity of formulations may be due to inhibition of free radical generation and subsequent tissue damage induced by alloxan or potentiation of plasma insulin effect by increase either pancreatic secretion of insulin from existing beta cells or its release from bound form as indicated by significant improvement in glucose and protein level because insulin inhibit gluconeogenesis from protein.

In comparative evaluation all brands found to be safe as they did not shown any sign of acute toxicity. The formulation HP was found to be more efficacious as compare to other.

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