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A Study on Evaluation of Antidiabetic Activity of A Polyherbal Formulation (Diabestop)

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ABSTRACT
A polyherbal anti-diabetic formulation was prepared from leaves of Momordica chirentia, Ocimum sanctum, Azadirachta indica and buds of Piper longum. The blood glucose lowering capacity of the formulation was studied at 100mg/kg dose at white albino rats of wistar strain and compared with standard drug Glibenclamide at 5mg/kg. Anti-diabetic activity was analyzed in intraperetoneally injected alloxan (150 mg/kg) induced diabetic rats. The various biochemical parameters studied were serum glucose, urea, creatinine, cholesterol, triglycerides, SGOT and SGPT level. The formulation was found to be effective in lowering blood glucose level and in normalizing other organ function significantly.

INTRODUCTION

Diabetes mellitus recognized as early as 1500 B.C. by Egyptian physician, described disease associated with "The passage of much urine"[1]. Diabetes mellitus (DM) is one of the most common endocrine diseases. It describes a group of disorders of varying etiology and pathogenesis usually characterized by elevated blood glucose concentration, reduced insulin action or insulin deficiency[2]. It is associated with abnormalities of glucose, lipid and protein metabolism and the development of both acute and long term complications. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunctioning and failure of various organs like eyes, kidney, nerves, heart and blood vessel[3]. The purpose of therapy in Diabetes mellitus is to restore the metabolism to normal, avoid symptoms due to hyperglycemia and glucoria, prevent short term complication (infection, ketoacidocis etc.) and long term sequels (cardiovascular, retinal, neurological, renal etc.). Oral hypoglycemia agents are useful in the treatment of DM and overcome the limitation of insulin therapy, but their use is restricted by the pharmacokinetic properties, secondary failure rates, and accompanying side effects[4]. The World Health Organization expert committee has announced that the use of alternate medicines and traditional methods of treatment should further be investigated[5-9]. Plants chosen Momordica chirentia, Ocimum sanctum, Azadirachta indica and Piper longum are known to exhibiting antidiabetic effect and anti-oxidant potential[10-13]. Therefore looking into the reported therapeutic potential of these plants present study was aimed to prepare a formulation which will have anti-diabetic potential superior to that of plant if taken individually. Different extracts from plant materials were prepared and the aqueous extracts were combined in different ratios to get best antidiabetic effect. The so formed effective combination was then transformed to the best suitable dosage form which was a suspension and was named as Diabestop. The Diabestop was further evaluated for the anti-diabetic action in white albino rats of wistar strain.

MATERIALS AND METHODS

The polyherbal Anti-diabetic formulation, Diabestop, was prepared in laboratory of Bihar, India. The standard drug for treatment was Glibenclamide, (HiMedia), Alloxane monohydrates (Loba Chemie), Cholesterol, triglyceride, SGOT, SGPT, creatinine and Urea kits (Biosoft Pvt. Ltd).

Procurement of Plant Materials and Selection of Animals: The leaves of Momordica chirentia, Ocimum sanctum, Azadirachta indica and Piper longum bud were procured from Herbal Garden in Bihar. Fresh materials were then left as such for shade drying and then dried in tray dryer at a temperature at a temperature not more than 50°C for 2 hrs. The well dried plant materials were the subjected to their extraction and pharmacological extras of all plants were then combined in different ratios to get maximum antidiabetic results. White albino rats of either sex of Wistar strain having body weight 100-200g and mice with body weight 20-30g free from all disease were selected for the study. The present study, "Antidiabetic Activity of A Polyherbal Formulation (DIABESTOP)" was approved by institutional animal ethics committee (C.P.E.S.E.A.). Albino rats were housed in polyacrylic cages (5 animals per cage) and maintained under standard laboratory conditions (temp 24-28°C and relative humidity (55±5%). They were fed commercially rat feed and water, ad libitum[14].

Selection of Does: For the assessment of hypoglycemia potential and other physiological action the suitable does level of Diabestop was 100 mg/kg which was 1/25th of the maximum does during acute toxicity study[15].

Acute Toxicity Study: 30 mice were subjected to acute toxicity study. The total number of animals were grouped in five different groups having six animals each. The animals were fasted overnight and different dosage of Diabestop (viz., 500mg, 1kg, 1.5kg, 2kg and 2.5kg per kg body weight) were administered to different group of animals.

Hypoglycemia Study and Other Biochemical Parameter Determination:

Experimental Setup and Grouping of Animals[16]: Albino rats of either sex weighing 100-200g were taken. Animals were fasted overnight prior to the single intraperetoneally injection of freshly prepared alloxan (150 mg/kg) to three groups of animals. The animals were then left aside for next three days under ordinary laboratory conditions. Induction of diabetes was confirmed after three days by estimation of fasting blood glucose level. Rats having blood glucose level more than 200 mg/kg were considered as diabetic one and grouped in following groups,

Group I: Kept as normal controlled, not diabetic and not receive any treatment.

Group II: Positive control, receive only 1% tween 80.

GROUP III: Treated orally with standard oral hypoglycemia agents, Glibenclamide (@ 5mg/kg)

Group IV: Treated orally with Diabestop (@ 100mg/kg)

Determination of Oral Hypoglycemia Activity: Treatment of hyperglycemia was carried out after 3rd day of alloxan administration. Group III and IV received different drugs for 15 days and blood glucose level was analyzed on 1st, 5th, 10th and 15th day of treatment by rat's tail pricking method.

Biochemical Determination: After completion of study (15th day) blood samples were collected from tail of each animals of every group. The serum was separated and analyzed for urea, creatinine, cholesterol, triglycerides, SGOT and SGPT level using standard kit supply.

Statistical Analysis[16]: The data were expressed as mean±SEM. The anti-diabetic potential were analyzed by one-way analysis of variance (ANOVA). A $p<0.05$ was considered as statistically significant.

RESULT AND DISCUSSIONS

Acute toxicity studies on the mice showed no mortality at a dose level of 2,500 mg/kg during a time period of 72 hrs. During the study no noticeable change were observed in the behavior of mice. This may lead to a conclusion that the formulation so preparared is safe to use and bears no toxicity. Administration of alloxan monohydrates (125 mg/kg) led to elevation of blood glucose level. The anti-hyperglycemia effect of the Diabestop (100 mg/kg) and glibenclamide (5 mg/kg) on blood sugar levels of diabetic rats were shown in (Table 1). The percent reduction of hyperglycemia was insignificant on the 1st day of the treatment by Diabestop and Glibenclamide. The percent reduction of hyperglycemia was significant ($p<0.01$) on 5th, 10th and 15 th day after treatment of the Diabestop which was 18.97, 39.82 and 59.60% respectively. The percent reduction of blood glucose levels were significant ($p<0.01$) 26.27, 51.47 and 62.86% on 5th, 10th and 15th day respectively after treatment by glibenclamide 5 mg/kg (Table 1). At the end of 15 days of treatment the various biochemical parameters were screened to evaluate the overall performance of the Polyherbal formulation Diabestop against diabetes. The normal function of kidney was assessed as serum urea level and serum creatinine level in normal, diabetic and treated animals. The results of serum urea and creatinine level indicates that the animals administered with Diabestop in diabetic rats reduced the urea and creatinine levels dose dependently ($p<0.01$). The percent reduction of blood urea and creatinine level was 61.85% and 37.89% with the treatment of Diabestop at the dose of 100 mg/kg and that of glibenclamide was reduced to 54.31% and 10.50% in comparison with the diabetic control group. The extent of gluconeogenesis and ketogenesis was assessed by estimation of cholesterol, SGOT and SGPT level in normal, diabetic and treated animals, after 15th day of test drug treatment. The levels of cholesterol, SGOT and SGPT level are shown in (Table 2). The levels of cholesterol, SGOT and SGPT were increased very significantly in diabetic rats as compared to normal rats. There was very significant ($p<0.01$) reduction in the levels of these parameters on treatment with Diabestop and glibenclamide. The blood cholesterol was reduced significantly ($p<0.01$) by Diabestop and this reduction was slightly higher than the that of standard, which reflects the potential hypolipidemic effect of the Diabestop. The percent reduction of blood cholesterol was 48.58% after treatment of alloxan induced diabetic rats by Diabestop and glibenclamide reduced blood cholesterol 53.19% in comparison with the diabetic group.

The treatment of alloxan induced diabetic rats by Diabestop decreased SGOT and SGPT levels significantly ($p<0.01$) and their reduction was slightly higher than the standard, which indicates the prevention of gluconeogenesis, ketogenesis and normal liver function. The percent reduction of SGPT was 58.00% and SGOT was 42.89% after the treatment of Diabestop and glibenclamide reduced 63.10% of SGPT and 47.04% of SGOT in comparison with the diabetic group. The triglyceride level was also found to be decreasing by 62.02% on treatment with Diabestop however this was 54.19% in glibenclamide treated diabetic rats.(Table 2). N = 5, *$p<0.05$, **$p<0.01$ vs Diabetic control (ANOVA followed by Dunnet's Test), Value expressed in mean±SEM. Type 2 DM constitutes the majority of the diabetic cases. Unlike type I DM, a disease of insulin shortage, victims of type II DM usually have insulin in their bloodstream. In fact, insulin levels in type II diabetics are sometimes even higher than those in non-diabetic individuals. However, since the cells of a type II patient do not respond to insulin by binding it and utilizing blood glucose as normal cells do. Thus type II DM patients may have hyperglycemia in spite of high insulin levels. Oral hypoglycemia drugs are used for the treatment of type 2 DM but because of some serious side effects associated with them these are not always recommended for prolong therapy[18,19]. Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of diabetes in many parts of the world. In present times, available evidence suggests a high prevalence of utilization of alternative medicine for the treatment of diabetes in some regions of the world[20-23]. The present study showed that aqueous extract a polyherbal formulation from the combination of aqueous extracts of leaves of Momordica chirentia, Ocimum sanctum, Azadirachta indica and buds of Piper longum reflects a good the anti-diabetic potential.

Table1: Effect of Drugs on Fast Blood Glucose Level

	Serum Glucose Level (mg/dl) in Days			
Group	**1st**	**5th**	**10th**	**15th**
Normal Control	85.6±4.2	85.9±7.2	83.6±12.9	80.5±7.3
Diabetic Control	302.5±7.9	315.1±7.7	315.9±12.2	322.3±4.4
Diabestop	311.4±9.2	255.3±12.4	190.1±5.4	130.2±5.1
Glibenclamide	309.4±2.7	232.3±13.3	153.3±3.8	119.69±11.3

Table2: Effect of drug on various kidney and liver functional parameters

	Kidney Function (mg/dl)		Liver Function (mg/dl)			
Group	**Urea**	**Creatinine**	**Cholesterol**	**SGOT**	**SGPT**	**Triglycerides**
Normal Control	34.41±4.95	0.98±0.08	85.91±3.12	55.56±10.93	42.31±7.17	82.27±7.03
Diabetic Control	111.88±2.54	2.19±1.01	188.70±3.93	109.13±7.37	117.42±3.71	192.92±5.22
Diabestop	42.68±5.23	1.36±0.82	97.02±5.19	62.32±5.31	49.31±7.73	77.13±3.32
Glibenclamide	51.11±2.52	1.96±0.77	88.33±6.82	57.79±6.92	43.32±5.12	88.37±7.99

Thus their combination shows synergistic approach in treatment of diabetes. However the anti-diabetic principal(s) and exact mechanism of their hypoglycaemic action requires further investigation.

CONCLUSION

To conclude, the Polyherbal formulation Diabestop prepared from Momordica chirentia, Ocimum sanctum, Azadirachta indica and Piper longum was proven to have good antidiabetic potential which was measure in terms of fast blood glucose reduction capacity and restoring biochemical parameters and thus this formulation can be used as an alternative remedy for the treatment of diabetes.

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