Pak. J. Chem. 1(1):32-37, 2011 Full Paper
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2011.v01.i01.p06

Evaluation of Herbal Drug for Hypoglycemic Activity in Normal, Hyperglycemic and Alloxan-Induced Diabetic Rabbits

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ABSTRACT
The herbal drug formulation (Diabrid) was evaluated for hypoglycemic activity in normal, glucose-fed hyperglycemic and alloxan-induced diabetic rabbits. Fasting blood glucose level was determined prior to the drug administration in each group. Blood sugar was also determined at various time intervals after oral drug administration and compared with the reference drug, Gliben clamide. The data was statistically analyzed. No significant hypoglycemic effect was produced by the herbal test drug in alloxan-induced diabetic rabbits. However, fasting blood glucose levels were significantly decreased in the normal animals within one hour after drug administration. The maximum hypoglycemic effect was noted within two hours. A significant hypoglycemic effect was observed in glucose-fed hyperglycemic rabbits. Diabrid at a dose of1000 mg/kg produced approximately the same effect as 3.3 mg/kg Glibenclamide. The herbal drug did not produce any toxic effect at higher doses. The study suggests that Diabrid is a safe potential herbal medicine in the amelioration of hyperglycemia in Type-II diabetes. It has not shown any significant effect in insulin dependent diabetes mellitus (IDDM) animal model.

Keywords: Hypoglycemia, Herbal Drug, Antidiabetic, Glucose over load, Diabetes mellitus

1. INTRODUCTION
An important and active area of modern research on medicinal plants is to analyze and identify the biologically active compounds and their molecular structures, which would provide data to formulate and manufacture the man-made drugs of high economic value. Although medicinal herbs are used since centuries for the treatment of various ailments, but most of them are still being used without any scientific validation for their safety and therapeutic efficacy. Scientific studies would not only confirm the therapeutic value of these plants, but would also help to develop new innovative drug formulations of high efficacy and low toxicity.

A number of medicinal herbs are described for the care and treatment of diabetes in ancient folklore medicine. Still today several herbal medicines are being prescribed for the management of diabetes mellitus in many developing countries of the world. Most of the people still rely on the traditional system of medicine, because of their therapeutic efficacy, minimum side effects and affordable cost. However, most of these herbal drugs are widely prescribed without any scientific knowledge about their therapeutic and toxic effects. Although a number of herbs have been scientifically studied and proved to be hypoglycemic in man and animals by various authors[1-5], but only very few standard herbal antidiabetic drug formulations have been developed and evaluated for efficacy and safety[6,7]. The search for effective and safe herbal drug formulations is the need of the day as many of the presently available allopathic drugs have side effects in the long run.

The present study is an attempt to evaluate a poly-herbal preparation, Diabrid, for its antidiabetic activity using rabbit as the diabetic animal model. This drug has been developed in our laboratory and is based on four medicinal plants having reported hypoglycemic activity. Each plant was, however, individually evaluated to confirm its efficacy and safety before the drug was formulated. The main herbal ingredients of Diabrid are: *Gymnemasylvestre Roxb*[8-10], *Momordicacharantia Linn*[11-14], *Trigonela foenum-graceum Linn*[15,3] and *Eugenia jambolana Lam*[16-19] .Clinical trials of Diabrid have also been undertaken on diabetic patients to confirm its efficacy and safety, the results of which would be communicated in near future.

2. EXPERIMENTAL

2.1 *Preparation of Herbal Formulation (Diabrid)*
The leaves of *Gymnemasylvestre R. Br.*(Asclepideceae)commonly known as Gurmar, the fruits of *Momordicacharantia* (Cucurbitaceae),a common vegetable, known as bitter gourd or Karela, seeds of *Eugeniajambolana* (Myrtaceae) commonly known as Jaman and seeds of *Trigonelafoenum*-graeceum (apilinaceae) known as Mathi, were obtained from the local dealers of medicinal plants and were authenticated by the taxonomists of Applied Biology Research Centre of PCSIR Laboratories Complex, Karachi. All the ingredients were cleaned and well dried in shade at room temperature in the laboratory. The dried parts of the plants were grinded into fine powder separately and passed through a sieve of 70 mesh size. The residual coarse material was re-grinded and again sieved

*Corresponding Author Received January 25th 2011, Accepted February 20th 2011

through a 70 mesh size sieve. The fine powders of *Gymnemasylvestre* leaves, *Momordica charantia*fruits, *Eugenia jambolana* seeds and *Trigonelafoenum*-graeceum seeds were accurately weighed, mixed thoroughly in 3:3:3:1 ratio, dried at 40ºC, cooled and kept in air tight containers.

2.2 *Standardization of Diabrid*

Authenticated sample of the dried Diabrid powder (200g) was extracted by Soxhlet extraction procedure with Hexane, Ethyl acetate, Chloroform and Methanol using 1 L of solvent in each case. The aqueous extract was prepared through maceration. The solvent was concentrated under reduced pressure and the extracted material was dried and weighed. The extracts were separated on pre-coated Silicagel $60F_{254}$ TLC plates and qualitative tests were performed to detect the presence of various compounds. The quantity of extract obtained with each solvent and the compounds detected are as under:

2.2.1 *Hexane Extract*

3.5 g (1.75 % of dry wt):Gave positive test for triterpenes, steroids and fatty acids. No nitrogenous compound was detected

2.2.2 *Ethyl Acetate Extract*

3.5 g (1.75 % of the dry wt.): Gave positive test for nitrogen containing compounds, steroids and alkaloids

2.2.3 *Chloroform Extract*

1.6 g (0.8 % of dry wt.): Gave positive test for alkaloids and amine like compounds

2.2.4 *Methanol Extract*

2.9 g (1.45% of the dry wt.): Gave positive test for carbohydrates, glycosides, alkaloids and amino acids

2.2.5 *Aqueous Extract*

4.4 g (2.2 % of the dry wt.): Gave positive test for saponin, monosaccharides, amino acids

2.2.6 *Ash Content*

6.528 %

2.2.7 *Trace Elements*

Mn (128.96 ppm), Zn (12.07 ppm), Cr (1.29 ppm),Cu (6.83 ppm) and Fe (334.22 ppm)

2.2.8 *HPLC Fingerprinting:*

The ethanol-water (1:1 v/v) extract was prepared and subjected to HPLC (Shimadzu) analysis using C_{18} column with a UV detector set at 280 nm. The spectrum was maintained as reference standard for batch comparison.

The ethanol extracts of each individual plant, different solvent extracts of dried Diabrid powder and the crude poly-herbal Diabrid powder, were also tested for their efficacy. It was observed that all plant extracts have shown glucose lowering activity up to certain extent (relatively higher in aqueous extract), but the highest activity was observed in the crude dried mixture of herbal formulation. This suggests that the active ingredients of plants, work synergistically towards glucose lowering effect in their natural environment, while a part of the activity is lost during extraction process. Therefore, the whole part of the plant was used in the herbal preparation.

2.3 *Experimental Animals*

The hypoglycemic studies of Diabrid were conducted on male adult, healthy rabbits weighing between 1.4–1.7 kg. They were fed cucumber, carrots, lucerne, grains and water adlibitum. All the experimental animals were kept in standard conditions in the animal house of Pharmaceutical Research Centre of PCSIR Laboratories Complex, Karachi in accordance with international Guidelines (NRC 1996). The animals were kept under quarantine for two weeks before starting the experiment. Fasting animals were deprived of food for at least 16 hours, but allowed free access to water. For toxicity experiments, male and female albino Wistar rats (*Rattusnorvegieus*) were used. They were maintained at standard environmental conditions of temperature, relative humidity and light according to NRC Guidelines (1996) and kept under observation for two weeks before the experiment was started. They were fed standard diet and water adlibitum.

2.4 *Acute Toxicity Study*

Any toxicity associated with the drug action of “Diabrid” was evaluated in normal adult albino Wistar rats. Thirty (30) normal, healthy adult albino Wistar rats (15 ma1es and 15females) weighing about 150-180 g were selected for the

study. They were kept under observation for 15 days prior to the start of the toxicity experiments. The animals were divided into five groups each having six animals (3 male and 3 female).After an overnight fast (approx. 16 hrs), the drug suspended in 10 ml distilled water was orally fed at a dose of 250, 500,1000 and 1500 mg/kg body weight in animals of Group-1 to Group-IV respectively. The animals of Group-V were kept as control and fed 10 ml distilled water as vehicle. The feeding was continued for 3 consecutive days and observed daily for any sign of toxic symptoms or any gross physical and behavioral changes, morbidity and mortality. They were kept in separate cages and further observed for 15 days. No sign of any toxic effect was observed.

2.5 *Determination of Blood Glucose*

Blood glucose was determined by the Test Strip Method using One Touch Blood Glucometer (Lifescane, Johnson & Johnson Co., California). It works on colorimetric method based on the enzyme glucose oxidase-peroxidase system and gives direct reading of glucose in mg% after calibration with standard strips provided with the equipment.

2.6 *Effect of Diabrid in Normal Rabbits*

Eighteen (18) healthy male adult rabbits were used for the experiment. The animals were divided into three groups, each consisted of six rabbits. Group-I was kept as vehicle control (negative control) and received 10 ml distilled water, while the animals of the Group-II and III received herbal drug (Diabrid) orally at a dose of 500 mg and 1000 mg/kg body weight respectively. All the animals were kept on fast overnight (16 hours approx.) and blood samples were collected before the commencement of an oral drug administration. The required dose for each animal was suspended in 10 ml distilled water and administered orally through feeding tube. Blood samples (0.2 ml) were collected just prior to (0 hour) and after 1, 2, 3 and 4 hours of drug administration for determination of blood glucose.

2.7 *Effect of Diabrid on Glucose-Fed Hyperglycemic Rabbits*

Twenty four (24) healthy male adult rabbits were used for the experiment. They were divided into four groups (I-IV) having six animals in each group. After an overnight fast blood samples were collected from the ear vein of the animals for determination of fasting blood sugar (0 hour). Glucose at a dose of 1.5 g/kg body weight was given orally to the animals of all groups. Group-I was kept as untreated Diabetic Control. Group-II received reference drug, Glibenclamide, at a dose of 3.3 mg/kg body weight (Positive Control),while Group-III and IV received test drug, Diabrid, at a dose of 500 mg/kg and 1000 mg/kg body weight respectively. Blood samples were collected after 1, 2, 3, 4 and 24 hours for determination of blood glucose. The percent glycemic change with respect to fasting blood sugar level was calculated for each group.

2.8 *Effect of Diabrid on Alloxan-induced Diabetic Rabbits*

Twenty four (24) animals were used for the experiment. Diabetes was induced in all rabbits by intra-peritoneal administration of alloxan monohydrate ata dose of 150 mg/kg body weight in sterile normal saline, which causes permanent necrosis of ß-cells of pancreas. After eight days blood samples were drawn and glucose levels were determined to confirm the development of diabetes. Animals with marked hyperglycemia, having fasting blood glucose levels greater than 350 mg/dl were selected and used for the study.

Table-1: Effect of herbal drug formulation (Diabrid) on blood sugar level of normal rabbits

Groups	Weight of animals (g)	Oral Dose	Blood Sugar Level (mg/dl)				
			0 h	1 h	2 h	3 h	4 h
Group 1 (Control)	1525 ± 30.96	10 ml distilled water	108.67 ± 2.56	107.67 ± 3.16 (- 0.92) NS	105.33 ± 3.92 (- 3.07) NS	[c]103.33 ± 4.43 (- 4.91)	[b]101.00 ± 3.82 (- 7.06)
Group II (Test-1)	1625 ± 30.96	Diabrid 500 mg/Kg body weight	104.00 ± 1.46	[a]93.00 ± 1.44 (- 10.58)	[a]90.00 ± 1.77 (- 13.46)	[a]91.50 ± 0.99 (- 12.02)	[a]90.66 ± 1.36 (- 12.83)
Group III (Test-2)	1600 ± 28.87	Diabrid 1000 mg/Kg body weight	105.17 ± 1.90	[a]94.17 ± 0.98 (- 10.46)	[a]88.50 ± 0.76 (- 15.85)	[a]87.17 ± 1.30 (- 17.12)	[a]85.00 ± 1.59 (- 19.18)

Values are Mean ± SEM of six replicate samples. Values in parentheses represent% glycemic change compared with zero hour reading

a = highly significant ($p < 0.001$) compared to the initial zero hour reading , b = significant ($p < 0.01$) compared to the initial zero hour reading

c= significant ($p < 0.05$) compared to the initial zero hour reading, NS= Non-significant,10 ml distilled water was used as vehicle

Table-2: Effect of herbal drug formulation (Diabrid) on blood sugar levels in glucose-fed hyperglycemic rabbits

Groups	Weight of Animals (g)	Oral Dose	Blood Sugar Levels (mg/dl)					
			0 h	I h	2 h	3 h	4 h	24 h
Group-I (Negative Control)	1543 ± 35.28	1.5 g Glucose	118.00 ± 1.73	[a]175.67 ± 1.31 (+48.87)	[a]161.33 ± 1.58 (+36.72)	120.17 ± 2.56 (+1.84) NS	[a]75.33 ± 1.98 (-36.16)	[a]*66.66* ± 1.68 (-43.51)
Group-II (Positive Control)	1675 ± 21.41	1.5 g Glucose +3.3 mg Glibenclamide	109.50 ± 0.88	[a]132.17 ± 1.08 (+20.70)	108.00 ± 0.58 (-1.37) NS	[a]78.33 ± 0.61 (-31.17)	[a]53.33 ± 0.66 (-51.30)	[a]62.33 ± 1.08 (-43.08)
Group-III (Test-1)	1552 ± 21.51	1.5 g Glucose + 500 mg Diabrid	112.33 ±1.05	[a]159.00 ± 1.24 (+41.55)	[a]147.5 ± 1.71 (+31.31)	[b]95.67 ± 1.82 (-14.83)	[a]83.66 ± 1.80 (-25.52)	[a]99.83 ± 1.27 (-11.13)
Group-IV (Test-2)	1605 ± 28.61	1.5 g Glucose +1000 mg Diabrid	96.17 ±1.74	[a]133.00 ±1.65 (+38.30)	[a]122.00 ±1.24 (+26.86)	[a]75.17 ±1.54 (-21.83)	[a]53.66 ±1.28 (-44.20)	[a]67.83± 1.58 (-29.47)

Values are Mean ± SEM of six replicate samples. Values in parentheses represent% glycemic change compared with zero hour reading

a = highly significant ($p < 0.001$) compared to the initial zero hour reading, b = significant ($p < 0.01$) compared to the initial zero hour reading

c= significant ($p < 0.05$) compared to the initial zero hour reading, NS= Non-significant,10 ml distilled water was used as vehicle

The rabbits were divided into four groups; each group consisted of six animals. After obtaining the fasting blood sugar values, Group-I was maintained as Control Diabetic Group and received 10 ml distilled water. The animals of Group-II and III were administered herbal drug Diabrid at a dose of 500 mg and 1000 mg/kg body weight respectively. Group-IV (Positive Control) was given subcutaneous soluble insulin at a dose of 4 IU/Kg body weight. Blood samples (0.2 ml) were collected at 0, 1, 2, 3 and 4 hours after the oral administration of drug for determination of blood glucose.

2.9 *Statistical Analysis*

The results are represented as mean ± S.E.M. The data were statistically analyzed by paired sample T-Test at 95 % confidence interval of the difference to determine the level of significance. P values ≤ 0.05 were considered significant. Statistics were calculated using SPSS for windows 14.0 software.

3. RESULTS AND DISCUSSION

The herbal drug, Diabrid, did not produce any morbidity or mortality on oral administration up to a dose of 1500 mg/kg body weight. All the animals were found active and showed normal physiological activity during 72 hours of observation period. There was no gross behavioral change in any animal for further 2 weeks of observation. These results indicate that the drug is well tolerated by animals and no acute toxicity was observed up to a dose of 1500 mg/kg body weight. The therapeutic dose of 1000 mg/kg body weight is considered to be safe for the management of diabetic condition. Much higher doses of similar other herbal products have been recommended due to their high tolerance and safety profile[7,20].

The test drug produced significant hypoglycemic activity in normal rabbits within one hour of its administration (Table-1). The blood glucose levels were further reduced in 2h, which were maintained in the subsequent hours and no hypoglycemic state was observed. The reason may be the normal self regulatory mechanism of the body to keep the carbohydrate level in homeostasis. This is a desirable feature as accidental overdose would not produce any serious hypoglycemic condition. The hypoglycemic activity exhibited by Diabrid is, however, time and dose dependent.

The glucose lowering effect in normal animals, up to certain limit, was also observed by other research groups in many individual plants[16, 2,19] and herbal formulations[6]. It seems that this herbal drug formulation may either possess insulin like activity; possibly due to *Momordicacharantia* component[21]or it stimulates the ß-cells of islets of Langerhans in pancreas to produce insulin, which in turn lowers blood glucose level. Similar observations were also reported by other research groups[4, 22].

The effect of the herbal preparation in diabetic animal model is provided in Table-2. In control group the fasting blood sugar level was increased up to 48.87% after glucose administration, while the increase was 38.30% in Diabrid (1000 mg/kg) treated group and 20.70% in Glibenclamide (Aventis Pharma) treated rabbits. The blood sugar returned to slightly above normal level in 3 hours in Control Group, in 2 hours in Glibenclamide treated group and shortly after 2 hours in Diabrid treated animals, which was maintained upto 24 hours. These studies suggest that the drug not only resists the initial increase of blood sugar after glucose administration but also brings down the increased sugar level in the normal range in about two hours which is maintained up to 24 hours and no hypoglycemia is observed. The hypoglycemic effect of Diabrid at a dose of 1000 mg/kg can be compared to that of well known oral anti-diabetic drug, Glibenclamide (belonging to the group of sulfonylurea), administered similarly by oral route in glucose-fed hyperglycemic rabbits. However, its dose is relatively higher as compared to the pure active compound, as it is a crude preparation, but it may be taken safely without producing any toxic effect. It is interesting to note that the therapeutic dose of herbal drug was effective to decrease the hyperglycemic level without producing any hypoglycemic state; whereas Glibenclamide induced hypoglycemic condition.

The herbal drug, Diabrid, fails to decrease blood glucose levels in alloxan induced diabetic animals, whereas insulin produced a typical hypoglycemic response (Table-2). Alloxan destroys β-cells of the islets of Langerhans in pancreas almost completely[23] and therefore, the availability of insulin is cut down, which creates a state of permanent diabetes. This suggests, that the herbal drug (Diabrid) works in the presence of insulin and decrease blood glucose level through stimulation of β-cells of the islets of Langerhans, if intact, to produce more insulin. Diabrid seems to work on the same mechanism as that of clinically used suphonylurea drug like Glibenclamide, which decrease blood glucose level by stimulating the β-cells to release insulin, by reducing the hepatic release of glucose or increasing the glycogen deposits in the liver and by diminishing the insulin resistance in normal animals.

Diabrid contains *Gymnemasylvestre*leaves, *Momordicacharantia*fruits, *Eugenia jambolana*seeds and *Trigonelafoenum*-graeceum seeds as its main ingredients. *Gymnemasylvestre* contains gymnemic acid, which is used to inhibit the adrenohypophyseal stress response[24] and the hyperglycemic response to adrenalin[25, 26] and growth hormone[24]. It may also help by increasing peripheral utilization of glucose. *Eugenia jambolana* seeds promote the release of insulin from pancreas[15] increasing the level of insulin and decreasing its degradation[15]. *Momordicacharantia* fruits have been shown to increase peripheral utilization of glucose perhaps due to its insulin-like factor[21]. The seeds of *Trigonelafoenum*-graeceum are also used for the treatment of Diabetes mellitus, although its mechanism of action is unknown.

Although the exact mechanism of hypoglycemic effect of Diabrid is not known, it can be suggested, that the herbal ingredients may work synergistically to reduce blood glucose level. The possible mechanism by which this herbal drug produces hypoglycemic effect may be either through improving the insulin secretion capacity of the β-cells of pancreas or by enhancement of peripheral metabolism of glucose. It may also reduce the blood glucose levels indirectly through decreasing hepatic release of glucose or increasing glycogendeposits in liver, as well as inhibiting the adrenocorticotropic hormones antagonist to insulin. Whatever the mechanism of action may be, the present study suggests the potential use of herbal drug, Diabrid, in the treatment of non-insulin dependent diabetes mellitus (NIDDM) or Type-II maturity on set diabetes mellitus and holds immense potential for its standardization and clinical trials in type-II diabetic patients as a new herbal anti-diabetic agent[27, 28].

Animal toxicity study has demonstrated that this herbal drug is a non-toxic material and can be used safely up to dose level 1000 mg orally as therapeutic agent for the treatment of Type-II Diabetes. The study further confirms the claims of traditional system of medicine, that the plants used in this preparation possess hypoglycemic activity and may be used for the treatment of diabetes. Further chemical, pharmacological and clinical studies are being carried out to isolate the active constituents and to elucidate the exact mechanism of anti-diabetic action of this poly-herbal drug.

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