Neutraceutical approaches to control diabetes: A natural requisite approach

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Abstract

Objective: The aim of this study is to screen the polyherbal preparation for antidiabetic activity in rats. Materials and Methods: The blood glucose lowering activity of the polyherbal preparation-I (1:1:1 of wheat germ oil, Coraidrum sativum, and Aloe vera) was studied in normal rats after oral administration at doses of 1.0 ml/kg and 2.0 ml/kg and polyherbal preparation-I, II (wheat germ oil, fresh juice of C. sativum, and A. vera in the ratio of 2:2:1), and III (wheat germ oil, fresh juice of C. sativum and A. vera in the ratio of 1:2:2) on alloxan-induced diabetic rats, after oral administration at doses of 1.0 ml/kg and 2.0 ml/kg. Blood samples were collected from the tail vein method at 0, 0.5, 1, 2, 4, 8, 12, and 24 h in normal rats and in diabetic rats at 0, 1, 3, 7, 15, and 30 days. Blood plasma glucose was estimated by the GOD/POD (glucose oxidase and peroxidase) method. The data were compared statistically by using the one-way ANOVA method followed by the Dunnett multiple component test. Statistical significance was set at P < 0.05. Results: The polyherbal preparation-I produced significant (P < 0.05) reduction in the blood glucose level of normal rats and polyherbal preparation-I, II, and III produced significant (P < 0.01) reduction in the blood glucose level of diabetic rats during 30-day study and compared with that of control and glibenclamide. Conclusion: The polyherbal preparation-I showed a significant glucose lowering effect in normal rats and polyherbal preparation-I, II, and III in diabetic rats. This preparation is going to be promising antidiabetic preparation for masses; however, it requires further extensive studies in human beings.

Key words: Alloxan-induced diabetes, blood plasma glucose level, effect of polyherbal preparation

INTRODUCTION

Every time we eat sugary or starchy food, the amount of glucose available to the body rockets. Yet the levels of glucose in the bloodstream are maintained within narrow limits by two key hormones—insulin and glucagon—working to prevent hyperglycemia (abnormally high glucose levels) or hypoglycemia (low glucose).[1] Insulin helps the sugar to leave the blood and go into our body cells, where it is used as a kind of fuel. When this happens the way it should, the level of sugar in the blood goes down and our bodies have the energy for a full and active life. In people with diabetes, this system does not work. When you have diabetes, your body cannot make energy from the food you eat. Sugar stays in the blood instead of going into the cells of the body. Diabetes is a lifelong serious disease and should be treated accordingly. If left unchecked, it shortens life. It is not a condition that goes away.[2] Diabetes is defined as a state in which homeostasis of carbohydrate, lipid, and protein metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normal and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus.[3]

On perusal of the literature survey, we found that in the indigenous Indian system of medicine (Ayurveda) a mention was made on a good number of plants for the cure of diabetes but the bioreactivity of single plant preparation is...
disappointing whereas a combination of substances is likely to contain several bioreactive components which act together and elicit additive or synergistic effects which shows optimal therapeutics response that are not apparent when the single plant preparations are used. Therefore, we have selected three different plants products in our study, namely wheat germ oil, fresh juice of Coriandrum sativum, and Aloe vera. A. vera has been reported to have hypoglycemic activity. Hence, specific objectives aimed in the present work are as follows:

To ascertain the effectiveness of polyherbal preparation for hypoglycemic activity in normal rats.

To explore the effect of polyherbal preparation for antidiabetic activity in diabetic rats.

MATERIALS AND METHODS

Materials
The polyherbal preparation which was used in the study has been prepared by using wheat germ oil which has been provided by Bhaiji Attarwali Perfumers (P) Ltd., Delhi. A. vera gel was collected from A. vera leaf which has been provided by Sree Siddaganga College of Pharmacy botanical garden, Tumkur, and the fresh juice of Coriander leaves was collected from Tumkur district, and were authenticated by Prof. K. Siddappa, Head, Department of Botany, Sree Siddaganga Boy’s College, Tumkur. A. vera leaves were washed properly to remove the dirt. After washing, the skin of leaves was removed, gel was separated and collected. The fresh juice of C. sativum obtained by crushing leaves in a mortar and squeezed the crushed material by means of a fine cloth to separate the juice and collected the fresh juice of leaves. Alloxan, sodium chloride, and acacia were obtained from S.D. Fine-Chem Limited, Mumbai. Glibenclamide tablet (5 mg) was obtained from Nicholas Piramal India limited. The glucose estimation kit was supplied by Qualigens Diagnostics.

Methods

Preparation of solutions and test samples

Preparation of alloxan monohydrate 5% solution
Alloxan monohydrate, 250 mg, was dissolved in 5.0 ml of 0.9% sodium chloride to give 5.0% of alloxan solution and injected immediately through an i.p. route within 5 min to avoid degradation.

Glibenclamide solution
Five milligrams of glibenclamide tablet was dissolved in 83.33 ml of distilled water to give 60 µg/ml solution. This solution was administered at a dose of 600 µg/kg body weight using a clean and dry oral feeding needle for 30 days.

Preparation of polyherbal preparation I, II, and III

Polyherbal preparation-I
It consists of wheat germ oil, fresh juice of C. sativum, and A. vera in the ratio of 1:1:1. Formulation was prepared by using acacia as a binding agent. An aliquot of 2.0 ml of wheat germ oil is taken and transferred it into a dry mortar. Then, 500 mg of gum acacia powder was added, and mixed thoroughly by trituration. The Coriander juice, 1.0 ml, was added at the time of continuous trituration until a light green product with clicking sound is produced. Primary emulsion was formed. A. vera gel 2.0 ml was added with the remaining 1.0 ml of coriander juice separately. This solution was added at a time to the primary emulsion with continuous and rapid trituration. The emulsion was used for the study.

Polyherbal preparation-II
It consists of wheat germ oil, fresh juice of C. sativum, and A. vera in the ratio of 2:2:1. Formulation was prepared by using acacia as a binding agent. An aliquot of 2.0 ml of wheat germ oil was taken and transferred it into a dry mortar. Then, 500 mg of gum acacia powder was added and was mixed thoroughly by trituration. Coriander juice 1 ml was added at the time of continuous trituration until a light green product with clicking sound is produced. Primary emulsion was formed. A. vera gel 1.0 ml was added with the remaining 1.0 ml of coriander juice separately. This solution was added at a time to the primary emulsion with continuous and rapid trituration. The emulsion was used for the study.

Polyherbal preparation-III
It consists of wheat germ oil, fresh juice of C. sativum, and A. vera in the ratio of 1:2:2. Formulation was prepared by using acacia as a binding agent. An aliquot of 1.0 ml of wheat germ oil was taken and transferred it into a dry mortar. Then, 250 mg of gum acacia powder was added, and mixed thoroughly by trituration. Coriander juice 1 ml was added at the time of continuous trituration until a light green product with clicking sound is produced. Primary emulsion was formed. A. vera gel 2.0 ml was added with the remaining 1.0 ml of coriander juice separately. This solution was added at a time to the primary emulsion with continuous and rapid trituration. The emulsion was used for the study.

Experimental animals

Animals
For pharmacological experiments, Wistar albino rats (160–250 g) of either sex were used. The animals were obtained from animal house of Sree Siddaganga College of Pharmacy, Tumkur [Ref: 123/1999/CPMSEA dated 19/5/99]. The protocol of the experiments and animal usage were discussed in the Institutional Ethical Committee meeting and permission has been obtained to carry out the parameters selected for the study (See Annexure -II) [Ref: SSCPT/ IAEC. Clear/41/2006-07 dated 25/11/06].

Housing of animal
Animals were maintained in the suitable nutritional and
environmental condition throughout the experiment. They were provided with food, water ad libitum. The bedding material of the cages was changed every day. The animals were maintained under natural day and night cycle.

Experimental method

Experimentally induced diabetes mellitus

Wistar rats (160–250 g) were fasted for 14 h before challenging with single injection (i.p.) of alloxan monohydrate 5.0%, freshly prepared, and injected within 5 min of preparation to prevent degradation at a dose of 150 mg/kg, i.p. After administration of alloxan, the animals had free access to feed and water ad libitum. The blood glucose was measured 72 h of alloxanisation. After one week when the condition of diabetes was stabilized, the animals were fasted again for 14 h before blood collection withdrawal from retro orbital plexus. The rats with the fasting serum glucose level of above 200 mg/dl were considered diabetic and included in the study.

Experimental design

The experimental setup consists of twelve groups, each group consists of five animals.

- **Group-I**: Vehicle control received saline solution (0.9% NaCl).
- **Group-II**: Normal rats received glibenclamide as standard, single dose. (Dose: 600 μg/kg).
- **Group-III**: Normal rats received polyherbal preparation-I, single dose. (Dose: 1.0 ml/kg).
- **Group-IV**: Normal rats received polyherbal preparation-I, single dose. (Dose: 2.0 ml/kg).
- **Group-V**: Alloxan induced rats received saline solution (0.9% NaCl).
- **Group-VI**: Diabetic rats given glibenclamide as standard, orally once daily. (Dose: 600 μg/kg).
- **Group-VII**: Diabetic rats given polyherbal preparation-I, orally once daily. (Dose: 1.0 ml/kg).
- **Group-VIII**: Diabetic rats given polyherbal preparation-I, orally once daily. (Dose: 2.0 ml/kg).
- **Group-IX**: Diabetic rats given polyherbal preparation-II, orally once daily. (Dose: 1.0 ml/kg).
- **Group-X**: Diabetic rats given polyherbal preparation-II, orally once daily. (Dose: 2.0 ml/kg).
- **Group-XI**: Diabetic rats given polyherbal preparation-III, orally once daily. (Dose: 1.0 ml/kg).
- **Group-XII**: Diabetic rats given polyherbal preparation-II, orally once daily. (Dose: 2.0 ml/kg).

Care of diabetic animals

Since diabetic animals drink a large amount of fluid and produce large volume of urine, the bedding is changed frequently, usually every day, and, in some circumstances, more than once per day. Diabetic rats should have sufficient food and water; therefore only three diabetic rats have been housed per cage to avoid competition for feed and water.

Collection of blood plasma samples

The rat was placed on the working table, a mortar was inverted on it, and no gap was maintained in between the mortar and the edge of the working platform. The tail was pulled out from the mortar’s beak gap and the tail was depilated. The tail was cleaned with spirit and allowed to dry; tail vein was dilated by focusing a low voltage electric lamp at the tip of tail for few seconds. After the dilation of the vein the blood was drawn by the tail vein method from rats (fasted for 14 h) on different occasion, i.e., at 0, 0.5, 1, 2, 4, 8, 12, and 24 h after oral administration of drug in groups I to IV, whereas in groups V to XII at 0, 1, 3, 7, 15, and 30 days. The blood samples were collected in a centrifugation tube and add heparin (0.2 ml for 1.0 ml blood) as an anticoagulant. The plasma was obtained by centrifuging the blood samples at 3000 rpm for 10 min, decanting supernatant fluid into the clean, dry test tube. Ten microliters of the plasma was collected, and the blood glucose level was estimated by the GOD/POD method.

Parameter analyzed

Fasting blood plasma glucose estimation

Blood plasma glucose was estimated by the GOD/POD (glucose oxidase and peroxidase) method using the Glucose–GOD/POD kit (GSK Qualisystems; AR106) [Table 1].

GOD/POD method

The older methods were based on the reducing property

| Group | Details |
|-------|---------|
| Group-XII | Diabetic rats given polyherbal preparation-II, orally once daily. (Dose: 2.0 ml/kg). |

Table 1: General procedure of GOD/POD for the estimation of glucose in plasma

| Content | Blank | Standard | Test |
|---------|-------|----------|------|
| Working reagent enzyme (ml) | 1.0 | 1.0 | 1.0 |
| Distilled water (µl) | 10 | 10 | 10 |
| Standard glucose | – | 10 µl | – |
| Serum (sample) | – | – | 10 µl |

GOD/POD: Glucose oxidase and peroxidase
of glucose. However, these methods do not measure the true glucose because of interferences. Subsequently, other chemical and enzymatic methods were developed to overcome this problem. The GOD/POD method is one such a evolved method by Trinder in 1964. This method is precise, simple, and single stepped, rapid, safe and reliable. Hence, in this study, we have adopted this method. Trinder's method (1964) utilizes two enzymes GOD and POD along with the chromogen 4-amino antipyrine and phenol. This method is intended for in vitro quantitative determination of glucose in serum/plasma and CSF. There was no interference due to the substances such as creatinine, fructose, galactose, reduced glutathione, ascorbic acid, and xylose. Hemoglobin or bilirubin up to 10 mg% does not affect this test.\[14\]

Glucose is oxidized by the enzyme GOD to give d-gluconic acid and hydrogen peroxide. Glucose present in the blood is oxidized by the enzyme GOD to give d-gluconic acid and hydrogen peroxide. Hydrogen peroxide further reacts with 4-aminoantipyrine and phenol in the presence of the enzyme POD to undergo oxidation to produce a red-color quinoneimine dye. The intensity of the color produced is directly proportional to the glucose concentration in the sample.

### Preparation of the working reagent

One vial (3 ml) of enzyme powder was transferred to one bottle (100 ml) of buffer solution and mixed gently to dissolve which is ready to use. Glucose (100 mg/dl) was used as the standard. All the reagents were stored at 2–8 °C, which is stable till the expiry date, reagents were not freeze. Capped reconstituted reagents are stable for 30 days at 2–8 °C. Reconstituted enzyme reagents when stored at 2–8 °C develop a slight pink color; however, this does not affect the performance of the enzyme reagent. The reagents should not be used if caking is observed due to possible moisture penetration.\[^{15,16}\]

### Procedure

The solutions were pipetted out into clean, dry test tubes and labelled as blank (B), standard (S), and test (T). Distilled water was considered as the blank solution. To 1.0 ml of the reagent, 10 µl of standard glucose (100 mg/dl) was added and incubated for 15 min at 37 °C. This incubated mixture was aspirated, and the concentration of standard was calibrated to show a value of 100 mg/dl. This process is duplicated to confirm the calibration value and was considered as correct if the value was within 5% of the original value. The fasting blood plasma glucose was estimated by adding 10 µl of the plasma sample to 1.0 ml of the reagent, mixed well, and incubated at 37 °C for 15 min. This incubated mixture was aspirated and the absorbance was recorded against a reagent blank at 510 nm using Photometer (GSK Qualisystems; AR106).

### Statistical analysis

The values are expressed as mean ± SEM. The data were analysed by using Student’s t-test using one-way analysis of variance (ANOVA) followed by the Dunnett multiple component test. Statistical significance was set at \( P < 0.05 \).

### RESULTS

#### Fasting blood plasma glucose

The fasting blood plasma glucose of the different groups of normal animals during the period of study is presented in Figure 2, which shows that the mean (±SEM) fasting plasma glucose values of the normal control group of rats was 96.4 ± 1.364, 104.2 ± 1.497, 102 ± 1.342, 97.4 ± 1.470, 99.8 ± 1.625, 91.6 ± 1.503, 100.6 ± 0.95, and 98.0 ± 1.125 on day 0, ½, 1, 2, 4, 8, 12, and 24 h, respectively. The above values show that the fasting plasma glucose in the normal group of rats was maintained within the normal range throughout the period of study.

The glibenclamide (600 µg/kg) treated normal rats show a mean (±SEM) fasting plasma glucose of 104.2 ± 2.835 mg/dl on 0 h which was reduced to 88.0 ± 2.302 mg/dl on ½ h, 85.6 ± 2.713 mg/dl on 1 h, 82.4 ± 2.441 mg/dl on 2 h, 77.8 ± 2.782 mg/dl on 4 h, 72.4 ± 2.542 mg/dl on 8 h, 75.16 ± 2.212 mg/dl on 12 h, and 86.83 ± 3.554 mg/dl on 24 h. These changes in fasting plasma glucose values illustrate that the normal rats treated with glibenclamide show a progressive and significant \( (P < 0.01) \) reduction in fasting plasma glucose, during the 24 h on the single dose treatment period in comparison to the normal group of rats. This indicates that the glibenclamide treatment of normal rats is able to reduce the fasting plasma glucose levels below the normal up to 8 h, but it further increases on 12 h and 24 h of the study period.

The polyherbal preparation-I (1.0 ml/kg) treated normal rats show a mean (±SEM) fasting plasma glucose of 100.8 ± 1.114 mg/dl on 0 h which was reduced to 97.2 ± 1.319 mg/dl on ½ h, 93.2 ± 1.114 mg/dl on 1 h, 90.4 ± 1.166 mg/dl on 2 h, 87.0 ± 1.265 mg/dl on 4 h, 81.0 ± 1.342 mg/dl on 8 h, 83.83 ± 1.108 mg/dl on 12 h, and 86.33 ± 1.687 on 24 h [Figure 1]. These changes in fasting plasma glucose values illustrate that the normal rats treated with polyherbal preparation-I show a progressive and significant \( (P < 0.01) \) reduction in fasting plasma glucose, during the 24 h on the single dose treatment period in comparison to the normal group of rats. This indicates that the polyherbal preparation-I treatment of normal rats is able to reduce the fasting plasma glucose levels below the normal up to
8 h but it further increases on 12 h and 24 h of the study period [Figure 2].

The polyherbal preparation-I (2.0 ml/kg) treated normal rats show a mean (±SEM) fasting plasma glucose of 99.6 ± 1.369 mg/dl on 0 h which was increased to 100.2 ± 0.8602 mg/dl on ½ h, and later reduced to 88.2 ± 1.562 mg/dl on 1 h, 81.4 ± 1.860 mg/dl on 2 h, 75.6 ± 2.561 mg/dl on 4 h, 69.4 ± 2.441 mg/dl on 8 h, 88.0 ± 1.581 mg/dl on 12 h, and 91.8 ± 0.7348 mg/dl on 24 h. These changes in fasting plasma glucose values illustrate that the normal rats treated with polyherbal preparation-I show a progressive and significant ($P < 0.01$) reduction in fasting plasma glucose, during the 24 h on the single dose treatment period in comparison to the normal group of rats. This indicates that the polyherbal preparation-I treatment of normal rats is able to reduce the fasting plasma glucose levels below the normal up to 8 h, but it further increases on 12 h and 24 h of the study period.

The fasting plasma glucose of the different groups of diabetic animals during the period of study Figure 3 shows that the mean fasting plasma glucose (±SEM) in the diabetic control group of rats was found to be 270.6 ± 3.140, 270.6 ± 3.140, 270.6 ± 3.140, 266.8 ± 2.905, 269.2 ± 2.653, and 261.4 ± 5.683 mg/dl on days 0, 1, 3, 7, 15, and 30, respectively. These elevated fasting plasma glucose levels were found to have been maintained throughout the 30 days of treatment period indicating that the rats are rendered diabetic.

The glibenclamide (600 µg/kg) treated diabetic rats show a mean (±SEM) fasting plasma glucose of 289.4 ± 1.691 mg/dl on day 0, 269.8 ± 3.497 mg/dl on day 1, 227.0 ± 1.378 mg/dl on day 3, and 212.8 ± 1.020 mg/dl on day 7 which was reduced to 178.0 ± 1.000 mg/dl on day 15 which reduced further to 132.6 ± 1.691 mg/dl on day 30. These changes in fasting plasma glucose values illustrate that the diabetic rats treated with glibenclamide show a progressive and significant ($P < 0.01$) reduction in fasting plasma glucose, during the 4 weeks of the treatment period in comparison to the diabetic group of rats. This indicates that the glibenclamide treatment of diabetic rats is able to bring back the fasting plasma glucose levels nearer to the
normal range in the 4 weeks of the study period.

The polyherbal preparation-I (1.0 ml/kg) treated diabetic rats show mean (±SEM) fasting plasma glucose of 272.2 ± 2.223 mg/dl on day 0, 267.2 ± 2.223 mg/dl on day 1, 256.8 ± 2.154 mg/dl on day 3, and 238.8 ± 2.059 mg/dl on day 7 which was found to have been reduced to 212.4 ± 1.661 mg/dl on day 15 and 202.2 ± 1.655 mg/dl on day 30. These changes in fasting plasma glucose values illustrate that the diabetic rats treated with polyherbal preparation-I (1.0 ml/kg) show a progressive and significant ($P$ < 0.01) reduction in fasting plasma glucose during the 30 days of treatment period in comparison to the diabetic group of rats.

The polyherbal preparation-I (2.0 ml/kg) treated diabetic rats shows mean (±SEM) fasting plasma glucose of 279.4 ± 3.696 mg/dl on day 0, 269.2 ± 3.652 mg/dl on day 1, 247.4 ± 3.076 mg/dl on day 3, 221.2 ± 2.577 mg/dl on day 7 which was reduced to 199.6 ± 2.943 mg/dl on day 15 which reduced further to 180.4 ± 2.619 mg/dl on day 30. These changes in fasting plasma glucose illustrate that the diabetic rats treated with polyherbal preparation-I (2.0 ml/kg) show a progressive and significant ($P$ < 0.01) reduction in fasting plasma glucose during the 30 days of the treatment period in comparison to the diabetic group of rats. The above observations show that the treatment of diabetic rats with polyherbal preparation-I reduces the fasting plasma glucose of diabetic rats at all the tested dose levels, but the high dose of polyherbal preparation-I (i.e. 2.0 ml/kg of polyherbal preparation-II) was able to reduce fasting plasma glucose which was comparable with the reduction caused by glibenclamide treatment during the 30 days of treatment.

The polyherbal preparation-II (1.0 ml/kg) treated diabetic rats show mean (±SEM) fasting plasma glucose of 274.2 ± 3.625 mg/dl on day 0, 267.0 ± 3.271 mg/dl on day 1, 224.0 ± 3.317 mg/dl on day 3, and 205.0 ± 2.608 mg/dl on day 7 which was reduced to 180.6 ± 1.913 mg/dl on day 15 which reduced further to 117.6 ± 1.887 mg/dl on day 30. These changes in fasting plasma glucose values illustrate that the diabetic rats treated with polyherbal preparation-II reduces the fasting plasma glucose of diabetic rats at all the tested dose levels, but the high dose of polyherbal preparation-II (i.e. 2.0 ml/kg) was able to reduce fasting plasma glucose which was comparable with the reduction caused by glibenclamide treatment during the 30 days of treatment.

The polyherbal preparation-II (1.0 ml/kg) treated diabetic rats show mean (±SEM) fasting plasma glucose of 272.0 ± 3.406 mg/dl on day 0, 257.0 ± 3.114 mg/dl on day 1, 216.8 ± 2.888 mg/dl on day 3, and 211.4 ± 2.337 mg/dl on day 7 which was found to have been reduced to 178.6 ± 2.522 mg/dl on day 15 and 137.0 ± 2.775 mg/dl on day 30. These changes in fasting plasma glucose values illustrate that the diabetic rats treated with polyherbal preparation-II (1.0 ml/kg) show a progressive and significant ($P$ < 0.01) reduction in fasting plasma glucose during the 30 days of the treatment period in comparison to the diabetic group of rats.

The polyherbal preparation-II (2.0 ml/kg) treated diabetic rats show mean (±SEM) fasting plasma glucose of 277.0 ± 3.536 mg/dl on day 0, 260.8 ± 1.744 mg/dl on day 1, 217.6 ± 1.208 mg/dl on day 3, and 182.8 ± 1.393 mg/dl on day 7 which was found to have been reduced to 161.4 ± 1.990 mg/dl on day 15 and 137.2 ± 2.775 mg/dl on day 30 [Figure 4]. These changes in fasting plasma glucose illustrate that the diabetic rats treated with polyherbal preparation-II (2.0 ml/kg) show a progressive and significant ($P$ < 0.01) reduction in fasting plasma glucose during the 30 days of the treatment period in comparison to the diabetic group of rats. The above observations show that the treatment of diabetic rats with polyherbal preparation-II reduces the fasting plasma glucose of diabetic rats at all the tested dose levels, but the high dose of polyherbal preparation-I (i.e. 2.0 ml/kg of polyherbal preparation-II) was able to reduce fasting plasma glucose which was comparable with the reduction caused by glibenclamide treatment during the 30 days of treatment.

The polyherbal preparation-III (1.0 ml/kg) treated diabetic rats show mean (±SEM) fasting plasma glucose of 271.6 ± 4.13 mg/dl on day 0, 253.8 ± 3.813 mg/dl on day 1, 202.0 ± 3.27 mg/dl on day 3, and 175.8 ± 2.615 mg/dl on day 7 which was found to have been reduced to 158.4 ± 2.839 mg/dl on day 15 and 132.6 ± 1.806 mg/dl on day 30 [Figure 5]. These changes in fasting plasma glucose illustrate that the diabetic rats treated with polyherbal preparation-III (1.0 ml/kg) show a progressive and significant ($P$ < 0.01) reduction in fasting plasma glucose during the 30 days of the treatment period in comparison to the diabetic group of rats. The above observations show that the treatment of diabetic rats with polyherbal preparation-III reduces the fasting plasma glucose of diabetic rats at all the tested dose levels, but the high dose of polyherbal preparation-II (i.e. 2.0 ml/kg) was able to reduce fasting plasma glucose which was comparable with the reduction caused by glibenclamide treatment during the 30 days of treatment.

The polyherbal preparation-III (2.0 ml/kg) treated diabetic rats show mean (±SEM) fasting plasma glucose of 277.4 ± 2.221 mg/dl on day 0, 267.0 ± 2.223 mg/dl on day 1, 247.4 ± 3.076 mg/dl on day 3, and 221.2 ± 2.577 mg/dl on day 7 which was reduced to 199.6 ± 2.943 mg/dl on day 15 which reduced further to 180.4 ± 2.619 mg/dl on day 30. These changes in fasting plasma glucose values illustrate that the diabetic rats treated with polyherbal preparation-III (2.0 ml/kg) show a progressive and significant ($P$ < 0.01) reduction in fasting plasma glucose during the 30 days of the treatment period in comparison to the diabetic group of rats.
levels, but the high dose of polyherbal preparation-III (i.e. 2.0 ml/kg of polyherbal preparation-I) was able to reduce fasting plasma glucose which was comparable with the reduction caused by glibenclamide treatment during the 30 days of treatment.

**DISCUSSION**

Diabetes mellitus is possibly the world’s largest growing metabolic disease, and the knowledge on the heterogeneity of this order is advanced, the need for more appropriate therapy increases. Traditional plant medicines are used throughout the world for the range of diabetic complications as an alternative for conventional hypoglycaemic agents because hypoglycemic or antidiabetic drugs in NIDDM or insulin in IDDM have a limited role to play. The risk of drug tolerance is high with oral hypoglycaemic agents, thereby causing a raise in dosage or a change of drug. Ayurvedic medicines may help as “potentiators” for these drugs or play a supportive role in regulating the dosage of hypoglycemics and maintain the quality of the diabetic life.

In the indigenous Indian system of medicine (Ayurveda) a mention was made on a good number of plants for the cure of diabetes, but the bioreactivity of single plant preparation is disappointing and most of these remedies have moderate potency as well as toxicity thus are not fast acting nor overtly dangerous whereas in the polyherbal preparation forms, they are likely to contain several bioreactive components which can act together and elicit additive or synergistic effects which shows optimal therapeutics response that are not apparent when the bioreactive compounds are used independently. Various herbal formulations such as diamed, coagent db, hyponidd and SMK001 are reported in the literature as an antidiabetic formulation while we have made an attempt to use three different plants products in our study namely wheat germ oil, fresh juice of C. sativum, and A. vera to prepare polyherbal preparation.

In this study, we have found that glibenclamide (600 µg/kg) treated normal group of rats showed significant ($P < 0.001$) and progressive reduction in fasting plasma glucose levels during the period of study when compared with the normal untreated rats. We have also observed that Ph p-I treated normal group of rats produce a progressive and significant reduction in the fasting plasma glucose level during the period of study when compared with normal untreated rats at all the selected dose levels, i.e. at 1.0 ml/kg and 2.0 ml/kg but the higher doses (2.0 ml/kg) have shown more constituent results throughout the study period.

In the study, the group of diabetic rats showed elevated fasting plasma glucose levels. This observation suggests that single i.p. infection of alloxan (150 mg/kg) produced a reproducible and consistent diabetes mellitus and appears to be a suitable model of diabetes in our laboratory conditions.

The glibenclamide (600 µg/kg) treated diabetic group of rats showed significant ($P < 0.001$) and progressive reduction in fasting plasma glucose levels during the 30 days of the study period when compared with the diabetic group of rats. In agreement with the present results, several studies have shown antidiabetic activity upon glibenclamide treatment. The Ph p-I, II, and III treated groups of rats shown progressive and significant ($P < 0.001$) reduction in fasting plasma glucose of diabetic rats when compared with untreated diabetic rats at all selected dose levels (1.0 ml/kg and 2.0 ml/kg), but the higher dose 2.0 ml/kg have shown more consistent results through the 30 days period of study.

In this study, it can be postulated that Ph p-I significantly reduces the normal blood glucose level and Ph p-I, II, and III significantly reduce the elevated blood glucose level in diabetic rats. This indicated possible antidiabetic effects of polyherbal preparation.

Experimental diabetes is suggested to result from alloxan, a β-cytotoxin, a broad spectrum antibiotic has been widely used for inducing the diabetes mellitus in a variety of animals. Following its administration, alloxan is concentrated in the islets and it is reduced to dialuric acid. This acid is unstable in aqueous solutions and undergoes oxidation back to alloxan, accompanied by generation of O$_2^−$, hydrogen peroxide, and hydroxyl radicals by the Fenton type reaction. The islet cells have low concentrations of super oxide dismutase (SOD), catalase, and glutathione POD and it causes a massive destruction of β-cells of islets of langerhans by accumulation of cytotoxic free radicals resulting in reduced synthesis and release of insulin which paves the ways for the decreased utilization of glucose by the tissues and induce experimental diabetes mellitus. It is well established that sulfonureas produce hypoglycemia by increasing the secretion of insulin from pancreas, and these compounds are active in mild alloxan-induced diabetes whereas they are inactive in intense alloxan diabetes (nearly all β-cells have been destroyed). Since our results showed that glibenclamide reduced blood glucose levels in hyperglycemic animals, the state of diabetes is not severe. Alloxan-treated animals receiving the polyherbal preparation showed reduction of blood glucose levels in comparison to control, and this could be due to the possibility that some β-cells are still surviving to act upon by Ph p to exert its insulin releasing effect. Moreover, like sulfonureas oral administration of Ph p produced hypoglycemia in normal animals. This suggests that the
mode of action of the active ingredients of polyherbal preparation is probably mediated by an enhanced secretion of insulin, such as sulfonylureas.

The possibility of antidiabetic effects of polyherbal preparation may be due to the effect of active constituents of different plants, namely vitamins such as E, minerals such as chromium, manganese, zinc, and hydroxylated fiber glucomannan from A. vera, vitamin E (tocopherols) from wheat germ oil, and vitamin A from C. sativum.

Ascorbic acid is a reducing agent and can reduce in cells by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins and thereby neutralize reactive oxygen species such as hydrogen peroxide which is known to mediate the glycation-dependent degradation of several proteins and is widely involved in the damage of various β-cells and decreasing the β-cell mass in type-2 diabetes; however, it can also reduce metal ions which leads to the generation of free radicals through the Fenton reaction.

\[
2\text{Fe}^{3+} + \text{Ascorbate} \rightarrow 2\text{Fe}^{2+} + \text{Dehydroascorbate}
\]

\[
2\text{Fe}^{2+} + 2\text{H}_2\text{O}_2 \rightarrow 2\text{Fe}^{3+} + 2\text{OH}^- + 2\text{OH}^-
\]

Chromium increases the number of insulin receptors present in a target tissue and increases the binding of insulin to its receptors and regulates key reactions involving phosphorylation/dephosphorylation, which turn on and off insulin action. Chromium via the enzyme insulin receptor tyrosine kinase catalyses the phosphorylation in the presence of insulin. Additionally, chromium inhibits tyrosine phosphates, which is responsible for terminating the insulin receptor response. Thus, by both increasing activation and inhibiting termination of insulin receptor-mediated responses, chromium can significantly influence glucose utilization by peripheral tissues and regulate glucose levels. Magnesium is a co-factor in glucose oxidation, and modulates glucose transport across cell membranes. It may increase insulin secretion and/or improve insulin sensitivity/peripheral glucose uptake whereas glucomannan (hydroxylated fiber) delaying glucose absorption. Manganese, zinc, and iron are co-factors for superoxide dismutase (SODs) that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide which further reduce to give water and protect β-cells from the toxic effects of reactive oxygen species. Vitamin E is lipophilic and inhibits lipid peroxidation which occurs in the plasma membrane and damages the membrane structure and permeability, scavenging lipid peroxyl radicals to yield lipid peroxides and the tocophrophyl radicals and protects the membrane from oxidation. In other words, inhibition of lipid peroxidation by antioxidants and vit E improves β-cells function which might influence protein glycation, lipid oxidation, and insulin secretion/sensitivity. It may also affect nonoxidative glucose metabolism. Besides these active chemical constituents of different plants polyherbal preparation also possesses vit A, vit B₁₂, digestive enzymes such as amylase and lipase that can offset the toll blood glucose level.

Therefore, polyherbal preparation is potent in preserving β-cell function in diabetes by scavenging free radicals liberated by alloxan in diabetic rats and also regulate key reactions involves in turn on and turn off insulin action as well as in glucose utilization. On the basis of above results, it could be concluded that polyherbal preparation, a combination of three herbal plants exert a significant antidiabetic effect. This could be due to different types of active principles, each with a single or a diverse range of biological activities, which serves as a good adjuvant in the present armamentarium of antidiabetic drugs.

CONCLUSION

This study on the polyherbal preparation-I, II, and III formulated from three different plants, i.e. wheat germ oil, fresh juice of C. sativum, and A. vera gel in three different ratios were started with an expectation and objective to explore the possibility of the drug to exert antidiabetic effect. In this study, alloxan-induced diabetic animals were used. Antidiabetic animals were treated with different doses of polyherbal preparation-I, II, and III to assess the effect of the drugs. After treatment, to analyze the effect of the drug glucose estimation test was performed. The data obtained were satisfactory and conclusive so as and to accomplish our objectives. In conclusion, the present data indicated that the polyherbal preparation-I, i.e. wheat germ oil, fresh juice of C. sativum and A. vera in the ratio of 1:1:1 showed significant glucose lowering effect in normal rats and polyherbal preparation-I, II, i.e. wheat germ oil, fresh juice of C. sativum, and A. vera in the ratio of 2:2:1 and polyherbal preparation-III, i.e. wheat germ oil, fresh juice of C. sativum, and A. vera in the ratio of 1:2:2 showed significant glucose lowering effect in alloxan-induced diabetic rats. This study suggests that polyherbal preparation-I, II, and III possess antidiabetic activity and is going to be a promising antidiabetic preparation for masses. An exact mechanism underlying this effect is not clear, but apparently may be due to preserving β-cell function in diabetic rats. Further studies are needed to elucidate the mechanism of action and to know the active principle/s involved in producing the effect.

Keeping in view the tremendous pharmacological activities these polyherbal preparations possess, it may be utilized to alleviate the symptoms of diabetes; however, it has to
be confirmed by clinical trials before put into the therapy.

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