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Evaluation of Hypoglycaemic Activity of Some Medicinal Plants of Satpuda Forest on Alloxan Induced Diabetes in Rats

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Abstract

Diabetes mellitus is a common endocrine disorder characterized by insufficient or lack of insulin and impaired carbohydrate metabolism in the body leading to long-term complications affecting the eyes, nerves, blood vessels, skin, and kidneys. There is an estimated 143 million people worldwide suffering from diabetes: a figure which is almost five times as much as the estimates ten years ago. In spite of the presence of antidiabetic drugs in pharmaceutical market, there is an increasing demand by patients to use the herbal products having hypoglycaemic activity. There have been reports on the hypoglycaemic activity of *Gymnema sylvestre* (Gurmar), *Eugenia jambolana* (Jamun), *Momordica charantia* (Karela) and *Alstonia scholaris* (Satwan) on individual plant basis. The present work was undertaken to study the antidiabetic effect of the individual plant extract and the composite of *G. sylvestre*, *E. jambolana*, *M. charantia* and *A. scholaris* on healthy and alloxan induced diabetic rat models. In the *in vivo* experiments, oral administration of individual plant extract and their composite in an appropriate concentration in normal and alloxan induced diabetic rats were evaluated. Blood samples from normal and experimental animals were collected and assayed for blood glucose level (BGL) and plasma insulin level (PIL). Results showed that there was significant reduction in blood glucose level and increase in plasma insulin level due to oral administration of extracts derived from *G. sylvestre*, *E. jambolana*, *M. charantia* and *A. scholaris* in alloxan induced diabetic rat models on individual plant basis but not in normal rats ($p<0.05$). As compared to administration of individual plant extract, the composite of all four plants shown highly significant decrease in BSL and augmentation in PIL in alloxan induce diabetic rats. It is concluded that the combined extracts from four plants proved its potentially excellent hypoglycaemic activity and such polyherbal therapy could be use in the management of diabetes.

Key Words: Hypoglyceamic activity, *Gymnema sylvestre*, *Eugenia jambolana*, *Momordica charantia*, *Alstonia scholaris*, Alloxan, Diabetes

Introduction

One of the commonest endocrine disorders is diabetes mellitus that is characterized by insufficient or lack of insulin and impaired carbohydrate metabolism in the body leading to excretion of sweet urine and long-term complications affecting the eyes, nerves, blood vessels, skin, and kidneys. It appears that rich food and sedentary lifestyles have created a worldwide epidemic of this disease. As per the current estimate, there are about 143 million people worldwide suffering from diabetes: a figure which is almost five times as much as the estimates ten years ago. In the global scenario, diabetic patients may rise to 300 million in 2025 [7]. The disease is affecting at an alarming rate to both rural and urban populations in India [6 and 9].

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human disorders. Medicinal plants have formed the basis of health care throughout the world and have considerable importance in international trade [1]. In India there are about 45 000 plant species and among them, several thousands have been claimed to possess medicinal properties. In the traditional system of medicine many plants have been claimed to be useful in the treatment of diabetes mellitus [4]. In spite of the presence of large number of pharmaceutical drugs for the treatment of diabetes, there is a continuous increasing demand by the patients for herbal products that show potential hypoglycaemic activity [8].

There have been reports on the hypoglycaemic activity of *Gymnema sylvestre*, *Eugenia jambolana*, and *Momordica charantia*, which vary in regard to the plant species, the part of the plant used, and in the preparation of extracts as well as the animal models. There has been a scarcity of literature in relation to anti-diabetic potential of *Alstonia scholaris.* The present work was undertaken to study the hypoglycaemic effect of *Gymnema sylvestre*, *Eugenia jambolana*, *Momordica charantia*, and *Alstonia scholaris* on healthy and alloxan induced diabetic rat models on the basis of individual plant extract and more trace has been given to workout the synergic action of combined mixture to combat the diabetes.

Materials and Methods

Plant materials and preparation of extracts:

Matured leaves of *Gymnema sylvestre* (Family Asclepiadaceae, Gurmar in Hindi that means sugar destroyer and Periploca of the wood in English) and *Alstonia scholaris* (Family Apocynaceae, Satwan in Hindi or Saptparni in Marathi) were collected from Satpura Forest near the Northwest Maharashtra- Madhya Pradesh border, India. Fruits of *Eugenia jambolana* (Family Myrtaceae, Jamun in Hindi and Black Berry in English) and Momordica charantia (Family Cucurbitaceae, Karela in Hindi and Bitter Gourd in English) were collected form local market. Plant materials were identified by Ayurvedarchaya Dr. Vikas Gulve, Bhusawal, Jalgaon district, Maharashtra, India. Leaves of *G. sylvestre* and *A. scholaris* were cleaned and washed with water. Then 50 g of selected leaves were dried separately under shade at 25^0C for 5 days in absence of sunlight and grounded well to fine powder. Nearly 30 g powdered leaves were extracted with boiling water using soxhlet extractor. The extractions were continued up to 12 h. The extracts were then cooled and filtered using Whatman filter paper no.1. The filtrates were centrifuged at 10,000 rpm at room temperature and sediments in both preparations were discarded. Supernatants were concentrated up to 100 mL on rotavapor under reduced pressure. The concentrated crude extracts were lyophilized into powder for both plants. Dried seeds of *E. jambolana* and whole fruits of *M. charantia* were mechanically crushed and extracted with distilled water separately at the room temperature up to 48h. The extract was filtered and concentrated in rotary evaporator under reduced pressure to obtain semisolid material, which was then lyophilized to get a powder (yield: 13.8% w/w). The lyophilized powder for all plants were dissolved in distilled water (DW) and used for evaluating the hypoglycaemic activity on the individual plant basis and the mixture of all plants extracts on normal and alloxan induced diabetic rats.

Chemicals

Chemically, alloxan is 2,4,5,6 tetra oxo hexahydro pyrimidine. It is the most widely used chemical for diabetogenesis since it has the ability to destroy the beta cells in Islets of Langerhans in pancreas: the part responsible for secretion of hormone insulin. Alloxan was obtained from Himedia Laboratory Limited, Mumbai, Maharashtra, India. Alloxan can be administered virtually through all routes in the body to induce the diabetes.

Experimental animals:

The Wister strains of male albino rats (weighing between 100-150 g) were obtained from Raj Udyog, Pune, MS, India. The animals were housed in larger spacious cages and they were fed with standard rat pellet diet (Hindustan Lever Ltd. Mumbai, India) and had free access to water *ad libitum*. The animals were acclimatized to standard environmental conditions of temperature (25± 5^0C) and humidity (55 to 60%) and 12 h light dark cycles throughout the experimental period. The institutional Ethical committee has approved the study.

Induction of diabetes in rats:

The overnight fasted rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt. The normal untreated rats received the same amount of saline solution. After one hour of alloxan administration, animals were fed *ad libitum* and 1 mL of (100mg/mL) glucose i.e., combats ensuring severe hyperglycaemia. After 72 h of the alloxan injection, the animals were tested for evidence of diabetes by drawing the blood from near the ear with the help of hypodermal syringe using glucose estimation kit. The blood glucose level more than 150 mg/100mL of blood was criteria.

In the experiment, a total of 49 rats (42 diabetic surviving rats and 7 normal rats) were used. The rats were segregated into 7 groups with minimum 7 rats in each group.

Group I : Normal untreated rats
Group II : Diabetic induced rats
Group III : Diabetic rats treated with leaf extract of *G. sylvestre*,
Group IV : Diabetic rats treated with leaf extract of *A. scholaris*
Group V : Diabetic rats treated with seed extract of *E. jambolana*
Group VI : Diabetic rats treated with fruit extract of *M. charantia*
Group VII: Diabetic rats treated with mixed extracts of *G. sylvestre*, *E. jambolana*, *M. charantia* and *A. scholaris.*

Treatment protocol and collection of blood samples:

Test extracts (250 mg/ kg body wt.) and control (2 mL saline) were administered orally to rats at every 24 h. The experimental animals were carefully monitored everyday; no sign of toxicity was noticed on the behaviour and general health of the rats when exposed to plant extracts. Animals described as fasted were deprived of food for at least 12 h but allowed free access to drinking water. Blood samples form the rats belonging to each group were withdrawn at interval of initial, 1, 3 and 5^{th} h of administration of single dose. Blood was collected with EDTA as anticoagulant and centrifuged at 3000 rpm for 15 min to separate plasma.

Analytical procedures:

Estimation of blood glucose level (BGL) in all groups was done by glucose oxidase method using standard glucose estimation kit and plasma insulin level (PIL) was assayed by Enzyme Linked Immuno Sorbent Assay kit using human insulin as standard. Both the analytical kits were obtained from Span Diagnostic Private Ltd., Udhana, Surat, Gujrat, India.

Statistical analysis: All values were expressed as mean ± SD. Two-way analysis of variance (ANOVA) was performed using Graph Pad Prism 4.02 for Windows (Graph Pad Software, San Diego CA, USA). Differences were considered to be significant when $p<0.05$.

Results and Discussion

Results in Table 1 shows that there was significant elevation in the blood glucose levels in alloxan induced rats (group II) as compared to normal untreated rats (group I). After alloxan administration, blood glucose levels in diabetic rats were raised by 1.8 to 1.9 times the value of the normal untreated rats. The intraperitoneal injection of alloxan in experimental animals produced hyperglycaemia, impaired carbohydrate metabolism and insulin resistance. Among the administration of plant extracts to diabetic rats (group III, IV, V, and VI), all the four plants extracts shown decreasing trend in blood glucose levels in diabetic rats on the individual plant

basis. Particularly, the rats treated with leaf extract of *G. sylvestre* (group III) shown significantly higher reduction in BGL as compared to diabetic rats treated with other three plants extracts (group IV, V, and VI). In the present study, it is important to note that the diabetic rats treated with mixed extracts of *G. sylvestre*, *E. jambolana*, *M. charantia* and *A. scholaris* (group VII) bring drastic reduction in blood glucose levels as compared to impact of individual plant extract of four plants on the diabetic rats.

Table 1. Effect of plant extracts on blood glucose level (mg/100mL) of alloxan induced diabetic rats after single dose.

Parameter	Dose	Initial	1 h	3 h	5 h
Group I Normal untreated rats	2 mL saline	96.63 ± 0.045	94.33 ± 0.036	95.54 ± 0.023	97.42 ± 0.061
Group II Diabetic induced rats	2 mL saline	174.56 ± 2.64 a	178.35 ± 3.07 a	182.44 ± 4.91 a	184.72 ± 3.72 a
Group III Diabetic rats + leaf extract of *G. sylvestre*	250 mg/ kg body wt	155.85 ± 2.44 b	152.05 ± 3.06 b	151.43 ± 2.12 b	149.76 ± 2.63 b
Group IV Diabetic rats + leaf extract of *A. scholaris*	250 mg/ kg body wt	164.33 ± 3.08 b	162.04 ± 2.98 b	160.12 ± 3.81 b	158.98 ± 2.88 b
Group V Diabetic rats + seed extract of *E. jambolana*	250 mg/ kg body wt	159.49 ± 2.19 b	157.69 ± 2.44 b	154.91 ± 2.17 b	151.11 ± 2.98 b
Group VI Diabetic rats + fruit extract of *M. charantia*	250 mg/ kg body wt	168.63 ± 2.31 b	167.07 ± 2.53 b	165.53 ± 2.33 b	162.91 ± 1.90 b
Group VII Diabetic rats + mixed extracts of all four plants	250 mg/ kg body wt	142.65 ± 3.02 c	141.66 ± 2.36 c	134.32 ± 2.86 c	132.28 ± 2.25 c

Values are mean ± SD of six observations in each group. Values are statistically significant at $p < 0.05$. Statistical comparison within the groups as follows: a: Group II and Group I, b: plant extract treated rats (Group III, IV, V and VI) and alloxan induced diabetic rats (Group II), and c: diabetic rats treated with composite of all four plants (Group VII) and diabetic rats (Group II).

Table 2. Effect of plant extracts on plasma insulin level (µU/mL) of alloxan induced diabetic rats after single dose

Parameter	Dose	Initial	1 h	3 h	5 h
Group I Normal untreated rats	2 mL saline	12.19 ± 0.32	12.78 ± 0.25	13.55 ± 0.09	12.68 ± 0.85
Group II Diabetic induced rats	2 mL saline	5.51 ± 0.09 a	5.35 ± 0.16 a	6.04 ± 0.22 a	5.78 ± 0.12 a
Group III Diabetic rats + leaf extract of *G. sylvestre*	250 mg/ kg body wt	8.11 ± 0.12 b	8.15 ± 0.16 b	8.43 ± 0.18 b	8.97 ± 0.15 b
Group IV Diabetic rats + leaf extract of *A. scholaris*	250 mg/ kg body wt	7.37 ± 0.09 b	7.52 ± 0.18 b	7.42 ± 0.27 b	7.97 ± 0.32 b
Group V Diabetic rats + seed extract of *E. jambolana*	250 mg/ kg body wt	8.02 ± 0.10 b	8.19 ± 0.25 b	8.46 ± 0.55 b	8.72 ± 0.07 b
Group VI Diabetic rats + fruit extract of *M. charantia*	250 mg/ kg body wt	7.79 ± 0.12 b	7.77 ± 0.32 b	7.98 ± 0.14 b	8.03 ± 0.37 b
Group VII Diabetic rats + mixed extracts of all four plants	250 mg/ kg body wt	11.01 ± 0.08 c	11.69 ± 0.16 c	10.36 ± 0.52 c	11.28 ± 1.03 c

Values are mean ± SD of six observations in each group. Values are statistically significant at $p < 0.05$. Statistical comparison within the groups as follows: a: Group II and Group I, b: plant extract treated rats (Group III, IV, V and VI) and alloxan induced diabetic rats (Group II), and c: diabetic rats treated with mixed extracts of four plants (Group VII) and diabetic rats (Group II).

On administration of alloxan, the plasma insulin level (PIL) significantly decreased in diabetic rats (group II) as compared to normal untreated rats (group I). Alloxan induced diabetic rats shown drop in their PIL by 45% of its value in the normal untreated rats (Table 2). The intraperitoneal injection of alloxan in experimental animals caused severe destruction of beta cells in Islets of Langerhans in pancreas and thus prevented the synthesis of hormone insulin leading to drastic depletion in plasma insulin level.

In the diabetic rats administered with plant extracts of *G. sylvestre*, *E. jambolana*, *M. charantia* and *A. scholaris* belonging to group III, IV, V, and VI respectively shown increasing trend in plasma insulin levels in diabetic rats on the individual plant basis. Particularly, the rats treated with leaf extract of *G. sylvestre* (group III) shown significantly higher elevation in PIL as compared to diabetic rats treated with other three plants extracts (group IV, V, and VI). It is important to note synchronizing results that the diabetic rats treated with mixed extracts of *G. sylvestre*, *E. jambolana*, *M. charantia* and *A. scholaris* (group VII) tend to bring plasma insulin levels to the normal and at the same time they shown drastic reduction in blood glucose levels (Table 1) as compared to impact of individual plant extract of four plants on the diabetic rats. These findings clearly established that there is a synergic action of the all the constituents present in extracts of four plants that bring highly significant reduction in the blood glucose level of the diabetic rats due to rejuvenation of beta cells in pancreas that restored its ability to produce insulin. As a result there was increase in plasma insulin levels of diabetic rats belonging to group VII through out the experimental period under the influence of combined extracts of all four plants.

In results, the individual role of *G. sylvestre, E. jambolana*, and *M. charantia* as potent hypoglycaemic plant is in accordance with the observations reported by Mitra and Bhattacharya [5], Kar et al. [3], Shukla et al [10] and Grover et al. [2]. Possibly, mechanism of action of these plants is that they make the receptors of target cells (like muscles and fat tissues) more sensitive to insulin, and therefore, the cells increase their absorption of glucose. Within the treatment period, combined extracts of *G. sylvestre*, *E. jambolana*, *M. charantia* and *A. scholaris* shown positive synergy to bring elevation in plasma insulin level and depletion in blood glucose levels in diabetic rats. Thus, constituents present in extracts of selected plants in the present study tend to compliment each other thereby producing the desired hypoglycaemic effect. This observation may buttress the proposition of Tiwari and Rao [11] as per advantage of polyherbal therapies over monotherapy to treat the diabetes mellitus.

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