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Development and Evaluation of Polyherbal Formulation for Diabetes

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Abstract

Objective: Treatment of diabetes without any adverse effects is a big challenge to the medical fraternity. There is need for alternative drugs with no side effects. The present study is aimed to develop a polyherbal formulation for effective management of diabetes using some of the indigenous plants taking lead from ethnobotanical information. *Ajuga parviflora, Saraca asoca, Potentilla fulgens* and *Aconitum heterophyllum* were selected for the development of antidiabetic formulation. **Materials and Methods:** Preliminary phytochemical screening of selected plants were performed. *In-vitro* antidiabetic activity of plants and standard drug acarbose was evaluated by α-amylase inhibition assay. The polyherbal suspension prepared and its antidiabetic activity was evaluated in a streptozotocin-induced diabetic rat model. **Results:** Preliminary phytochemical screening revealed the presence of alkaloids, terpenoids, flavonoids, phenolics and tannins in the plants. The prepared formulation was brown, easily pourable from the container and have redispersibility property with optimum particle size distribution. Sedimentation studies showed that the sedimentation volume of a formulation is in-between a range of 1. Administration of polyherbal formulation at 200 mg/kg and 400 mg/kg dose for 21 days to diabetic rats decreased fasting blood sugar 159 ± 2.81 mg/dL and 147.74 ± 2.03 mg/dL respectively as compared to the diabetic control group 361 ± 3.89 mg/dL. **Conclusion:** The present findings indicated that developed polyherbal formulation at a dose of 200 mg/kg and 400 mg/kg showed significant antihyperglycemic activity (***p<0.001) in streptozotocin induced diabetic rat model.

Keywords: *Aconitum heterophyllum*, *Ajuga parviflora*, Diabetes, Polyherbal formulation, *Potentilla fulgens*, *Saraca asoca*

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Introduction

Diabetes mellitus is a group of metabolic disorders characterized by a chronic hyperglycemic condition with elevated blood glucose levels.[1] It may be either impaired insulin production or the inability of cells to respond properly to the insulin synthesized from beta cells.[2] The diabetic condition is described by fasting hyperglycemia alongside a danger of thrombolytic and atherosclerotic problems that predominantly influence the cerebral, fringe, and coronary veins. Diabetes is associated with various complications including microvascular and macrovascular complications such as retinopathy, nephropathy, atherosclerosis, endothelial dysfunction, and erectile dysfunctions.[3]

Oral hypoglycemic drugs are effective in the management of diabetes. In recent years, the most accepted drugs for the management of diabetes are insulin and oral hypoglycemic agents. There are several types of glucose-lowering drugs including insulin secretagogues (sulfonylurea and meglitinides), insulin sensitizers (biguanides, metformin, and thiazolidinediones), and α-glucosidase inhibitors (miglitol and acarbose).[4] A newer class of agent dipeptidyl peptidase-IV inhibitors is sitagliptin and vildagliptin. They promote blood glucose homeostasis by stimulating insulin secretion from pancreatic beta cells in a glucose-dependent manner.[5] However, most glucose-lowering drugs may have side effects, such as severe hypoglycemia, lactic acidosis, liver cell injury, permanent neurological deficit, digestive discomfort, headache, dizziness, and even death.[6]

Several plant species have been reported in the traditional system of medicine to treat diabetes. In recent years, there has been an increased inclination toward herbal formulations due to the trend toward natural sources and a healthy lifestyle. Moreover, the complexity, side effects, and costly treatment associated with the allopathic medicines have caused both the health-care practitioners and the majority of world populations to turn toward alternative therapies, more likely toward the herbal medicines since these systems are believed to be free from side effects and affordable. Various traditional medicinal plants and synthetic compounds derived from their active principles have been shown to possess antidiabetic properties. Several phytomolecules including flavonoids, alkaloids, glycosides, saponins, glycolipids, dietary fibers, polysaccharides, peptidoglycans, carbohydrates, amino acids, and others obtained from various plant sources have been reported as potent hypoglycemic agents.[7]

In the traditional system of plant medicine, it is usual to use plant formulation and combined extract of the plant as a drug of choice rather than individual ones, to get the advantage of synergism, and to find suitable antidiabetic and antioxidant combination therapy.[8] Nowadays, the polyherbal formulation concept is developed. These formulations were found effective in chronic illness. In diabetes treatment, many polyherbal

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formulations are available in the market and are very effective. Triphala, a polyherbal formulation found very effective in oxidative stress conditions and on the cell-mediated immune response. Dianex, a polyherbal formulation consisting of the aqueous extracts of eight medicinal plants, showed very effective results in diabetic rats.[9] Hyponidd is a herbomineral formulation composed of the extracts of 10 medicinal plants investigated for its possible antihyperglycemic and antioxidant effect in diabetic rats. The results showed that hyponidd exhibits antihyperglycemic and antioxidant activity in streptozotocin (STZ)-induced diabetic rats.[10]

The study aimed to develop antidiabetic polyherbal formulation from some Indian medicinal plants and scientifically investigate the hypoglycemic activity of the developed formulation in Wistar albino rats as diabetes is associated with increased blood sugar concentration.

Materials and Methods

Materials

The fresh leaves of *Ajuga parviflora* were collected from the Bheemtal. The roots of *Potentilla fulgens* were collected from the Uttarkashi. The flowers of *Saraca asoca* were collected from the Central Institute of Medicinal and Aromatic Plants, Lucknow. The fresh roots of *Aconitum heterophyllum* were procured from the Uttarkashi. All the plant samples were identified by Botanical Survey of India, Dehradun, Uttarakhand. 3,5-dinitrosalicylic acid (DNS) was procured from Sigma-Aldrich. α-amylase was purchased from MP Biomedicals Ltd. STZ was procured from Sisco Research Laboratories Pvt. Ltd. Glibenclamide was obtained as a gift sample from Bal Pharma Ltd., Bengaluru.

Extraction

Each plant powder was placed in a closed vessel. Ethanol (90%) was used as a solvent. The different plant materials were soaked in ethanol (90%) individually for 7 days in a closed vessel. The vessels were shaken occasionally. Extracts of different plant materials were filtered. Filtrates thus obtained were evaporated to obtain the dried extract. The percentage yield (% w/w) of each dried plant extract was calculated. Crude extracts thus obtained were stored in airtight container for further studies.

Preliminary Phytochemical Screening

The ethanolic extracts of all four plants were subjected to preliminary phytochemical screening for the detection of various phytoconstituents.

In Vitro Antidiabetic Activity of Different Plant Extracts

In vitro antidiabetic activity of different plant extracts was determined by α-amylase inhibition.[11] The extracts of different plant samples were subjected to α-amylase inhibitory assay. The α-amylase inhibitory assay was performed using DNS (Sigma-Aldrich). A 500 µL of test extracts of different concentrations were mixed with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) containing 0.04 units of α-amylase (MP Biomedicals Ltd.) solution. The solutions were incubated at 37°C for 10 min. After incubation, 500 µL of 1% starch solution (made in 0.02 M sodium phosphate buffer, pH 6.9) was added in each tube. One milliliter of DNS reagent was added in each tube to stop the reaction. The test tubes were placed for 5 min in a boiling water bath and kept at room temperature for cooling. Ten milliliters of distilled water were added into each tube for the dilution of the reaction mixture and the absorbance was taken at 540 nm. The control sample was prepared similarly except it consists of 500 µL of the solvent instead of extract. The absorbance of the control sample was determined at 540 nm. Acarbose (Glucobay Bayer Zydus Pharma) was used as the reference standard. The results were expressed as % inhibition of enzyme activity according to the following formula given below.

Inhibition activity (%) = ([Absorbance of control–Absorbance of test sample]/Absorbance of control) × 100

IC_{50} values of test samples and reference standard were calculated from the calibration curve by plotting the graph between % inhibition and different concentration of the sample.

Development of Polyherbal Formulation

The polyherbal suspension of all four plant extracts was prepared by the trituration method in mortar and pestle. The ethanolic extracts of *P. fulgens* roots, *A. parviflora* leaves, *A. heterophyllum* roots, and *S. asoca* flowers were mixed in an equal ratio of 1:1:1:1.[12] One hundred milliliters polyherbal suspension contains 10 g of bioactive extract. Tween 80 (0.1%) and sodium carboxymethyl cellulose (CMC) (2 g) were used as a suspending agent, propylparaben (0.20%) as the preservative, glycerine (1 mL) as a wetting agent, peppermint oil (0.15%) as a flavoring agent, and purified water (q.s) was utilized as a vehicle. A uniformly dispersed suspension was obtained.[13,14]

Evaluation of Polyherbal Formulation

The prepared polyherbal formulation was evaluated for organoleptic characters, sedimentation volume, density, viscosity, particle size, pH, and antihyperglycemic activity.

pH

The pH of the developed polyherbal formulation was determined using a digital pH meter.

Particle size determination

Particle size determination of formulation was done by optical microscopy. A drop of the suspension was placed on a glass slide with a coverslip and observed under 40×. Individual particle size was determined using an ocular micrometer calibrated with the help of a stage micrometer. The average particle size of the formulation was determined by calculating the means of 30 particle sizes.[15]

Density

The density of suspension was determined using a relative density bottle. The ratio of the density of the two liquids is the relative density of the first liquid to the second liquid.[16] The density of suspension is determined by the given formula.

$\rho_1 = (w_3\text{-}w_1/w_2\text{-}w_1) \times \rho_2$
ρ_1 = Density of unknown liquid (formulation)
ρ_2 = Density of water
$w_3\text{-}w_1$ = Weight of unknown liquid (formulation)
$w_2\text{-}w_1$ = Weight of water.

Viscosity

The viscosity of the formulation was determined at 25°C using a rotational viscometer.

Sedimentation volume

The homogenized suspension was taken in a stoppered measuring cylinder and stored at room temperature (27°C ± 1°C). The volume of sediment was observed at regular intervals of time. The sedimentation volume was calculated as the ratio of ultimate height (V_u) of the sediment to the initial height (V_o) of the suspension.[17]

$F = V_u$ (Final settled volume)/V_o (initial volume)

Antihyperglycemic Activity of the Polyherbal Formulation

Acute toxicity study

The acute toxicity study of the formulation was carried out in Wistar albino rats according to OECD guideline 423 adopted in December 2001.[18] The rats were administered an oral dose of herbal suspension up to 2000 mg/kg body weight. Mortality or behavioral changes were observed.

Animals

Antihyperglycemic activity of the polyherbal formulation was evaluated in a STZ -induced diabetic rat model. Healthy adult albino Wistar rats (180–200 g) of either sex were procured from the Indian Toxicology Research Centre, Lucknow (ITRC). Rats were housed in standard polypropylene cages lined with husk. The animals were kept in standard environmental conditions. Animals were maintained under controlled room temperature (22°C ± 2°C) and relative humidity (45 ± 5%) with a 12:12 h light and dark cycle. A standard pellet diet was given to the animals and water *ad libitum*. The animals were acclimatized to the laboratory conditions for 7 days before behavioral studies. All the experimental procedures used in the study were approved by the Animal Ethical Committee of the Institute (Reg. No. 809/PO/Re/S/03/CPCSEA).

Induction of diabetes

Healthy male Wistar albino rats of average bodyweight 180-200 g were selected for the study. The animals were fasted overnight and made diabetic by intraperitoneal injection of STZ at a dose of 45 mg/kg body weight. STZ was prepared in freshly prepared 0.1 M ice-cold citrate buffer (pH 4.5) solution. After 6 h of STZ administration, 5% dextrose solution given in feeding bottles was placed in the different cages for 24 h to control the starting hypoglycemia. After 48 h of STZ injection, the blood glucose level of animals was checked by a glucometer. Wistar rats having blood glucose levels above 250 mg/dL were selected for antihyperglycemic study. Animals not found diabetic after 48 h of STZ injection were not considered and omitted from the study.[19]

Treatment protocol

The rats were randomly divided into different groups and each group consisted of six rats. Animals received the following treatments up to 21 days.

1. Group I: Normal control rats treated with 0.1 % (w/v) CMC
2. Group II: Diabetic rats (STZ 45 mg/kg, i.p) treated with 0.1 % (w/v) CMC
3. Group III: STZ rats treated with polyherbal formulation (200 mg/kg, p.o)
4. Group IV: STZ rats treated with polyherbal formulation (400 mg/kg, p.o)
5. Group V: STZ rats treated with glibenclamide (5 mg/Kg, p.o).

Fasting blood glucose levels were checked regularly on 0 day (before administration of test sample), 7th day, 14th day, and 21st day. The blood glucose levels were measured with the help of an Accu-Chek glucometer by pricking the tail vein of the rat. Blood samples were collected from the tip of the tail of each rat under mild anesthesia and tested for glucose concentration.[20]

Table 1: Ethanol-soluble extractive value of different plant samples

Ethanolic extract	Color	Nature	% w/w Mean±SD
A. parviflora leaves	Blackish-Green	Viscous	18.42±0.98
P. fulgens roots	Dark brown	Gummy	19.16±0.79
A. heterophyllum roots	Muddy	Granular mass	27.76±0.54
S. asoca flowers	Rusty brown	Viscous	16.99±0.81

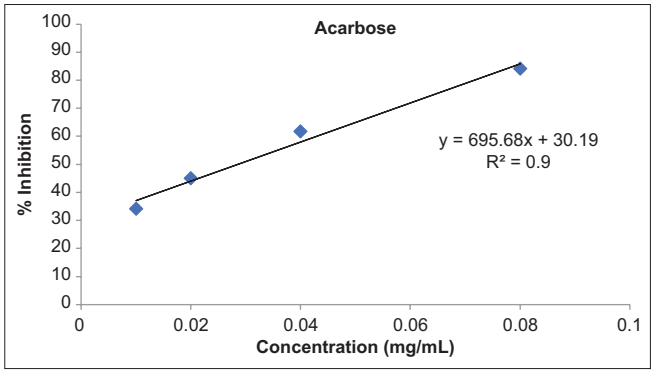

Figure 1: *In vitro* antidiabetic assay of acarbose by 3,5-dinitrosalicylic acid method color reagent

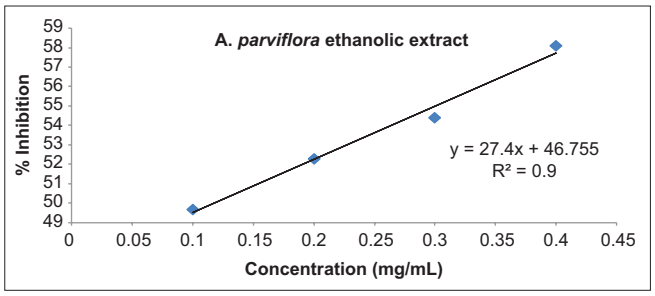

Figure 2: *In vitro* antidiabetic assay of *Ajuga parviflora* ethanolic extract by 3,5-dinitrosalicylic acid method color reagent

Results

Extraction

Ethanol-soluble extractive values of different plant samples were determined in triplicate and results are given as Mean ± SD in Table 1.

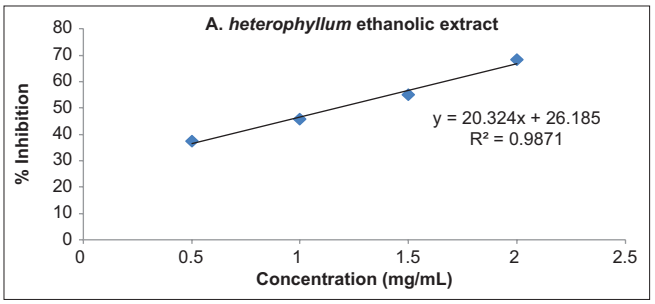

Figure 3: *In vitro* antidiabetic assay of *Aconitum heterophyllum* ethanolic extract by 3,5-dinitrosalicylic acid method color reagent

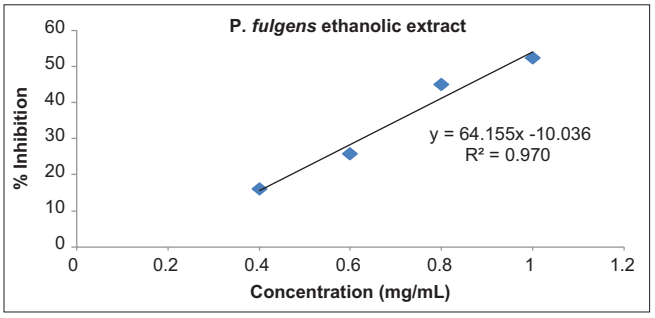

Figure 4: *In vitro* antidiabetic assay of *Potentilla fulgens* ethanolic extract by 3,5-dinitrosalicylic acid method color reagent

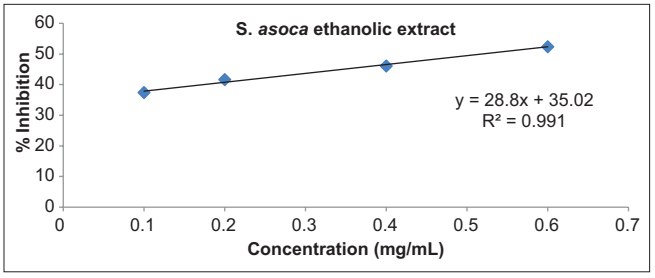

Figure 5: *In vitro* antidiabetic *assay of Saraca asoca* ethanolic extract by 3,5-dinitrosalicylic acid method color reagent

Table 2: Preliminary phytochemical screening of different plant samples

Plant constituents	Ethanolic extract			
	A. parviflora	*P. fulgens*	*A. heterophyllum*	*S. asoca*
Carbohydrates	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	–	–	–	–
Flavonoids	+	+	–	+
Phenolic and Tannins	+	+	–	+
Alkaloids	+	–	+	–
Saponin	–	+	–	–

Preliminary Phytochemical Screening

The preliminary phytochemical studies showed the presence of different chemical constituents that are responsible for the medicinal properties of plants. The results are tabulated in Table 2.

Table 3: Percentage of α-amylase inhibition and IC_{50} of acarbose at various concentrations

Concentration of acarbose (mg/mL)	% inhibition	IC_{50}
0.01	34.17	0.028 mg/mL
0.02	45.12	
0.04	61.70	
0.08	84.12	

Table 4: Percentage of α-amylase inhibition and IC_{50} of *A. parviflora* ethanolic extract at various concentration

Concentration of *A. parviflora* (mg/mL)	% inhibition	IC_{50}
0.1	49.67	0.11 mg/mL
0.2	52.27	
0.3	54.38	
0.4	58.1	

Table 5: Percentage of α-amylase inhibition and IC_{50} of *A. heterophyllum* ethanolic extract at various concentration

Concentration of *A. heterophyllum* (mg/mL)	% inhibition	IC_{50}
0.5	37.4	1.17
1.0	45.8	mg/mL
1.5	54.89	
2.0	68.25	

Table 6: Percentage of α-amylase inhibition and IC_{50} of *P. fulgens* ethanolic extract at various concentrations

Concentration of *P. fulgens* (mg/mL)	% inhibition	IC_{50}
0.4	16.12	0.94 mg/mL
0.6	25.8	
0.8	45	
1.0	52	

Table 7: Percentage of α-amylase inhibition and IC_{50} of *S. asoca* ethanolic extract at various concentrations

Concentration of *S. asoca* (mg/mL)	% inhibition	IC_{50}
0.1	37.42	0.52 mg/mL
0.2	41.6	
0.4	46.1	
0.6	52.4	

Table 8: Determination of density of the polyherbal suspension

Parameter	Value	Density of suspension $\rho_1 = (w_3 - w_1/w_2 - w_1) \times \rho_2$
Wt. of RD bottle (w_1)	13.5 g	1.43 g/cm^3
Wt. of RD bottle+Water (w_2)	23.5 g	
Wt. of RD bottle+suspension (w_3)	28.9 g	
Density of water at 25°C ρ_2	1(g/cm^3)	

In Vitro Antidiabetic Activity of Different Plant Extracts

The *in vitro* antidiabetic activity was assessed by α-amylase inhibition assay based on the DNS method. The results showed that as the concentration of inhibitors increased, the percentage of α-amylase inhibition increased resulting in the reduction of hydrolysis of starch [Figures 1-5]. The results are tabulated below in Tables 3-7.

Table 9: Determination of particle size of the polyherbal suspension

Particle size (μm)
14.2
28.4
21.3
35.5
42.6
14.2
28.4
21.3
42.6
35.5
21.3
14.2
28.4
42.6
35.5
21.3
14.2
35.5
28.4
42.6
49.7
42.6
35.5
21.3
28.4
35.5
14.2
21.3
28.4
49.7

The average size of particle=29.82 μm

Table 10: Determination of sedimentation volume of the polyherbal suspension

Time	Original volume of suspension before settling in mL (V_o)	Ultimate volume of sediment in mL (V_u)	Sedimentation volume $F=V_u/V_o$
10 min	100	98	0.98
20 min	100	95	0.95
30 min	100	90	0.90
40 min	100	88	0.88
1 h	100	83	0.83
4 h	100	67	0.67
12 h	100	63	0.63
24 h	100	55	0.55
36 h	100	48	0.48
48 h	100	44	0.44
72 h	100	44	0.44

Evaluation of Polyherbal Formulation

Organoleptic properties

The developed polyherbal suspension was brown in color, liquid in nature, agreeable odor, and having good redispersibility.

pH

The pH of the polyherbal suspension was found to be 5.1.

Viscosity

The viscosity of polyherbal suspension was found to be 40.901 Cps.

Density

The density of suspension was found to be 1.43 g/cm^3 [Table 8].

Particle size determination

The average size of particles of suspension was found to be 29.82 μm [Table 9].

Sedimentation volume

Sedimentation studies showed that the sedimentation volume of a formulation is in-between a range of 1 [Table 10].

Antihyperglycemic Activity of the Polyherbal Formulation

The results of the effect of polyherbal formulation made of ethanolic extract of *A. parviflora*, *A. heterophyllum*, *P. fulgens*, and *S. asoca* at 200 mg/kg and 400 mg/kg dose on fasting serum glucose levels in diabetic rats are given in Table 11.

Discussion

The extractive value gives an approximate amount of chemical constituent extracted out in a particular solvent used for extraction. The alcohol-soluble extractive value of plant powder was highest for *A. heterophyllum* (27.76 ± 0.54), followed by *P. fulgens* (19.16 ± 0.79) and *A. parviflora* (18.42 ± 0.98), and lowest in *S. asoca* (16.99 ± 0.81).

Table 11: Hypoglycemic activity of different plant extracts and polyherbal formulation

Treatment group	Fasting blood glucose level (mg/dL)			
	0 day	7th day	14th day	21st day
Control (0.1% w/v CMC)	113.6±3.14	108±4.37	108.11±3.53	105.26±2.27
STZ (45 mg/kg i.p)	301.3±5.67$^{###}$	312.17±4.48$^{###}$	326.6±5.16$^{###}$	361.3±3.89$^{###}$
STZ+Polyherbal formulation (200 mg/kg)	306.6±4.36	269.6±5.28	206.3±3.07**	159±2.81***
STZ+Polyherbal formulation (400 mg/kg)	300.46±4.13	253.28±3.38*	197±2.19**	147.74±2.03***
STZ+Glibenclamide (5 mg/kg)	302.12±2.18	247.38±1.93*	156.52±2.45***	126.56±1.79***

Values are given as Mean±SEM (*n*=6). Significant values were compared with ***P<0.001 versus STZ group, **P<0.01 versus STZ group, and *P<0.05 versus STZ group, $^{###}P$<0.001 versus control group, STZ: Streptozotocin, CMC: Carboxymethyl cellulose

The preliminary phytochemical studies showed the presence of different chemical constituents that are responsible for the medicinal properties of plants. Positive tests for carbohydrates, terpenoids, and steroids were given by *A. parviflora*, *P. fulgens*, *A. heterophyllum*, and *S. asoca* extract. Alkaloids detected in *A. parviflora* and *A. heterophyllum*. Flavonoid, phenolic, and tannins were detected in *A. parviflora*, *S. asoca*, and *P. fulgens*. Saponin is found only in *P. fulgens*.

Results of α-amylase inhibition by DNS showed that with the increase in the concentration of inhibitors, degradation of starch reduced and thus indicated the inhibition of enzyme activity. This indicated the α-amylase inhibition activity of plant samples. The results of plant samples were compared with α-amylase inhibitor antidiabetic drug acarbose. Acarbose exhibits 34.17–84.12% inhibition at a concentration of 0.01–0.80 mg/mL. IC_{50} of acarbose was observed at 0.028 mg/mL, whereas IC_{50} of *A. parviflora* was 0.11 mg/mL, followed by *S. asoca* (0.52 mg/mL), *P. fulgens* (0.94 mg/mL), and A. heterophyllum (1.17 mg/mL). The results indicated that *A. parviflora* exhibits the most promising *in vitro* antidiabetic activity [Tables 3-7].

The acute toxicity study showed that formulation is safe up to 2000 mg/kg dose as no sign of toxicity, adverse effects, and mortality observed in the treated animals up to 14 days. Based on the above study, 1/10th and 1/5th doses of 2000 mg/kg were chosen for antidiabetic study of polyherbal formulation. The 200 mg/kg and 400 mg/kg dose of polyherbal formulation was selected for the evaluation of the antidiabetic activity of the formulation.

Streptozotocin-induced diabetes in Wistar albino rats by harming Langerhans islet of β cells hence produce hypoinsulinemia and hyperglycemia. The fasting sugar level ($^{###}P<0.001$) in diabetic animals (STZ group) increased when compared to the control group. The 21 days treatment with polyherbal formulation significantly decreases the level of fasting blood sugar in polyherbal formulation treated diabetic rats in comparison to the STZ group, suggested the hypoglycemic activity of the formulation. The standard drug glibenclamide at 5 mg/kg dose significantly lowers the level of fasting blood sugar ($^{***}P<0.001$) in diabetic rats. The polyherbal formulation at 200 mg/kg and 400 mg/kg dose significantly reduced the glucose level ($^{***}P<0.001$) in diabetic rats. However, % lowering of fasting blood sugar was more at 400 mg/kg dose than 200 mg/kg dose. The antidiabetic drug glibenclamide declines the fasting blood sugar up to 58% in STZ -induced diabetic rats whereas the polyherbal formulation at 400 mg/kg dose reduced the glucose level up to 51% in diabetic rats. The abnormal range of blood sugar levels indicates a medical condition. A persistently high blood sugar level is known as hyperglycemia. The results indicated that polyherbal formulation has significant hypoglycemic activity.

The main objective of the study was to develop an antidiabetic polyherbal formulation. In this study, oral suspension was prepared and evaluated for safety and efficacy. The prepared formulation was brown, liquid in nature, easily pourable from the container and has redispersibility property with optimum particle size distribution. The pH of the formulation was 5.1. The viscosity of polyherbal suspension was found to be 40.901 Cps. The average size of particles was found to be 29.82 µm. The density of suspension was found to be 1.43 g/cm^3. Sedimentation studies showed that the sedimentation volume of a formulation is in-between a range of 1.

Conclusion

The present findings indicated that developed polyherbal formulation at a dose of 400 mg/kg showed significant antihyperglycemic activity than 200 mg/kg dose.

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