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Formulation and Evaluation of Antidiabetic and Antihyperlipidemic Activities of Polyherbal Formulation in Streptozotocin induced diabetic rat

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Abstract

Gymnema sylvestre, Trigonella foenum and *Phyllanthus emblica* are used for the management of diabetes. The mixing of these plant parts in different ratio may produce synergistic antidiabetic action. Hence the aim of the present study was to formulate polyherbal formulations containing various proportions of *Gymnema sylvestre* (Leaves)*, Trigonella foenum* (Seeds) and *Phyllanthus emblica* (Fruits) to investigate their antidiabetic and antihyperlipidemic activity in Streptozotocin induced diabetic rat. The physicochemical estimations namely total ash value, acid insoluble ash value, water soluble ash value, loss on drying, alcohol soluble extractive values and water soluble extractive value for plants parts were performed. The decoction of four different polyherbal formulations (HF1 to HF4) were prepared and antidiabetic activity was investigated in Streptozotocin induced diabetic rat. The physicochemical value of plant materials were under limits and acceptable. The administration of polyherbal formulation (HF1 to HF4) significantly decreased the blood glucose levels compared to control diabetic rats. The polyherbal formulation significantly lowered the elevated total cholesterol, triglycerides (TGL) and low density lipoprotein (LDL) level while increased the high density lipoprotein (HDL) indicates the antihyperlipidemic activity. The findings demonstrated the antidiabetic and antihyperlipidemic activity of polyherbal formulations, and HF2 produce higher protective effect from diabetes.

1 Introduction

Diabetes arises due to disturbances of carbohydrate, fat and protein metabolism. It is metabolic disorder causing insulin resistance, relative insulin deficiency and hyperglycemia. Diabetes has become a serious health problem with continuously increasing rates of incidence and mortality. The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. Even though the synthetic drugs, including insulin and oral hypoglycemic agents, control the blood sugar level as long as they are regularly administered, Cost, Complications, limited tolerability and other side effects reduces its wide acceptance. Further the herbal medicines could be used by minimizing above contests. This situation may be the main reason for the shift of common people form allopathic system to herbal drug nowadays[1-3].

The pharmacological activity of single plant is considered less compared to polyherbal formulation containing ratio of different plants. The polyherbal formulation produces synergistic action which is more potent, and also diminishes the concentrations of single herbs, thereby reducing adverse effects. In traditional systems of medicine, many plants have been documented to be useful for the management of diabetes. The various indigenous systems of medicines are more effective compared to synthetic medicines. The lack of comprehensive standardization of medicinal plant is one of the important challenges encountered by the traditional system of medicine. In ancient literature it has been validated the concept of polyhedral formulation, and is more potent compared to single plant[4]. Therefore it was planned to formulate antidiabetic polyherbal formulation containing *Gymnema sylvestre* (Leaves)*, Trigonella foenum* (Seeds) and *Phyllanthus emblica* (Fruits).

2 Material and Methods

2.1 Plant material

The leaves of *Gymnema sylvestre*, seeds of *Trigonella foenum* and fruits of *Phyllanthus emblica* were selected for the proposed study. *Gymnema sylvestre*, *Trigonella foenum* and *Phyllanthus emblica* were identified at Department of Pharmacognosy, TIT College of Pharmacy, Bhopal (M.P.), India. The plant materials were dried under shade, reduced to coarse powder and stored in airtight container till further use.

2.2 Physicochemical evaluation

Air-dried powdered material was subjected to qualitative physicochemical estimations for total ash value, acid insoluble ash value, water soluble ash value, loss on drying, alcohol soluble extractive values and water soluble extractive value.

2.3 Preparation of formulations

The dried leaves of *Gymnema sylvestre*, seeds of *Trigonella foenum* and fruits of *Phyllanthus emblica* were powdered and passed through a 10-mesh sieve. The four formulations (HF1, HF2, HF3 and HF4) were made by mixing different ratio of plant powdered materials. The composition of different polyherbal formulation is given in table 1.

Table 1: Composition of Polyherbal formulations

Formulations	Plants (gm)		
	G. sylvestre	***T. foenum***	***P. emblica***
HF1	10	10	10
HF2	10	30	10
HF3	30	10	10
HF4	10	10	30

2.4 Preparation of decoction

20 g of mixture of each formulation with 150 ml of distilled water were macerated at ambient temperature for 24 h. After 24 h the drug macerate was boiled for 45 min and filtered through muslin cloth to get a decoction. The volume of the decoction was adjusted such that 20 g of mixture gave 50 ml of the decoction[5].

2.5 Antidiabetic activity

2.5.1 Oral glucose tolerance test of polyherbal formulation

The oral glucose tolerance test was performed in overnight fasted (18 hours) rats. The rats were divided into seven groups (n = 6).

- Group I served as normal control rats, administered drinking water daily
- Group II had glucose control rats
- Group III rats were administered standard drug Glibenclamide (0.5 mg/kg)
- Group IV rats were administered HF1 (20 ml/kg body weight)
- Group V rats were administered HF2 (20 ml/kg body weight)
- Group VI rats were administered HF3 (20 ml/kg body weight)
- Group VII rats were administered HF4 (20 ml/kg body weight)

Glucose (2 g/kg) was fed to rats of Group II to Group VII, 30 minutes prior to the administration of the extracts and standard drug. Blood was withdrawn from the retro-orbital sinus after 0, 30, and 90 minutes of extract and standard drug administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase–peroxidase glucose estimation kit[6-8].

2.5.2 Induction of non-insulin dependent diabetes mellitus

Non-insulin dependent diabetes mellitus was induced in overnight fasted adult rats weighing 170 – 220 g by a single intraperitoneal injection of 60 mg/kg Streptozotocin, 15 minutes after i.p. administration of 120 mg/kg of nicotinamide. Streptozotocin was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours and then on day 7, after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as > 126 mg/dl. Only those rats that were found to have permanent induction of non-insulin dependent diabetes mellitus were used for the study.

2.5.2.1 Evaluation of antidiabetic activity of polyherbal formulation

The animals were segregated into seven groups of six rats each. The polyherbal formulation was administered for 28 days.

- Group I served as normal control rats, administered drinking water daily for 28 days
- Group II had diabetic control rats, administered drinking water daily for 28 days
- Group III rats were administered standard drug Glibenclamide (0.5 mg/kg) daily for 28 days
- Group IV rats were administered HF1 (20 ml/kg body weight) daily for 28 days
- Group V rats were administered HF2 (20 ml/kg body weight) daily for 28 days

- Group VI rats were administered HF3 (20 ml/kg body weight) daily for 28 days
- Group VII rats were administered HF4 (20 ml/kg body weight) daily for 28 days

The fasting glucose levels were determined on days 0, 7th, 14th and 28th after herbal formulation administration[8,9].

2.5.2.2 Estimation of biochemical parameters

The biochemical parameters were determined on day 28 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were determined by the glucose oxidase method, using an auto-analyzer[10-12].

2.6 Statistical analysis

The results are expressed as mean ± SEM of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet's test. A $P < 0.05$ value was considered as statistically significant.

3 Results and Discussions

In the present study, we planned to developed antidiabetic polyherbal formulation containing *Gymnema sylvestre* (Leaves), *Trigonella foenum* (Seeds) and *Phyllanthus emblica* (Fruits).

3.1 Standardization of plant material

The physicochemical characters of plants were performed for standardization of crude drug. The physicochemical characters of *Gymnema sylvestre, Trigonella foenum* and *Phyllanthus emblica* studied, and the results are presented in tables 2.

The value obtained for the *Gymnema sylvestre, Trigonella foenum* and *Phyllanthus emblica* were around 8.6%, 5.1% and 7.1%, respectively as total ash (Table 2). The ash value study indicates the presence of inorganic materials, such as carbonate, silicates, oxalates, and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components.

The acid insoluble values of *Gymnema sylvestre, Trigonella foenum* and *Phyllanthus emblica* were 2.6%, 1.9% and 1.6%, respectively. The water soluble ash values of *Gymnema sylvestre, Trigonella foenum* and *Phyllanthus emblica* were 3.5%, 2.4% and 2.9%, respectively (Table 2). The findings of acid insoluble values indicate the presence of acid insoluble material in the drug material. The water soluble ash value demonstrated the existence of water soluble material in the plant material.

The loss on drying of the *Gymnema sylvestre, Trigonella foenum* and *Phyllanthus emblica* powder were 14.2%, 8.2% and 19.4%, respectively. The alcohol extractive values of *Gymnema sylvestre, Trigonella foenum* and *Phyllanthus emblica* were 23.1%, 17.3% and 20.7%, respectively. The water extractive values of *Gymnema sylvestre, Trigonella foenum* and *Phyllanthus emblica* were 27.9%, 20.5% and 24.2%, respectively (Table 2). The findings of extractive values indicate that all plant materials contains greater amount of highly water soluble phytoconstituents.

Table 2: Physicochemical parameters of various plant powders

Studied parameters	Observations (% w/w)		
	G. sylvestre	***T. foenum***	***P. emblica***
Loss on drying	14.2±0.52	8.2±0.36	19.4±0.78
Total ash value	8.6±0.07	5.1±0.02	7.1±0.03
Acid insoluble ash value	2.6±0.06	1.9±0.04	1.6±0.08
Water soluble ash value	3.5±0.04	2.4±0.09	2.9±0.05
Alcohol extractive value	23.1±0.32	17.3±0.62	20.7±0.59
Water extractive value	27.9±0.47	20.5±0.18	24.2±0.27

Values are mean ± SEM of three determinations

3.2 Antidiabetic activity of polyherbal formulation

3.2.1 Oral glucose tolerance effects of polyherbal formulation

The antidiabetic effect of the prepared polyherbal formulations on the blood glucose level are exhibited in table 3. The increase in glucose level in blood was seen in glucose control, polyherbal formulation (HF1 to HF4) treated and standard group animals after the administration of glucose.

Table 3: Antidiabetic activity of polyherbal formulations on oral glucose tolerance test

Group	Plasma glucose concentration (mg/dl)		
	0 min	**30 min**	**90 min**
Normal Control	80.2±3.4	75.1±4.5	77.5±4.8
Glucose control	78.6±5.1	195.1±3.9[a]	136.2±4.1[a]
Glucose+ Glibenclamide (0.5 mg/kg)	74.3±2.7	105.2±4.6*	75.3±6.7*
HF1 (20 ml/kg body wt.)	77.9±3.2	133.4±3.2*	92.1±3.4*
HF2 (20 ml/kg body wt.)	76.6±5.3	110.3±6.2*	79.3±4.6*
HF3 (20 ml/kg body wt.)	80.4±2.7	118.7±5.7*	83.6±3.1*
HF4 (20 ml/kg body wt.)	75.7±3.7	126.1±2.4*	85.7±5.4*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at [a]P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

The significant reductions in glucose level in rat were observed after treatment with polyherbal formulation compared to control group rats. In addition significant decrease in plasma glucose level was noted in glibenclamide treated group.

3.2.2 Effect on non-insulin dependent diabetes mellitus of polyherbal formulation

The existence of high fasting glucose level demonstrated the stimulation of diabetes in rats. The antidiabetic activity of polyherbal formulation (HF1 to HF4) on serum glucose levels of normal and Streptozotocin-induced rats are exhibited in table 4. The streptozotocin treated rats indicates significant rise in serum glucose level on 0, 7th, 14th and 28th day compared with normal control group rats. The administration of polyherbal formulation (HF1 to HF4) to rats leads to significant decrease in in blood glucose level. The findings exhibited that HF2 produces more potent antidiabetic activity compared HF1, HF3 and HF4.

Table 4: Antidiabetic activity of polyherbal formulations on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	Day 7th	Day 14th	Day 28th
Normal Control	76.1±5.2	79.6±2.8	77.4±4.7	81.2±2.7
Diabetic control (Streptozotocin)	162.4±3.8^{a}	214.7±5.6^{a}	286.1±5.4^{a}	302.5±4.8^{a}
HF1 (20 ml/kg body weight)	145.1±4.1	139.2±4.8*	125.9±3.7*	90.4±3.4*
HF2 (20 ml/kg body weight)	139.8±5.3	120.4±3.7*	105.7±6.7*	80.3±5.1*
HF3 (20 ml/kg body weight)	145.2±3.7	128.1±5.3*	116.2±4.1*	85.9±3.7*
HF4 (20 ml/kg body weight)	148.3±4.5	136.7±3.4*	118.4±5.1*	86.1±6.2*
Diabetic + Glibenclamide (0.50 mg/kg)	141.3±6.4	102.8±4.2*	88.1±3.9*	76.4±4.6*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at ^{a}P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

3.2.3 Anti-hyperlipidaemic effect of polyherbal formulation

The regulation of lipid profiles in different groups of rats is exhibited in table 5. The significant increment in TGL, total cholesterol and LDL while increase in HDL observed in diabetic control rats compared with normal rats. The polyherbal formulation and standard drug treated rats significantly decreased in TGL, total cholesterol, LDL, and increased HDL compared to diabetic control group rats. The results of study indicate the antihyperlipidaemic activity of polyherbal formulations. The HF2 produces extreme antihyperlipidaemic activity compared with HF1, HF3 and HF4.

Table 5: Biochemical parameters after treatment with polyherbal formulations

Group	Lipid Profile (mg/dl)			
	Triglyceride	Total Cholesterol	HDL	LDL
Normal control	76.1±2.7	85.3±6.4	69.1±4.5	52.7±3.4
Diabetic control (Streptozotocin)	201.8±4.3^{a}	188.3±4.8^{a}	23.7±6.2^{a}	193.8±5.6^{a}
HF1 (20 ml/kg body weight)	86.3±3.8*	96.4±5.2*	59.7±4.6	56.8±3.7*
HF2 (20 ml/kg body weight)	79.0±4.9*	84.3±4.9*	75.6±3.8*	50.7±2.8*
HF3 (20 ml/kg body weight)	82.3±5.7*	92.1±3.7*	65.1±5.3*	55.9±5.5*
HF4 (20 ml/kg body weight)	83.7±3.4*	87.5±5.4*	68.7±3.7*	56.1±4.9*
Diabetic + Glibenclamide (0.50 mg/kg)	78.1±4.1*	84.6±3.6*	71.3±5.7*	51.7±2.8*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at ^{a}P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

The streptozotocin reduced the functioning of pancreatic β cells due to its alkylating properties. The streptozotocin modifies the biological macromolecules, fragment DNA, and destroy the β cells, causing insulin-dependent diabetes.

The reduction in glucose levels may be due to increase in plasma insulin levels or enhanced transport of blood glucose in the peripheral tissue. Our study gives evidence that the polyherbal formulation decrease the plasma glucose levels and has promising antidiabetic activity.

The diabetic hyperglycemia induced by streptozotocin causes altered of plasma biochemical levels of triglyceride, total cholesterol, HDL, and LDL, which are considered as significant markers of diabetes. The polyherbal formulation treated animals reversed the effect of streptozotocin on the lipid profie[5]. This may be due to the antidiabetic mechanism of the individual herbs present in the polyherbal formulation.

4 Conclusion

The polyherbal formulation (HF1 to HF4) incorporating different ratio of *Gymnema sylvestre* (Leaves)*, Trigonella foenum* (Seeds) and *Phyllanthus emblica* (Fruits) exhibited strong antidiabetic activity. The antidiabetic potential of the polyherbal formulation is comparable with that of glibenclamide, which is evidenced by decreased levels of blood glucose, total cholesterol, triglyceride, LDL and cholesterol, and increase in HDL. The HF2 produced maximum antidiabetic activity compared to other polyherbal formulations.

5 Conflict of interests

The authors have no conflict of interests.

6 Author's contributions

SSS, AM and BD performed whole experimental procedures. All authors read and approved the final manuscript.

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