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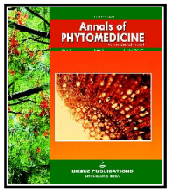

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Evaluation of antidiabetic efficacy of polyherbal formulations in experimentally induced hyperglycemic rats

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Abstract

Polyherbal formulations, as the name suggests, include numerous components of various herbal origins. The plant secondary metabolites might have a broad range of pharmacological activities. Polyherbal formulations are primarily used to boost the activity of chemicals derived from other plants or to prevent their harmful effects. Owing to the inclusion of numerous secondary metabolites, these formulations may have a synergistic, potentiated, agonistic/antagonistic pharmacological impact. These mixtures contain numerous active ingredients with varied mechanisms of action that can work together to combat various diabetic problems. The focus of this research is to create a polyherbal formulation and test its anti-diabetic properties in animal models. The study results showed the acute toxicity trials of the polyherbal formulation at dosages up to 2000 mg/kg over 14 days revealed no adverse effects. The crude extract of polyherbal formulation at 200 mg/kg lowers the fasting blood glucose levels however, the higher dose, 400 mg/kg, lowers the blood glucose levels, significantly when compared to diabetic control groups. Whereas, the decoction of polyherbal formulation at 200 mg/kg produced a less significant reduction and the higher dose at 400 mg/kg produced a significant reduction in blood glucose level. Besides, the powder of polyherbal formulation at a high dose (400 mg/kg) produced a less significant reduction in blood glucose level on the seventh day, when the lower dose did not show any effect on the blood glucose level.

1. Introduction

Diabetes mellitus (DM) is a chronic condition due to an underlying or acquired deficiency in insulin levels by the pancreas or by the lack of effectiveness of insulin production. This insufficiency results in increased blood glucose levels that cause damage the many organ functions, particularly the blood vessels and nerves (Syed *et al*., 2005). The percentage of diabetes is increasing rapidly worldwide. The World Health Organization (WHO) has foretold that the number of adults with diabetes by 2030 will have almost doubled worldwide, from 177 million in 2000 to 370 million. The major causes of death in diabetes are cardiovascular diseases like atherosclerosis. A marked geographical variation in the prevalence of type 2 diabetes exists the highest rates are found in Americans of Arizona and in the inhabitants of the south pacific island of Nauru, where half the adult population has diabetes. In the rural communities of China and Chile, the prevalence is less than 1%. The rates of type II DM are higher in urban populations than in rural communities. Type I DM accounts for up to the 10% of all cases of diabetes is rising. Across Europe, the average annual increase in the incidence in children under 15 years old is 3.4%, with a steep rise in those under 5 years old. Type II DM accounts for around 90% of all cases of diabetes. There are approximately 1.4 million people with diagnosed type II DM. incidence of diabetes increases with age, with most cases being diagnosed after the age of 40 years (Shipra *et al.*, 2009).

The heritability of type II DM is nearer than for type I DM, and is estimated to account for 40-80% of total disease susceptibility many patients will have a family history of diabetes, and twin studies show a high concordance rate (60-90%) in monozygotic twins the maternal history of diabetes confers a higher risk of type II DM in the offspring than paternal history. Overweight and lack of physical activity are the two most major environmental risk factors for diabetes. It is estimated that up to 80% of all new cases of diabetes can be contributed to obesity in the UK. The average body mass index (BMI) of a person with type II diabetes is 30.0 kg/m^2; in the USA, 67 % of more than 27 kg/m^2 and 46% have a BMI of more than 30 kg/m^2. The risk of developing type II increases across the normal range of BMI, such that the risk in the middle ages women whose BMI is over 35 mg/m^2 is 93.2 times greater than in a woman whose BMI is below 22.5 kg/m^2 (Shipra *et al.*, 2009).

In worldwide, only India having the highest number of diabetic patients, the disease is becoming an enormous health problem in the country; due to this reason now India calling as “the capital of the diabetic world”. The International Diabetic Federation (IDF) was predicted that the number of patients with diabetes mellitus in India more than double from 19 million in 1995 to 40.9 million in 2007. It is estimated by 2025 will increase to 69.9 million. At present, in India up to 3% of the rural population and 11% of the city population above the age of 15 has diabetes. The WHO estimated that mortality from diabetes and heart disease costs India about 210 billion dollars every year and is expected to increase by 335 Billion Dollars in the next 10 years (Singh *et al.*, 2003).

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In diabetes, the major complication is hyperglycemia and hyperlipidemia. Hyperglycemia is characterized by elevated levels of blood glucose, this leads to the generation of secondary complications like atherosclerosis, hyperosmolar nonketotic coma, diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy as well as the hyperlipidemia is characterized by elevated levels of cholesterol, triglycerides, phospholipids, and changes in lipoprotein composition. The most common lipid abnormality in diabetes is hypertriglyceridemia, which is associated with metabolic consequences of coagulability, hyperinsulinemia, insulin resistance, and insulin intolerance (Rang *et al*., 2006).

Around the world and especially in developing countries, about 80% of the people have been using herbal remedies. The herbal products are thought to be less harmful, safer, and more culturally acceptable, as well as having more effectiveness, strength, and fewer side effects (Latha *et al.*, 2021). According to the WHO report, over 21000 plant resources have been used for medical purposes across the world. Herbal remedies are largely has been using by Indian people. Its climate is very favorable to the growth of medicinal plants, around 45000 plant species, of which 15000 plants are flowering plants having about 1000 species identified as medicinal plants (Divya and Sanjeev, 2021). Due to this reason, India is also called the "Medicinal Garden of The World". Only 40 plant species are currently used by pharmaceutical companies (Mohamed *et al*., 2006).

Several treatments have been available for alternative therapy of diabetes. These can decrease the hyperglycemic condition but not identify the exact mechanism of action. Antioxidants relatively have the potential to inhibit the oxidation chain reactions at even small concentrations also. Several plant antioxidants are experimentally proved and used as effective protective agents against oxidative stress. It has been recognized that the antihyperglycemic effect of these plants either may be due to stimulation of pancreatic β cells or the facilitation of metabolite in insulin-dependent process or inhibit the intestinal absorption of glucose (Mahendran *et al*., 2014; Akanksha *et al*., 2010).

2. Materials and Methods

2.1 Plant collection and treatment

The plants were collected in Vaikaalmedu and authenticated in the Department of Pharmacognosy, Nandha College of Pharmacy, Erode, Tamilnadu. The collected plant material was washed in running water, shade dried, and pulverized to get the powdered drug. The material was subjected to further experimental purposes.

2.2 Experimental animals

Swiss Albino mice and Wistar rats were purchased from The Animal House, Department of Pharmacology, IRT Perundurai Medical College, Erode, Tamilnadu. Animals were housed in the Animal House, Nandha College of Pharmacy and Research Institute, Erode, Tamilnadu. The animals were placed randomly in polypropylene cages with paddy husk as bedding and housed at standard condition maintained a temperature of 24 ± 2°C and relative humidity of 30-70%. A 12 h light and dark cycles were strictly followed throughout the study. The animals had free access to a standard animal pellet diet (Sukumar Agro Industries, Pune) and water. The Animal Ethical Committee of Nandha College of Pharmacy, Erode, Tamilnadu, approved our research proposal and presented it by CPCSEA guidelines.

2.3 Chemicals and drugs

The chemicals and drugs were purchased as LR and AR grade, those were alloxan monohydrate (Sigma Chemicals, Bangalore), formalin solution (Nice Chemicals, Cochin), carboxy-methylcellulose, and Diethyl ether (Nice Chemicals, Cochin).

2.4 Preparation of polyherbal formulations

The composition and ratio of herbal ingredients are selected according to the potency of antidiabetic activity, which was stated in previous references, those were powder form of polyherbal formulation, decoction of polyherbal formulation, and crude extract of polyherbal formulation.

i. Preparation of powder of polyherbal formulation (PPHF)

The coarsely powdered plant materials were sieved through sieve no. 60. It helps to get the uniform-sized powder and according to the weight of individual powder, which is present in Table 1 has taken into a beaker and mixed well by using a suitable blender to get homogenous powder formulation. Then it was packed in a suitable container and kept in a dry place.

Table 1: Composition of polyherbal formulation

S.No.	Plant name	Weight percentage (%)
1	*Aegle marmelos*	2.66
2	*Annona squamosa*	9.32
3	*Bougain villia*	2.66
4	*Cassia auriculata*	6.66
5	*Emblica officinale*	8.00
6	*Ficus carica*	13.30
7	*Hibiscus rosa-sinensis*	6.66
8	*Psidium guajava*	6.66
9	*Stevia rebaudiana*	9.32
10	*Tea leaves*	2.66
11	*Tenospora cardifolia*	10.66
12	*Terminalia chebula*	8.00
13	*Zingiber officinale*	13.30

ii. Preparation of decoction of polyherbal Formulation (DPHF)

All individual powders were taken in a beaker and mixed well with a suitable blender until to get a uniform powder formulation. Then packed the formulation in water diffusible paper to make easily dip in the hot water for making of decoction.

iii. Crude extract of polyherbal formulation (EPHF)

The polyherbal formulation is a mixture of all the individual different parts of plant extracts. It contains *Aegle marmelous* (leaves), *Annona squamosa* (leaves), *Bougan villia* (leaves), *Cassia auriculata* (flowers), *Emblica officinale* (fruits), *Ficus carica* (fruits), *Hibiscus rosa-sinensis* (flowers), *Psidium guajava* (leaves), *Stevia rhubidiana* (leaves), *Camellia sinensis*, *Tenospora cardifolia* (leaves), *Terminalia chebula* (fruit), *Zingiber officinale* (rhizome).

This extraction is carried out by the maceration process for 24 h. All the thirteen dried and pulverized plant ingredients were taken in individual conical flasks. Then added some amount of water up to rinse the powder, gently agitated for the first 6 h, and kept aside for the remaining 18 h. After 18 h, filtered them individually, finally evaporated the solvent at 100^0C up to get a paste-like preparation. Then, the different weights of the extracts, according to Table 1, taken into a beaker, mixed them properly with a little amount of water, and evaporated the water up to get a paste-like preparation and kept in the refrigerator after being packed in a suitable container (Sengottuvelu *et al*., 2008).

2.5 Pharmacological study

2.5.1 Acute oral toxicity study

Acute oral toxicity refers to those adverse effects occurring due to oral administration of a single dose or multiple doses are given within 24 h. For every phase, three animals were employed, and the beginning dose is chosen from among the four predetermined values of 300, 1000, and 2000 mg/kg body weight. The use of the increased upper dosage level of 5000 mg/kg body weight may be explored in rare situations and only when warranted by special regulatory reasons.

2.5.2 Acute toxicity studies

Three mice (25-30 gm) were maintained under standard laboratory conditions. A total of 3 animals have to be used, which received a single oral dose of 2000 mg/kg body weight of polyherbal formulation. Animals were kept overnight fasting before the drug administration. After the administration of 3 doses of 3 formulations (extract, decoction, and powder) of the polyherbal formulation, food should be held for a further 3-4 h. Animals have to be observed individually once during the first 30 min after the dose given, predominantly during the first 24 h and daily for 14 days. Daily, once cage-side observations have to be done to find out the changes in skin, eyes, mucous membrane (nasal), respiratory rate, circulatory (heart rate and BP), anatomically (salivation, lacrimation, perspiration, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors, and convulsions) changes.

2.5.3 Screening of antihyperglycemic activity

Healthy Wistar albino rats of either sex weighing 150-250 gm were used and before initiating the experiment, the animals were acclimatized in laboratory condition for seven days. The animals were kept in polypropylene cages, provided with a standard animal pellet diet (Pranav Agro Industries, Pune) and water and *libitum*. The animals were divided into nine groups of six each after overnight fasting all of the animals, except the normal control group, were injected with alloxan monohydrate suspension intraperitoneally, at a dose of 150 mg/kg body weight. The animals were allowed to stay for 72 h provided and diabetes was conformed, if the blood glucose levels exceed 200 mg/dl of blood. Drug doses were prepared by suspending the appropriate quantity of drug in 0.5% w/v of carboxymethylcellulose solution. The control animals were treated with 2 ml of vehicle daily through the oral route and the all other groups were daily orally administered with their corresponding dose levels according to their body weight for 7 days as follows.

Group I - Normal animals treated with vehicle (0.5 %w/v CMC).

Group II - Diabetic animals treated with alloxan150 mg/kg.

Group III - Diabetic animals treated with glibenclamide 5 mg/kg/day p.o).

Group IV - Diabetic animals treated with EPHF, 200 mg/kg p.o.

Group V - Diabetic animals treated with EPHF, 400 mg/kg. p.o.

Group VI - Diabetic animals treated with DPHF, 200 mg/kg. p.o.

Group VII - Diabetic animals treated DPHF, 400 mg/kg.p.o.

Group VIII - Diabetic animals treated with PPHF, 200 mg/kg. p.o.

Group IX - Diabetic animals treated with PPHF, 400 mg/kg.p.o.

The animals fasting blood sugar levels were measured using a glucometer (Model- Accucheck, Roche Germany) after 1, 3, 5, and 7 days of treatment plans. Blood was collected from the tail tip of the animals after 3 h of drug administration, and the tail wound is applied with povidone-iodine ointment to prevent any infection. The fasting blood glucose levels in various polyherbal formulations treated animals were compared with that of diabetic control group animals (Bhise *et al.*, 2009; Chandra *et al.*, 2007).

2.6 Statistical analysis

The data pertaining to proper statistical testing, including one-way ANOVA (Analysis of Variance) and a Dunnett's post hoc test. Less significant, significant, and more significant were defined as $p>0.05$, $p>0.01$ and $p>0.001$, correspondingly. Graph pad prism software, version 4, was used to do the study.

3. Results

3.1 Acute oral toxicity of polyherbal formulations in mice

In Table 2, the represented data indicates the changes in behavioral and physiological activities of mice with different doses of polyherbal formulation. The mice in the acute toxicity study daily received polyherbal formulations at dose levels of 300, 1000, and 2000 mg/kg. The duration of this study is 14 days. Observed behavioral changes in mice with polyherbal formulations are given in Table 2. By comparing with the control group, it was observed that extract of polyherbal formulation at doses 1000 and 2000 mg/kg showed drowsiness. Except this, it was not shown any effect on the function of kidney, CNS, Liver, gastrointestinal tract, respiratory system, and color of skin, so no behavioral changes and no mortality was observed in mice with polyherbal formulations.

3.2 Result of antihyperglycemic activity

The effects of polyherbal formulations (EPHF, DPHF, and PPHF) on fasting blood glucose levels in alloxan-induced diabetic rats were given in Table 3 and Figure 1. On the seventh day of the treatment protocol, the lesser dose of both EPHF and DPHF at 200 mg/kg lowers the fasting blood glucose levels, whereas there was a significant reduction in blood glucose level observed at 400 mg/kg when compared to diabetic control groups. However, the PPHF at a high dose at 400 mg/kg only produced a decreased reduction in blood glucose level.

Table 2: Results for acute oral toxicity study of polyherbal formulation

Group	Treatment	Dose (mg/kg)	Salivation	Lacremation	Mucous	Urinary	Defecation	Tremors	Drowsiness
I	CMC	1 ml	–	–	–	–	–	–	–
II	Extract	300	–	–	–	–	–	–	–
III	Extract	1000	–	–	–	–	–	–	+
IV	Extract	2000	–	–	–	–	–	–	+
V	Powder	300	–	–	–	–	–	–	–
VI	Powder	1000	–	–	–	–	–	–	–
VII	Powder	2000	–	–	–	–	–	–	–
VIII	Decoction	300	–	–	–	–	–	–	–
IX	Decoction	1000	–	–	–	–	–	–	–
X	Decoction	2000	–	–	–	–	–	–	–

Table 3: The effect of different polyherbal formulations on fasting blood glucose level

Group	Treatment (mg/kg)	1st day	3rd day	5th day	7th day
I	Normal control (CMC-0.5 %)	081.5 ± 1.15	81.6 ± 1.20	82.6 ± 1.70	83.4 ± 0.90
II	Diabetic control (alloxan-120)	211.7 ± 3.50**	237.7 ± 2.90**	249.4 ± 3.80**	268.7 ± 3.20**
III	Positive control (glibenclamide-5)	206.4 ± 1.70	177.6 ± 2.06**	137.2 ± 0.88**	109.5 ± 0.50**
IV	EPHF (200)	197.5 ± 2.90	186 ± 0.50*	165.3 ± 2.00*	152.3 ± 1.70*
V	EPHF (400)	206.5 ± 3.05	182 ± 1.50*	152.5 ± 2.00**	126.8 ± 1.70**
VI	DPHF (200)	208.6 ± 5.30	192.3 ± 1.30	173.3 ± 2.08	165.7 ± 1.10*
VII	DPHF (400)	218.3 ± 0.80	184.6 ± 1.30	161.3 ± 1.40*	149.6 ± 1.70**
VIII	PPHF (200)	214 ± 2.60	204.1 ± 3.20	188.3 ± 2.30	175.2 ± 2.00
IX	PPHF (400)	221.6 ± 3.40	205.3 ± 2.10	174.6 ± 2.18	168.3 ± 5.30*

All values are mean ± SEM, n = 3. One-way Analysis of Variance (ANOVA), followed by Dunnett's test was performed as the test of significance.

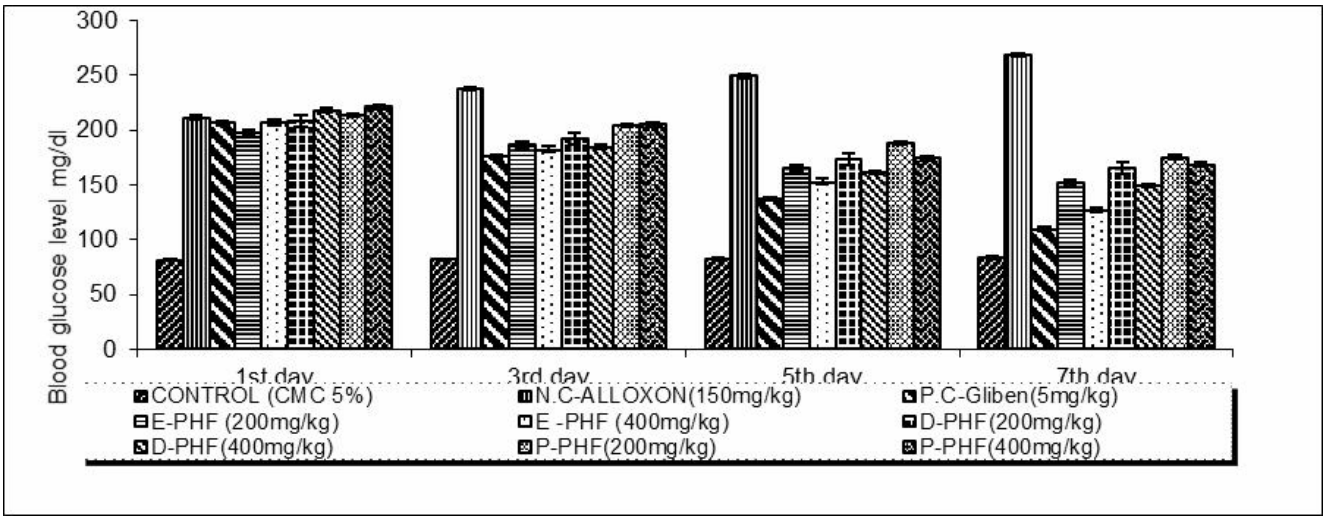

Figure 1: The fasting blood glucose level after the treatment protocol of polyherbal formulation in diabetic rats.

4. Discussion

Diabetes mellitus (DM) is a prevalent endocrine illness that affects more than 150 million people globally, with that figure expected to rise to 300 million by 2025, out of which the Indians are accounting for more than one-fifth of those affected. India has been designated as the world's diabetes center by the International Diabetes Federation (Satyanarayana *et al.*, 2010). Synthetic drug therapies for diabetes are now developed however, the prolonged use of those drugs could cause greater side effects to lead to death. Hypoglycemic episodes, nausea, dermatitis, diarrheas', jaundice, vision abnormalities, anemia,

and other side effects have been reported with glibenclamide, metformin, repaglinide, and pioglitazone, among others. Polyherbal treatments contain synergistic, potentiating, agonistic, and antagonistic pharmacological substances that operate in harmony to generate treatment effectiveness with minimal adverse effects (Nupur, 2021).

The previous studies for the treatment of diabetes with polyherbal formulation exert good acceptable results (Petchi *et al.*, 2014; Kaleem *et al.*, 2008). The polyherbal formulation is assessed in diabetic rats at 500 mg/kg showed its effectiveness in oral glucose tolerance test and antidiabetic activity, but it does not produce a hypoglycemic effect. The efficacy of polyherbal formulation in obesity-related diabetes was studied by patil *et al.* (2010). The antidiabetic and antiobesity effect of the formulation was found to be nearly similar to that observed for glibenclamide and sibutramine, respectively.

The toxicological evaluation of polyherbal formulation is useful to evaluate its toxic effects which were produced on long-term administration. In the acute toxicity study of polyherbal formulations, mortality was observed at any dose. At dose levels of 1000 and 2000 mg/kg P.O. of extract of polyherbal formulation only produces drowsiness, except this, no behavioral changes were observed at different doses of polyherbal formulations. The symptoms which were observed in acute toxicity represent the safety and no toxicity of polyherbal formulation (Sabu *et al.*, 2009).

Alloxan induces a wide variety of animal species by damaging the insulin-secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release, which paves the way for the decreased utilization of glucose by the tissues (Choudhury *et al.*, 2017). β cells' destruction is happened by the free radicals, which are generated in the fenton reaction of alloxan metabolism. The formation of hydroxyl radicals (·OH-) from the hydrogen peroxide H_2O_2 is called fenton. Due to this, blood glucose levels of rats were increased significantly. Glibenclamide is a standard drug that will significantly decrease the raised blood glucose levels. In β-cells of the pancreas, Na+ ion channels are responsible for depolarization which will increase the production of insulin and K^+ ion channels are responsible for repolarisation which will decrease the production of insulin. Glibenclamide increases the production of insulin by inhibiting the K^+ ion channels (Punith *et al.*, 2019).

In the present experiment, administration of polyherbal formulations for seven days prevented a significant elevation of glucose levels in diabetic rats. This could be due to the result of improved glycemic control produced by the formulation. When related to other dosages of decoction and powder formulation, the extract of polyherbal formulation at a dose of 400 mg/kg P.O showed a substantial drop in fasting blood glucose levels. The pronounced antihyperglycemic effect may be because of the synergistic effect of various active principles in the ingredients of polyherbal formulation. The reduced fasting blood glucose level may be either due to the increase in glycogenesis, decrease in glycogenolysis, or increase in the entry of glucose molecules to various skeletal muscles (Petchi *et al.*, 2014).

5. Conclusion

Diabetes is a chronic clinical syndrome little is talked about in aspects of prevention and curation, but rather the management, there is an increased focus on herbal medicines in the search for appropriate hypoglycemic and hyperglycemic agents. Many medicinal plants are effective in diabetes mellitus; acute toxicity study helps to improve the acceptability and authenticity of the herbal plants. The polyherbal formulation consists of thirteen plant-origin ingredients, which are individually used traditionally in the treatment of diabetes mellitus. Each plant act by different mechanisms to treat diabetes.

Based on the above results, it could be concluded that extract of polyherbal formulation exerts significant hypoglycemic and anti-oxidant activities. When compared to extract formulation, the decoction of polyherbal formulation and powder formulation had less potency on diabetic activity. Due to hyperglycemia, several chronic secondary complications will generate like diabetic ketoacidosis, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, and hyperosmolar nonketotic coma. The polyherbal formulation has capable of control of hyperglycemia. As a result, it may be concluded that polyherbal formulation treats diabetes' secondary problems gradually. Continued research is needed to determine the actual underlying mechanisms of antidiabetic efficacy of polyherbal formulation, as well as the involvement of the exact active secondary metabolites present in it.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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