Research Article

Hypoglycaemic Potential of Polyherbal Formulation in Alloxan Induced Diabetic Rats

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ABSTRACT

Catharanthus roseus, Andrographis paniculata, and *Ipomoea batatas* are familiar herbs that are widely available all over the India and have a notable application in treating a variety of diseases, including diabetes mellitus. Whole plant of all three species are well known for hypoglycaemic potential, but the additive or synergistic effects are unknown. The concept of polyherbalism is mentioned in the Sharangdhar Samhita, an Ayurvedic text from 1300 AD. Polyherbal formulations improve therapeutic action while lowering single herb concentrations, reducing adverse events. The current study's goal is to create a polyherbal formulation and test its hypoglycaemic potential in an alloxan-induced animal model. The polyherbal formulation was created by combining ethanolic extracts of *Catharanthus roseus*, *Andrographis paniculata*, and *Ipomoea batatas* leaves in a 1:1:1 ratio. The finished product's quality was assessed using the World Health Organization's guidelines for quality control of herbal materials. The polyherbal formulation showed no toxic symptoms in acute oral toxicity studies at doses up to 5000 mg/kg over 14 days. The oral hypoglycaemic activity of the polyherbal formulation (300 and 500 mg/kg) was tested in rats against alloxan (150 mg/kg) induced Diabetes mellitus. The investigational new polyherbal formulation was administered for 20 consecutive days, and the effect of the polyherbal formulation on blood glucose levels and body weight was studied at regular intervals. Polyherbal formulations demonstrated significant antidiabetic activity at 300 mg/kg (P <0.005**) and 500 mg/kg (P<0.001***) respectively, and this effect was comparable to that of standard metformin hydrochloride (120mg/kg) (P<0.001***). The high dose (500mg/kg) of polyherbal formulation have shown better therapeutic results than that of standard metformin hydrochloride. The body weight of animals is in control with all the treatment groups. Through these reports it can be concluded that the new polyherbal formulation have a good potential to control diabetes mellitus and have great scope to develop the product for further use.

Keywords: Diabetes mellitus, hypoglycaemia, Alloxan, Metformin, polyherbal formulation.

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INTRODUCTION

Diabetes is considered as metabolic disorder characterised by hyperglycaemia caused by abnormalities in insulin secretion, action, or both. Diabetes long-term complications include retinopathy, which can result in vision loss, neuropathy, which causes dysfunction of one or more peripheral nerves, and nephropathy, which can result in renal failure. Diabetes patients are more likely to develop other comorbidities like atherosclerosis, coronary artery diseases, peripheral arterial diseases and cerebrovascular disease. Diabetes is frequently associated with hypertension and abnormalities in lipoprotein metabolism.[1]

Among the global population,4% of people got effected with Diabetes mellitus. Diabetes was estimated to affect 77 million people in India in 2019, with that figure expected to rise to more than 134 million by 2045. Approximately 57% of these people are still undiagnosed. Conventional medical treatments for diabetes, such as injections/oral hypoglycaemic medications, have a number of negative side effects, and are subject to financial constraint etc. Many researchers focus on finding medications using natural items or Traditional Chinese system of Medicine (TCM).[2]

Polyherbal formulations are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines, minerals and their combinations have a great treatment value in different chronic diseases like diabetes, asthma and so on.[3]

Andrographis paniculata (King of Bitters) belongs to Acanthaceae family which is also known as Kalmegh. It has been used to treat hepatitis, gastroenteritis, upper respiratory tract infections, high fever, herpes, and a variety of other chronic and infectious diseases. Andrographolide is an important constituent that is present in the leaves of *Andrographis paniculata* is mainly responsible for anti-diabetic potential.[4,5]

Ipomoea batatas (sweet potato) is a member of the convolvulaceae family. Several studies have found that

sweet potato leaves inhibit the mutagenicity and growth of cancer cells, increase faecal bile acid excretion and lower hepatic cholesterol in rats, and inhibit the oxidation of low-density lipoprotein in humans.[6,7] The presence of anthocyanin's (Flavonoids) an important constituent of the leaves of *Ipomoea batatas* has a significant effect on blood glucose level control.[8]

Catharanthus roseus (Vinca rosea) is a member of the Apocynaceae family. It is grown primarily for its alkaloids, which have anticancer properties.[9] The presence of alkaloids and flavonoids constituents in Vinca rosea leaves and flowers has a significant effect on blood glucose control.[10] Hence these herbs are used in combination to improve therapeutic action by showing synergistic effects and reduce the adverse effects.

MATERIALS AND METHODS

Animal selection

Adult Wister strain albino rats weighing between 150g to 200g were employed in the investigation. A minimum of 20 adult albino rats were divided into four groups with 5 rats in each group. They were kept in clean, dry cages for a week before the experiment began, so they could get used to it. The animals were fed regular pellet food and kept in an environment at a constant temperature of 21°-23°C with a 12-hour light-dark cycle. Animals were carefully selected to ensure that they were only used for one reaction when they were not required for another. Each experimental group had their own set of animals. All the experiments were carried out in accordance with the ethical standards.[11]

Housing

5 rats were placed in each neat cage. The bedding in the cages were removed and replaced three times a week with fresh material to keep the animals clean and dry. In order to avoid animals coming into contact with watering pipes while moving in between. When the cage is changed, enough bedding material is used. Drinking tubes were periodically replaced and checked if they were working properly.[11]

Chemicals and test kits

Alloxan monohydrate was obtained from ultrapure-lab chem industries LLP, India.

Metformin hydrochloride was a product of USV Private Ltd, Himachal Pradesh, India.

Glucometer assay kit used produced from Roche diabetes care India pvt. Ltd.

Sodium chloride was obtained from Molychem, Mumbai, India.

The remaining reagents were all of analytical grade.

All solutions were prepared using distilled water.

Disposable insulin syringe and oral catheter.

Test Drug

The fresh leaves of *Catharanthus roseus, Andrographis paniculata* and *Ipomoea batatas* were obtained from the home gardens in Mangalore and authenticated by Botanist Dr. Krishnakumar G, Professor and Chairman, Department of Applied Botany, Mangalore University.

Preparation of Extracts

The plant leaves of *Catharanthus roseus, Andrographis paniculata* and *Ipomoea batatas* was authenticated and the sample was kept in the herbarium department of Mangalore university. Plants are collected, leaves are removed from stems, and leaves are air dried separately at room temperature in the shade. Using a mixer grinder, the dried plant leaves were ground into a powder. It was then sieved through mesh number 18 to make a fine powder.

Crude alcoholic extracts were made by macerating 100g of each leaf powder with 500ml of ethanol individually for 72 hours, throughout the maceration process the extract was shaken intermittently (5-6 times in a day). Whatman filter paper was used to filter the extract after each extraction. The filtrate was dried using a rotary evaporator under reduced pressure after filtration. At room temperature, the semisolid extract was dried further and stored in an air tight container. The organoleptic properties and percentage yield of the extract was done and the extract was refrigerated until it could be reconstituted in saline solution and used to treat diabetic rats.[11,12]

Acute toxicity study

According to OECD 423 acute oral toxicity studies were performed in which the mice weighing 20-25g were made to fast for 12 hours prior to the commencement of the study. Four female mice were used and each were given a single dose of 200, 500, 2000, and 5000 mg/kg of Polyherbal formulation (P.O). They were strictly and individually observed for the first 0.5, 4, and 24 hours, then daily for 15 days. They were then evaluated for altered autonomic functions like lacrimation, salivation, piloerection, central nervous system effects like tremors, convulsions, drowsiness, skin like fur condition, body weight, feed and water consumption, and mortality.[13]

Phytochemical analysis of the ethanolic crude extract

Extract was tested for the presence of active principles such as alkaloids, flavonoids, saponins, terpenoids, tannins, cyanogenic glycosides, phenols, lipids, coumarins, carbohydrates, resins and glycosides.[14]

Induction of diabetes

Diabetes was induced by single intraperitoneal injection of Alloxan monohydrate (150mg/kg body weight). Alloxan monohydrate was obtained from ultrapure-lab chem industries LLP, India.) dissolved in 0.8ml of normal cold saline solution given to overnight fasted rats. The rats were then kept in their cages for the next 24 hours on 10%

glucose solution bottles to prevent hypoglycemia. After 72 hours, blood was drawn from the animal's tail tip to be measured with a glucometer (Glucometer assay kit procured from Roche diabetes care India private limited). Diabetic animals with blood glucose levels of more than 200mg/dL were included in the study.[11]

Experimental procedure

Twenty diabetic animals were randomly divided into four groups (n=4)

Group 1: DM control treated with 1ml of saline water (0.9%w/v)

Group 2: DM treated with metformin hydrochloride at a dose of 120mg /kg

Group 3: DM treated with ethanolic extract of polyherbal formulation at a dose of 300mg/kg

Group 4: DM treated with ethanolic extract of polyherbal formulation at a dose of 500mg/kg

As the reference mentioned above, the drug was administered every morning respectively through compulsory oral intubation before meals. The treatment was continued for 20 consecutive days.[11]

Blood Sample Collection for Glucose Analysis

Blood samples for glucose evaluation have been collected from the tail vein of the rat by pricking with the needle. These rats were kept for fasting for 12 hours and treated with alloxan (150 mg/kg) and supplied with glucose water for 72hours. Then the animals were conducted for their first blood test and served with normal pellet diet and water. The first blood drop was wiped away and the second blood drop was dropped on the glucose strip and kept inside the Accu-Check glucometer, to get the glucose reading. The tails have been then rubbed with ethanol to save from infection. Along with the blood glucose values body weight of every experimental rat have been recorded daily.[11,15]

Statistical analysis

All data were represented as mean ± SEM by One-way analysis of variance (ANOVA) in graph pad prism 7.05. $p < 0.05$ considered as statistically significant.[15,16]

RESULTS

The percentage yield of ethanolic extract

The percentage yield of ethanolic extract of polyherbal formulation is given below

Weight of the dry plant powder – 300gm

Weight of the dry extract – 12gm

Percentage yield = Weight of dry extract × 100/ Weight of the dry plant powder

= 12×100/300

= 4%

Phytochemical screening of crude extract

Phytochemical tests were performed to determine the presence of chemical constituents in the ethanolic extract of polyherbal formulation. The presence of phytoconstituents were given in table 1.

Table 1: Results of phytochemical screening of the polyherbal extract

Phytochemical test	Result
Carbohydrates	+
Alkaloids	+
Flavonoids	+
Tannins	+
Proteins and Amino acids	+
Resins	–
Glycosides	+
Cyanogenic glycosides	+
Phenols	+
Saponins	+
Terpenoids	+
Coumarin	–

The symbol + denotes the presence of chemical constituents.
- denotes a lack of chemical constituents

Acute oral toxicity studies

The ethanolic extract of polyherbal formulation was subjected for acute oral toxicity studies. For the present study, the 4 dose levels i.e., 200 mg/kg, 500 mg,2000 mg/kg and 5000 mg/kg bodyweight were selected. The animals were found to be stable after 14 days of administration. The extract was found to be nontoxic up to 5000mg/kg body weight when given orally. There was no mortality or any signs of toxicity have been observed. Hence, the leaf extract is found to be safe.

Effects of polyherbal formulation on blood glucose level in alloxan induced diabetic rats

Diabetic control animals showed severe hyperglycaemia compared to other groups of animals. It was observed that the standard drug metformin hydrochloride lowered the blood glucose level, whereas the ethanolic extract of polyherbal formulation of test high dose significantly decreases the fasting blood glucose level in diabetic rats as compared to the standard group. The statistical data represented in Table 2. The changes in the blood glucose levels in different treatment groups were depicted in figure 1.

Effects of polyherbal formulation on body weight in alloxan induced diabetic rats

The diabetic control animals showed a considerable reduction in their body weight when compared to the animals which were undergoing treatment. However, polyherbal formulation and metformin maintained the body weight throughout the study. The statistical data represented in Table 3. Change in body weight of different treatment groups were depicted in figure 2.

Table 2: Blood glucose level during the treatment

Formulation	n	Day 0	Day 1	Day 4	Day 7	Day 10	Day 13	Day 17	Day 20
Control (Normal saline)	5	349±3.99	387.4±2.83	396.6±3.37	411.8±3.54	430.6±2.23	451.6±4.43	477.8±5.67	490.2±4.44
Standard dose (Metformin Hydrochloride)	5	366.4±2.83 ***	355.8±2.04 ***	320.6±1.84 ***	283.4±2.42 ***	240.2±2.61 ***	215.4±2.03 ***	181±2.92** *	128.6±2.60 ***
High dose (500mg/kg Extract)	5	362.6±4.44 ***	341.4±4.60 ***	316.4±1.53 ***	271.4±2.12 ***	232.2±1.86 ***	197.8±2.98 ***	157.8±2.33 ***	96.4±3.15* **
Low dose (300mg/kg Extract)	5	362.2±2.87 **	356.2±3.38 **	330.4±2.03 **	306.6±2.72 **	254.8±2.28 **	222.8±1.92 **	173.8±4.71 **	157.2±2.74 **

All values were expressed as Mean ± S.E.M and n indicates number of animals; **indicates p< 0.005 and *** indicates p< 0.001 when compared to the alloxan induced control group (One-way ANOVA method).

Table 3: Body weight of alloxan induced diabetic rats

Formulation	n	Day 0	Day 1	Day 4	Day 7	Day 10	Day 13	Day 17	Day 20
Control (Normal saline)	5	192.2±1.775	190.2±1.905	206.6±18.522	182.4±1.663	173.2±1.926	164±2.224	136.2±2.819	146.4±1.971
Standard dose (Metformin Hcl)	5	181.4± 1.930#	179±1.549#	175± 1.673#	187.6±2.342#	190.6±2.342#	193.6±2.237#	196.2±2.3048#	199±2.190#
High dose (500mg/kg Extract)	5	188.4±3.104*	184.6±3.001*	183± 2.993*	192.4±3.001*	195.8±3.192*	199.4±2.974*	202.8±2.834*	206.4±2.750*
Low dose (300mg/kg Extract)	5	185.8±2.748#	183.8±2.834#	183.2±2.718#	187.4±2.974#	191.8±2.862#	189±2.862#	193±2.771#	194.8±2.704#

All values were expressed as Mean ± S.E.M and n indicates number of animals; * indicates p< 0.05 and # indicates p value are non-significant when compared to alloxan induced control group by One-way ANOVA method.

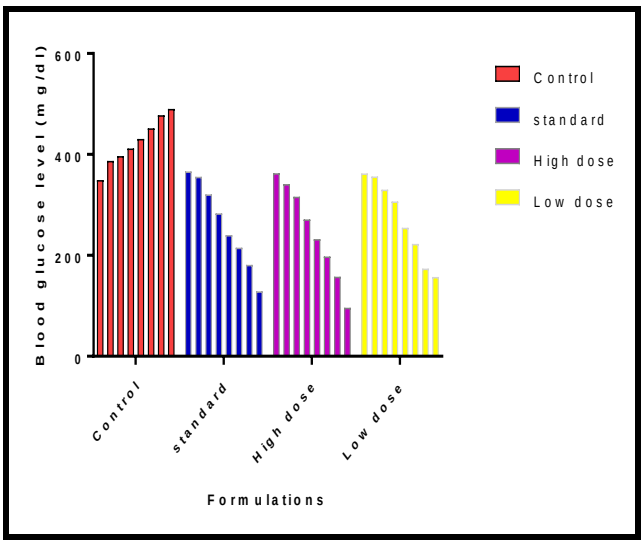

Figure 1: Representation of blood glucose level in various treatment groups

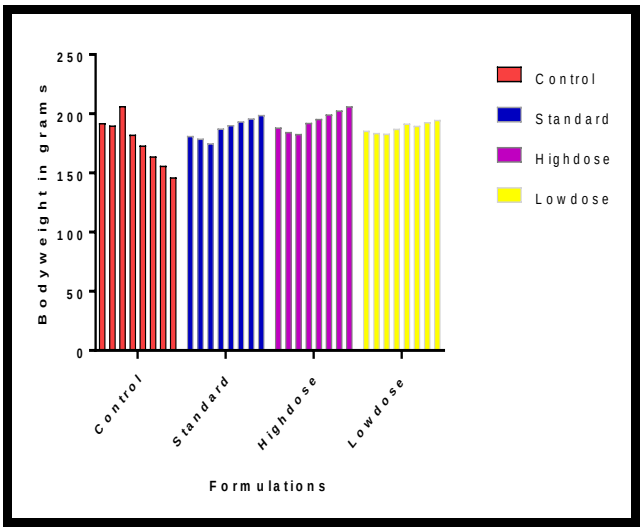

Figure 2: Representation of body weight in different treatment groups

DISCUSSION

Diabetes mellitus is a collection of syndromes which includes hyperglycaemia and impaired protein, lipid, and carbohydrate metabolism. Maintaining blood glucose level is one of the anti-diabetic methods. A variety of managements, such as natural or plant derived pharmaceuticals, nutritional aids, and synthetic drugs are employed to manage diabetes. Due to the benefits, people are turning to herbal remedies for disease treatment and prevention. With low side effects, Diabetes mellitus can be treated by using plant products that contain certain beneficiary phytochemicals. The phytochemicals present in

the leaf extracts of plants, like alkaloids, flavonoids, glycosides, tannins have various effects on Diabetes mellitus. The polyherbal formulation prepared by using ethanolic extracts of the leaves *Catharanthus roseus, Andrographis paniculata* and *Ipomoea batatas* in 1:1:1 ratio was used in this present study.

In experimental animal models, alloxan is the most commonly used agent for inducing diabetes mellitus. Which induces Diabetes mellitus within 72 hours in rats. which is confirmed by checking blood glucose levels using a glucometer. In the present study normal blood glucose level rats were selected and alloxan was induced, after 72 hours of induction blood glucose level was checked, the rats which shows ≥200 mg/dL were selected and randomly divided into 4 groups namely Group 1, 2, 3 and 4, in which each group contains 5 animals. In the study, glucose level in experimental rat on day 0 were found to be 349 ±3.99, 366.4 ±2.837, 362.6 ±4.442 and 362.2 ±2.876 of group 1, 2,3 and 4 respectively. On twentieth day of treatment the blood glucose levels were 490.2±4.44, 128.6±2.60, 96.4±3.15 and 157.2 ± 2.748 of control, standard, low dose and heigh dose treated groups respectively. Here the blood glucose levels significantly decreased throughout the experimental period in group 2, 3, 4 as compared to group 1 animals. The standard drug metformin and the polyherbal formulations both reduced high blood glucose levels, but the higher dose of polyherbal formulation has a better therapeutic effect ($p<0.001$) when compared to the standard drug. Along with blood glucose levels, the body weight of the animals was monitored during treatment days. At day 0 body weight of group 1,2,3 and 4 was found to be 192.2± 1.775, 181.4±1.930, 188.4±3.014 and 185.8±2.748 respectively, and on day 20 body weight was found to be 146.4±1.971,199±2.190,206.4±2.750 and 194.8±2.704 respectively, Here the body weight was maintained throughout the study when compared to the control animals.

CONCLUSION

From the above results and discussions, the polyherbal formulation prepared from ethanolic extracts of *Catharanthus roseus, Andrographis paniculata* and *Ipomoea batatas* are rich in phytochemicals with excellent antidiabetic potential in rats. The polyherbal formulation at a dose of 500 mg/kg has shown better results compared to standard metformin by significantly maintaining body weight. As we know, heavy weight loss is one of the main symptoms of diabetes. While most conventional diabetes medications show weight gain as a major side effect, here in this present study-maintained weight in the treatment groups indicates that this formulation will combat the major side effect caused by many conventional oral hypoglycaemic drugs. Therefore, this study demonstrated that polyherbal formulation can be considered as treatment target for diabetic people. Based on the above results and considerations, we propose this research for future development to evaluate molecular level of drug action.

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