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Anti-Diabetic Activity Of Polyherbal Formulation On Alloxan Induced Diabetes.

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Abstract: *Diabetes is a metabolic disorder with major complication associated with hyperglycemia, inflammation, foot ulcer, Nerve disorders and sexual depression Plant medicines are readily used in combination rather than in a single form to get maximum benefit from their combined potential. . Therefore a polyherbal suspension containing alcoholic extract of rhizomes of Curcuma caesia, Roxb whole plant of Evolvulus alsinoide ,seeds of Citrullus lanatus , leaves of Gymnema sylvestre, stems of Tinospora cordiofolia, fruits of Withania coagulans and seeds of Caesalpenia bonduc was prepared and evaluated for its antidiabetic activity. Antidiabetic activity was studied in Alloxan induced diabetic rats. The standard drug used was Glibenclamide 10mg/kg. polyherbal suspension at both the doses 400mg/kg bw was having significant activity. The weight gain and decrease in blood glucose level was less than that of standard drug. Thus the prepared oral Polyherbal suspensionis safe for use with promising antidiabetic activity.*

Keywords: *Antidiabetic activity, polyherbal formulation, Alloxan induced, OGTT, medicinal plant*

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I. Introduction

According to WHO, it has been recently projected that a total number of patients diagnosed with type II diabetes will be more than 300 million before 2025. The increasing rate of mortality and morbidity due to diabetes is mainly because of the microvascular and macrovascular disease associated with diabetes, making it as one of the five leading cause of death in the world.[1,2] In the three countries that have the most people living with type 2 diabetes. In China about 90 M around 9.3% of its population, In India, about 61.3 million around 8.3% of its population and In the United States about 25.8 M around 8.3% of population of USA[3]. A large number of medicinal plants are used in the treatment of diabetes. There are numerous traditional plants mentioned in Siddha and Ayurvedic system of medicine which are used as antidiabetic agents.[4] Polyherbal formulations (PHFs) enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing the adverse events. Compared to the single herb, the PHF has better and multi-targeted therapeutic potential. WHO report 80% of the world population relies on the drug from natural origin. Diabetes is a metabolic disorder with associated with various ailments such as elevated hyperglycemia, inflammation, foot ulcer, Nerve disorders and sexual depression obesity. [5]. Plant medicines are readily used in combination rather than in a single form to get maximum benefit from their combined potential. Keeping the above information in view, an indigenous polyherbal preparation was developed containing the ethanolic extracts of rhizomes of *Curcuma caesia,* Roxb(Zingiberaceae) whole plant of *Evolvulus alsinoide* Linn. (Convolvulaceae),seeds of *Citrullus lanatus* Thunb. (Family: Cucurbitaceae), leaves of *Gymnema sylvestre* (Asclepidaceae), stems of *Tinospora cordiofolia* (Menispermiaceae), fruits of *Withania coagulans* Dunal (Solanaceae), and seeds of *Caesalpenia bonduc* (Caesalpiniaceae) were used to investigate their effect on blood glucose, in rat model of Alloxan induced diabetes.

II. Material Method

Collection of plant material

Air dried whole plant of *Evolvulus alsinoide* , leaves of seeds of *Citrullus lanatus, Gymnema sylvestra,* stems of *Tinospora cordiofolia* fruits of *Withania coagulans* Dunal (Solanaceae), seeds of *Caesalpenia bonduc* (Caesalpiniaceae) were collected from leghapani (hills of toranmal) in satpuda and rhizomes of *Curcuma caesia* were purchased from bhuveneshwar Orisa . They were authenticated by Dr. D.A. Patil, Head of Department of Botany, S.S.V.P.S. art science and commerce College, Dhule.

Preparation of Extracts [6,7]

The plant material first washed with water thoroughly to remove dirt and soil deposits and dried under shade until complete removal of moisture content, such dried plants were powdered by mechanically and passed through sieve no 80. About 100 grams(each plants) of rhizomes of *Curcuma caesia,* whole plant of *Evolvulus alsinoide* ,seeds of *Citrullus lanatus* , leaves of *Gymnema sylvestra*, stems of *Tinospora cordiofolia,* fruits of *Withania coagulance*, and seeds of *Caesalpenia bonduc* were powdered and subjected to extraction with various solvents such as petroleum ether(40-60) , ethanol by using soxhlet apparatus successively at room temperature. The extracts were filtered and concentrate at reduced pressure and lyophilized. The dried lyophilized extracts were stored carefully for further investigation.

Phytochemical investigation [8,9]

All the Preliminary qualitative phytochemical analysis of all the extracts were carried out by employing standard conventional protocols for preliminary phytochemical screening. The result of phytochemical investigation was shown in Table no.1

Preparation of polyherbal formulation [10,11]

The evaporated lyophilized residue alcoholic extracts of *Curcuma caesia, Evolvulus alsinoide* ,*Citrullus lanatus* ,*Gymnema sylvestra*, *Tinospora cordiofolia, Withania coagulance*, and *Caesalpenia bonduc* in the ratio of 1:1:1 were mixed in water and the different additives likeTween-80, Sodium CMC, sweetening agent (Sodium saccharin), Flavouring agent (Lemon oil) used for its better stability.

Animal.

Adult Wistar albino rats of either sex (180-250 g) were housed in standard temperature/humidity conditions and environment (12 h light/dark cycle) all sets of experiments. The rats were allowed to take standard pellet diet and water ad libitum all time except during the estimation of the behavioural parameters. The experimental protocols were approved by the Institutional Animal Ethics Committee of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dist-Dhule, Maharashtra, India which was registered with Committee for the purpose of control and supervision of experiments on animal (CPCSEA),Govt. of India.

Acute Toxicity Studies[12

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) guidelines 420. Animals were administered with single doses of in different groups and observed for the mortality during period of toxicity. In each steps three animals were used in each group. The animals were observed continuously for the 24 hours. A single oral administration of the dose from 300mg/kg body weight to 2000 mg/kg body weight in different group of mice. There was no mortality observed at 2000mg/kg for the formulation. Therefore 2000mg/kg dose was considered as cut off dose so 1/10th and 1/5th of maximum dose was selected.

Induction of diabetes[13,14]

Diabetes was induced in rats by administering alloxan monohydrate 150 mg/kg body weight freshly prepared in normal saline solution by intraperitoneal route. After one hour of alloxan administration, animals were given feed ad libitum and 1 ml of 100mg/ml glucose I.P. to confirm hypoglycemia after 72 hours of alloxan injection. Induction of diabetes confirmed on 3rd day by measuring glucose level. The blood glucose level more than 200 to 350 mg/ml of blood was selection of criteria for the experiment.

Oral Glucose Tolerance test[15]

The method followed by V.babu et al (2002) has been adopted. Four groups of 4 rats each were used for the study.

Group I – Normal (vehicle 1% acacia)

Group II- PHF 1 (100 mg/kg b.w).

Group III- PHF 2 (200 mg/kg b w)

Group IV- PHF- 3(400 mg/kg b w)

The rats of all group were loaded with 60% glucose (3gm/kg/oral) 30 min. after administration of polyherbal formulations. Blood glucose levels were measured prior to administration at 0 min, 30 min, 90min, and 150min. blood glucose levels were measured using glucometer (Accuchek) within range of 10-600mg/dl.

Evaluation of anti-diabetic activity of the Polyherbal formulation [16].

The Diabetic animals were randomly divided into eight groups with 8 rats in each group and treated as follows for 14 days continuously orally.

Experimental Group:

Rats were randomly divided into 6 groups containing 8 rat in each group.

I. Normal group: Saline (saline 1ml p.o)
II. Alloxan : (150mg /kg i.p one's daily dose)
III. Standard : (Glibenclamide 10 mg/kg bw)
IV. Test drug 1 : Diabetic rats+ (PHF 100 mg/kg bw p.o one's daily dose)
V. Test drug 2 : Diabetic rats+ (PHF 200 mg/kg bw p.o one's daily dose)
VI. Test drug 3 : Diabetic rats +(PHF 400 mg/kg bw p.o one's daily dose)

For acute study, the Blood Glucose Levels were monitored after of a single dose and at the end of 3, 9 and 14 days for prolonged treatments.

Collection of blood and detection of Serum Glucose

Blood samples of the fasted rats were collected at days 3,9,14 from Retro-orbital immediately with capillary tubes under ether anaesthesia. Serum was separated by centrifugation at 1500 rpm for 10 min. 30 µl of serum sample and 3 ml of working glucose reagent were taken in to a dry and clean test tube and incubated for 10 minutes at 37^{o} C. Blood glucose level was estimated at 3^{rd}, 9^{th} and 14^{th} day in serum sample according to manufactures protocol by using the standard kit glucose–oxidase–oxygen method.

III. Statistical Analysis

The values were expressed as mean±SEM. The data was subjected to the analysis of variance(one way ANOVA) to determine the significance of changes followed by Dunnet post hoc test. The statistical significance of difference between two independent groups were calculated for the determination.

IV. Result

The preliminary phytochemical investigation showed that all the drug extracts contain mainly glycosides, triterpenoid, flavonoids and alkaloids, steroid and tannins [Table 1].. In the acute oral toxicity studies, no mortality were observed for polyherbal formulation up to 2000mg/kg . In order to determine hypoglycemic effect of Polyherbal formulation on glucose tolerance in normal rats, variable doses (100, 200, and 400 mg/kg) of formulation examined. At 90 min interval, it was found that there is no significant reduction in blood glucose level in PHF 1and PHF 2. Polyherbal formulation at a dose of 400 mg/kg shows significant reduction the blood glucose level at (90 87.2±2.66) and 150 (81.4±3.2) min after glucose administration compare to glucose loaded(109.6±5.0) [Table 2].On 14 th day in diabetic control group there is decrease in the Body wt of rat was observed(186.0±4.45). There is no significant decrease in weight of rat(was observed in PHF 1 (100mg/kg).it was found that there is significant increase in body weight of rat(195±4.15)in PHF 3 (400 mg/kg) formulation as compare to Alloxan treated(186.0±4.45) [Table-3].In diabetic animal, on 9^{th} day there is no significant reduction of blood glucose level in PHF-1 as compared to alloxan treatd, PHF 2 and PHF 3 respectively. On continuous administration of polyherbal formulation up to 14 days it was found that there is significant reduction in the blood glucose level in PHF3(100.3±2.999) as compared to Alloxan treated (330.8±4.139) and Normal (96.42±6.147). So it was concluded that the anti-diabetic activity of polyherbal formulation in suspension form is nearly comparable with alloxan treated (330.8±4.139) at a dose of 400mg/kg body weight (100.3±2.999) than the dose of 200mg/kg body weight. [Table-4].

V. Discussion

The present manuscript discusses about the antidiabetic effects of the polyherbal formulation on normal and Alloxan-induced-diabetic rats. From the preliminary phytochemical screening, it is confirmed that the polyherbal formulation used in this study is a rich source of flavonoids and tritepenoids, steroide and alkaloid. These compounds are known to possess free radical scavenging effect rejuvenating potential, to increase in insulin level or inhibit the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes. Alloxan exerts its action by formation of superoxide radicals which undergo dismutation to hydrogen peroxide with a simultaneous massive increase in cytosolic calcium concentration due to which causes it rapid destruction of pancreatic cells [18,19]. Administration of Polyherbal formulation may exerts the potential to cause chemical or pharmacological interactions leading to possess its hypoglycemic action due to

number of mechanisms may be associated with selected herbal drug such as *Curcuma caesia* rhizome used traditionally for lowering blood glucose levels by the people of Manipur, *Evolvulus alsinoide* whole plant and *Citrullus lanatus* seeds are documented in srilankan ayurvedic treaties.[20,21], *Gymnema sylvestra* increasing serum insulin levels through repair/ regeneration[22], *Evolvulus alsinoides* extract reduces the lipid peroxidation level and increases the antioxidant level also prevents the pancreas by suppressing the oxidative stress[23], *Caesalpinia bonducella* extracts may be due to the blocking of glucose absorption,[24] *Tinospora cordifolia* extracts involved in carbohydrate metabolism through enzymetic control, [25] presence of alkaloide and steroids in extracts of fruits of *Withania coagulance*.[26] From above discussion we conclude that the hypoglycemic activity of PHF 3 (400mg/kg)formulation may be due to presence of various phytoconstituents and its mechanism in prolong treatment. The study, however, recommends undertaking of further research to establish the mechanism of the hypoglycemic activity to explore possibilities of developing a herbal formulation an ideal alternative for the existing synthetic formulation which can function by a similar mode of action.

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Table no.1: Phytochemical investigation.

Name of Phytoconstituents	Curcuma caesia		Evolvulus alsinoides		Citrullus lanata		Withania coaglancce		Tinospora cordifolia		Gymnema sylvestra		Caesalpenia bonduc	
	Pet Ether (40-60)	Ethanol	Pet Ether (40-60)	Ethanol	Pet Ether (40-60)	Ethanol	Pet Ether (40-60)	Ethanol	Pet Ether (40-60)	Ethanol	Pet Ether (40-60)	Ethanol	Pet Ether (40-60)	Ethanol
Alkaloides	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Glycosides	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Saponins	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve
Steroides	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Terpenoides	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	-ve
Flavonides	-v	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
Tannins	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Carbohydrate	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
Phenolic	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve
Protein	--	--		--	-ve	+ve	-ve	-ve	--	-ve	--	---	-ve	-ve

Table: 2Effect of Polyherbal formulation on blood glucose level on glucose tolerance in normal fasted rats

Group	Blood Glucose Level (mg/dl) At Various Time Intervals			
	0 min	30 min	90 min	150 min
Glucose loaded	55.1±3.5	132.5±3.2	124±4.0	109.6±5.0
Test 1	60.5±5.0	125.3±5.2	115±4.6	96.3±5.1
Test 2	57.6±4.0	107.6±2.5	111±3.3	84±3.4***
Test 3	58.4±2.4	110.4±3.16	87.2±2.66**	81.4±3.2***

Data was expressed as mean ± SEM (n =4). Statistical significances were determined using one-way analysis of variance (ANOVA) followed by Dunnet post hoc test. ###p<0.001 as compared to normal, **p<0.01, ***p<0.001 as compared to loaded

Table: 3Effect of Polyherbal formulation Body weight of Alloxan -induced diabetic rats during prolonged treatment

Group	Body Wt	
	Initial	14th day
Normal	194.5±5.25	198.7±5.28
Standard	197.8±6.68	205.3±5.54
Alloxan	204.0±5.60	186.0±4.45
Test 1	201.83±6.36	198.23±4.32
Test 2	188.8±2.39	192.8±2.23***
Test 3	190.5±2.39	195±4.15***

Data was expressed as mean ± SEM (n =8). Statistical significances were determined using one-way analysis of variance (ANOVA) followed by Dunnet post hoc test. ###p<0.001 as compared to normal, **p<0.01, ***p<0.001 as compared to alloxan

Table: 4Effect of Polyherbal formulation on serum blood glucose level of Alloxan -induced diabetic rats during prolonged treatment

Group	Blood glucose level (mg/dl)		
	3rd day	9th day	14th day
Normal	101.1±4.91	94.25±2.66	96.42±6.147
Standard	143.8±17.73	122.5±7.86	107.2±11.27
Alloxan	218.4±9.26###	220.7±4.95###	330.8±4.139###
Test 1	195.9±20.39	194.8±9.23	163.7±13.21***
Test 2	151.1±7.59***	140.0±7.16***	131.6±6.717***
Test 3	109.0±4.38***	102.2±3.83***	100.3±2.999***

Data was expressed as mean ± SEM (n =8). Statistical significances were determined using one-way analysis of variance (ANOVA) followed by Dunnet post hoc test. ###p<0.001 as compared to normal, **p<0.01, ***p<0.001 as compared to alloxan.

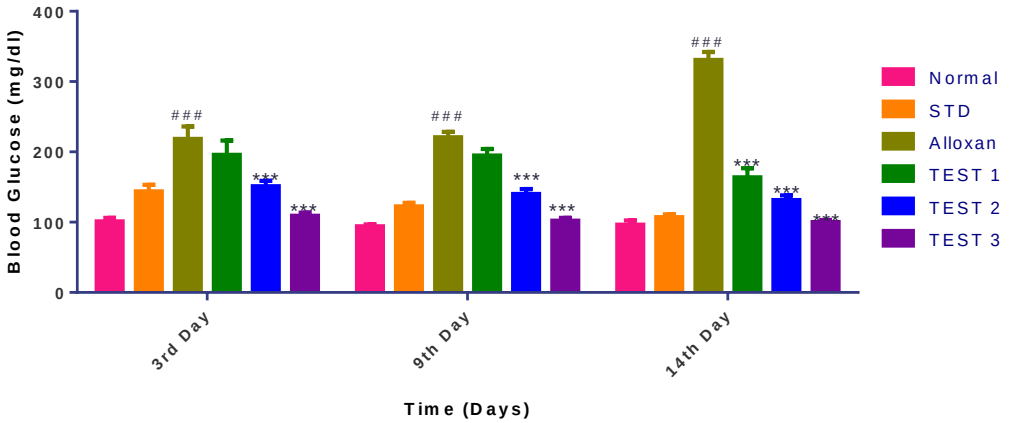

Fig: 1 Data was expressed as mean ± SEM (n =8). Statistical significances were determined using one-way analysis of variance (ANOVA) followed by Dunnet post hoc test. ###p<0.001 as compared to normal, **p<0.01, ***p<0.001 as compared to Alloxan Effect of Polyherbal formulation on serum blood glucose level of Alloxan -induced diabetic rats during prolonged treatment

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