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ANTI-DIABETIC ACTIVITY OF POLYHERBAL FORMULATION ON ALLOXAN INDUCED RATS

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ABSTRACT

The polyherbal anti-diabetic formulation is prepared in solution form by mixing the water and alcoholic extract of five medicinal plants indigenous in India. The polyherbal formulation was evaluated for its anti-diabetic effect at two different doses. The anti-hyperglycemic effect of formulation found nearly comparable with glibenclamide which is used as standard drug to compare the anti-diabetic effect of formulation at different doses by using the glucometer to measure the blood glucose level. It was found that the anti-diabetic activity of formulaion at a dose of 400mg/kg of body weight (294.5± 1.40) more effective than 200mg/kg of body weight (297.33 ±0.76) on wistar albino rats. So it was concluded that the anti-diabetic activity of polyherbal formulation in solution form is nearly comparable with standard drug glibenclamide(261.5 ±0.56) and the formulation at the dose of 400mg/kg was showed more significant anti-diabetic effect at 1st hour(359.16± 1.42) after drug administration and at the dose of 200mg was showed more significant effect at 2nd hours(354± 0.57) when its compare with the control group (363.83±0.5 and 376±0.63 for 1st and 2nd hours respectively) on wistar albino rats. It was also concluded that the formulation have significant anti-diabetic activity and also stable till the 3 month which was observed by the stability testing by using different parameters used for evaluation of solution.

Key words: Polyherbal formulation, Glibenclamide, Solution.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by chronic hyperglycemia due to deficient insulin secretion, factor opposing the tissue effects of insulin or both.[1] It has

recently broken the age barrier and appears even in younger people. It is debilitating metabolic disorder and robs persons of their energy and vitality.[2] The insulin therapy and oral hypoglycemic agents is the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and failed to significantly alter the course of diabetic complications.[3] Thus plants are a potential source of anti-diabetic drugs.[4]Therefore the search for more effective and safer hypoglycemic herbal formulation has continued to be as a aim of present study. Hence the polyherbal formulation was prepared in the form of solution by using the five different plants indigenous to India because the drug in solution form is immediately available for absorption.[5]

2. MATERIAL AND METHODS

2.1. Collection of plant material

Air dried barks of *Albizzia odoratissima*, barks of *Anoegeissus latifolia*, roots of *Chonemorpha fragrans*, barks of *Diospyros malabarica* and flower of *Woodfordia fructicosa* were collected from local market of Khari Babri, Dehli. They were authenticated by Dr. Seema Bhadhauria, Head of Department of Botany, R.B.S College, Agra and specimens of all plants were submitted to the R.B.S.College, Agra.

2.2. Preparation of extract [6,7,8]

About 800 grams(each plants) of air dried barks of *Albizzia odoratissima*, barks of *Anoegeissus latifolia*, roots of *Chonemorpha fragrans*, barks of *Diospyros malabarica* and flowers of *Woodfordia fructicosa* were powdered and subjected to extraction with various solvents such as water and alcohol at room temperature for seven days by simple maceration method. The extracts were filtered and concentrate to dryness at room temperature to avoid the decomposition of natural metabolites. The dried extracts were stored carefully for other investigation.

2.3. Preparation of polyherbal formulation [9, 10]

The evaporated residue extracts were mix in water and the different additives like antioxidant (Butylated hydroxyanisol), preservative (Sorbic acid), sweetening agent (Sodium saccharin), Flavouring agent (Chocolate) used for its better stability during shelf life of formulation. The residual extracts were mixed in the same ratio to make 4% w/v solution dosage form.

Table No. 1: General Formula adopted for Developed Polyherbal Formulation

SI.NO	Name of Ingredient	Quantity taken
1	Albizia odoratissima water extract	0.4gms
2	Albizia odoratissima alcohol extract	0.4gms
3	Anoegeissus latifolia water extract	0.4gms
4	Anoegeissus latifolia alcohol extract	0.4gms
5	Chonemorpha fragrans water extract	0.4gms
6	Chonemorpha fragrans alcohol extract	0.4gms
7	Diospyros malabarica water extract	0.4gms
8	Diospyros malabarica alcohol extract	0.4gms
9	Woodfordia fructicosa water extract	0.4gms
10	Woodfordia fructicosa alcohol extract	0.4gms
11	Butylated hydroxyanisol	0.2%w/v
12	Sorbic acid	0.2%w/v
13	Sodium saccharin	0.1%w/v
14	Chocolate flavor	q.s.
15	Purified water(Q.S.)	100ml

2.4. Maintenance of animals

Healthy wistar rats of either sex were used in the present study. They were housed in standard condition of temperature (25 ± 2^0) with 12 hour light per day cycle.

2.5. Acute toxicity study [11]

The acute oral toxicity studies were carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), draft guidelines 420.A single oral administration of the dose from 300mg/kg body weight to 2000 mg/kg body weight in different group of mice. In each steps three animals were used in each group. The animals were observed continuously for the 24 hours. There was no mortality observed at 2000mg/kg for the formulation. Therefore 2000mg/kg dose was considered as cutoff dose so $1/10^{th}$ and $1/5^{th}$ of maximum dose was selected i.e.200mg/kg and 400mg/kg, for anti-diabetic studies.

Table No. 2: Dose Selection and Finalising LD_{50} Cut-Off value of Polyherbal Formulation

Sr.No.	Name of formulation	LD_{50} Cut-Off mg/kg.b.w.	Therapeutic dose(effective dose)
1	Polar polyherbal formulation	2000mg/kg b.w.	$1/10^{th}$ of lethal dose 200mg/kg b.w.
2		2000mg/kg b.w.	$1/5^{th}$ of lethal dose 400mg/kg b.w.

2.6. Induction of diabetes [12,13,14]

The acclimatized animals were kept fasting for 24 hrs with water ad libitum and injected intraperitoneally at a dose of 150mg/kg b.w. of alloxan monohydrate (S.D. Fine Chemicals Ltd., Biosar) freshly prepared in normal saline solution. Before starting the experiment, animals were separated according to their body weight. After one hour of alloxan administration, animals were given feed ad libitum and 1 ml of 100mg/ml glucose I.P. to combat ensuring severe hypoglycemia after 72 hours of alloxan injection, the animal were tested for evidence of diabetes by estimating their blood glucose level by using Glucometer. The blood glucose level more than 250 mg/ml of blood was criteria.

Anti-diabetic activity: **The animals were grouped as follow**

a) Diabetic control: (Normal saline solution)

b) Formulation Group A: (200mg/kg)

c) Formulation Group B :(400mg/kg)

d)Standard group: Glibenclamide: (10mg/kg b.w. orally by dissolving in normal saline solution)

A 0.2 ml of blood was withdrawn at interval of initial (0 hr), 1^{st} ,2^{nd} ,4^{th} and 8^{th} hrs of administration of single dose for anti-diabetic activity and the blood glucose level was measured in all groups by using Glucometer (Pulsatum, Pulsatum Health Care Pvt.Ltd., Bangalore). The blood is again withdrawn at interval of 24^{th} hours followed by second dose administration (after twelve hours of first dose).

Table no.-3: Anti diabetic activity of polyherbal formulation against alloxan induced diabetic rats

Group	O hour	1hour	2 hour	4 hour	8 hour	24 hour
Diabetic control	367.83±0.32	363.83±0.57	376±0.63***	372.33±0.83***	391±0.37***	425±0.57
Formulation(200mg/kg)	370.66±0.88	363.33±0.57	354±0.57***	323.83±0.60***	316.5±0.76***	297.33±0.76***
Formulation(400mg/kg)	374.5±0.84	359.16±1.42***	349.50±0.84***	316.16±1.07***	301.83±0.83***	294.5±1.40***
Diabetic+Glibenclamide (10mg/kg)	344.33±0.53	323.83±0.53***	309±0.81***	302.33±0.88***	273±0.43***	261.5±0.56***

Values are the mean±S.E.M.,n=6,*P<0.05,**p<0.01,***p<0.001(vs.Control)

2.7. Statistical analysis

All the values were expressed as Mean ±S D. The data were statistically evaluated by using one way analysis of variance (ANOVA) followed by dunnetts test. Values of P<0.001 were considered as more significant.

2.8. Stability studies of polyherbal formulation

2.8.1. Physical tests for Polyherbal formulation

Table No. 4 : Physical tests for Polyherbal formulation

Sr.No.	Parameter	Initial	First Month		
			Room temperature	25^0C	45^0C
1	Nature (Appearance)	Liquid, like sweet and clear solution	Liquid, like sweet and clear solution	Liquid, like sweet and clear solution	Liquid, like sweet and clear solution
2	Color	Dark reddish brown	Dark reddish brown	Dark reddish brown	Dark reddish brown
3	Odor	Characteristic	Characteristic	Characteristic	Characteristic
4	Taste	Slightly sweet with bitterness	Slightly sweet with bitterness	Slightly sweet with bitterness	Slightly sweet with bitterness

The results were also same for the 2nd and 3rd month.

2.8.1. Accelerated stability studies [15]: The stability of the formulation was checked by using the different parameters like pH changes, light stability, suspendibility, viscosity, gas evolution.

2.8.1(a) pH changes: pH of the formulation was observed by using the Digital pH meter and the result was showed in Table no.5

Table No. 5: pH of the formulation

Sr. No.	pH of the formulation during storage		
1.	1st month	2 nd month	3th month
	6.9±0.66	6.8±0.45	6.8±0.90

2.8.1(b) Suspendibility of formulation

The supendibility of the formulation was checked by necked eyes in presence of day light for 1st,2nd and 3rd month during storage of the formulation. The results were showed in the Table no.6

Table No.6: Suspendibility of the formulation

Sr. No.	Suspendibility of the formulation during storage		
1.	1st month	2 nd month	3th month
	NO Particles settled (NO SUSPENDIBILITY)	NO Particle settled (NO SUSPENDIBILITY)	NO Particle settled (NO SUSPENDIBILITY)

2.8.1(c) Gas Evolution of formulation

The evolution of gas from the solution was observed by necked eyes and observation of hissing sound for 1st, 2nd and 3rd month.

Table No.7: Gas Evolution of formulation

Sr. No.	Gas evolution of the formulation during storage		
1.	1st month	2 nd month	3th month
	NO GAS EVOLUTION	NO GAS EVOLUTION	NO GAS EVOLUTION

2.8.1 (d) Light stability of formulation: The formulation was observed during day light.

Table No.8: Light stability of formulation:

Sr. No.	Light stability of the formulation during storage		
1.	1st month	2 nd month	3th month
	NO visible particles present	No visible particles are present	No visible particles present

RESULTS

The dried bark of *Albizzia odoratissima*, bark of *Anoegeissus latifolia*, roots of *Chonemorpha fragrans*, bark of *Diospyros malabarica* and flower of *Woodfordia fructicosa* were powdered and subjected to extraction with various solvents such as water and alcohol at room temperature for seven days by simple maceration method. The extracts were filtered and

concentrate to dryness at room temperature to avoid the decomposition of natural metabolites. The dried extracts were stored carefully for the phytochemical analysis.

The preliminary phytochemical investigation showed that all the drug extracts contain glycosides, triterpenoid, saponin, carbohydrates, flavonoids and alkaloids.
The formulation was developed by using the above mentioned formula in Table no.-1

Anti-diabetic activity: The anti-diabetic activity of polar polyherbal formulation at the dose of 400mg/kg body weight showed more significant activity at first hours but the formulation at the dose of 200mg/kg of body weight was showed more significant activity at 2nd hours when it compare with control group and it also showed more significant activity at 24 hrs after second dose administration of drug which may be nearly comparable with standard drug glibenclamide. So it was concluded that the polyherbal formulation at the dose of 400mg/kg b.w. showed more significant anti-diabetic activity than 200mg/kg b.w. and used for pharmacological screening results are shown in Table.no.-3.

The developed formulation was reddish brown in color, liquid in nature, somewhat bitters with sweet in taste, the texture was clear colored liquid.

4. CONCLUSION

The developed formulation possesses every quality of the formulation in solution form that is available in market and showed the significant anti-diabetic activity when it is compared with the standard drug glibenclamide. The stability of solution was best for three month.

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