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R E S E A R C H A R T I C L E

Anti-diabetic activity of a polyherbal preparation in Alloxan-induced diabetic rats

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A B S T R A C T

The present research work mainly focused on evaluation of anti-diabetic activity in poly herbal preparation in alloxan – induced rats. In this study using polyherbal preparations are *Momordica charantia, Eugenia jambolana and Ocimum sanctum*, it was prepared by in the ratio of 1:1:1. These herbal plants also exhibit the Antibacterial Activity, Antiviral activity, Anti-HIV activity, Antiherpes activity, Anti-poliovirus activity and Anticancer activity. *Momordica charantia* (fruits), *Eugenia jambolana* (seeds), *Ocimum sanctum* (leaves), Allium cepa (juice) have been used traditionally to alleviate increased blood glucose level.In the present study, we have measured the potential of Poly Herbal Preparation extract to inhibit lipid peroxidation in rat liver homogenate, induced by the $FeCl_2$–H_2O_2 system.Poly herbal xtracts having both hypoglycemic and antioxidative properties would be useful antidiabetic agents. Anti-diabetic activity in polyherbals were compard to the alloxan-induced diabetic rats. It is suggested that is helpful in maintaining the hyperglycaemia as well as the complication which are frequently seen in diabetic patients.

Keywords: Anti diabetic activity, Poly herbal, Alloxan, *Momordica charantia, Eugenia jambolana and Ocimum sanctum*

A R T I C L E I N F O

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CONTENTS

1. Introduction

Diabetes mellitus (DM) is the name given to a group of disorders characterized by chronic hyperglycemia, polyurea, polydipsia, polyphagia, emaciation and weakness due to disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and / or insulin action. DM is a

condition in which the sugar level is above the normal sugar level 80-120 mg/dl of the whole blood 1.

Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)

A. Immune-mediated

B. Idiopathic

Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretory defect with insulin resistance). Oral synthetic agents currently being used as anti-diabetic agents have severe undesirable side effects and has failed to correct the fundamental lesion and diabetic complication.This fact has provoked the WHO expert committee on DM to investigate for anti-diabetic agents from medicinal plants. The number of herbal drugs are used to treatment of Diabetis mellitus. The advantage of these drugs are;

- ✓ No side effects as such reported
- ✓ Easily available.
- ✓ Economical in use.

Based on literature surveyMomordica charantia (fruits), Eugenia jambolana (seeds), Ocimum sanctum (leaves),Allium cepa (juice) have been used traditionally to alleviate increased blood glucose level. But no such evidence regards the synergestic action of these plants when put in a single formulation is available.

2. Materials and Methods

Poly Herbal Preparation (Momordica charantia, Eugenia jambolana, Ocimum sanctum, Allium cepa): These plants were reported to have anti-diabetic activity with different mechanism. So we have aimed to prepare a polyherbal preparation which could be active against diabetes through different mechanism.

A. Momordica charantia

Family - Cucurbitaceae

Part Used - Dried fruits.

Chemical Constituents: Charantin (steroidal saponin), momordicin, carbohydrates, mineral matter, alkaloids, glucosides, saponins and mucilage.

Medicinal Action and Uses: Antibacterial Activity, Antiviral activity, Anti-HIV activity, Antiherpes activity, Antipoliovirus activity, Anticancer activity, Abortifacient, Antifertility, Anthelmintic study.

B.Eugenia jambolana

Family - Myrtaceae

Part Used - Dried seeds

Chemical Constituents: Contains approximately 70% eugenol, carvacrol and eugenol-methyl-ether.

Medicinal Uses: The fruits and seeds are sweets, acrid, sour, tonic, diarrhea, pharyngitis, splenopathy, urethrorrhea and ringworm.

C. Ocimumsanctum

Family - Lamiaceae

Part Used - Dried leaves

Chemical Constituents: It contains approximately 70% eugenol, carvacrol and eugenol-methyl-ether.

Medicinal Action and Uses: Used as stimulant, aromatic, anticatarrhal, spasmolytic and diaphoretic.

D.Allium cepa

Family - Liliaceae

Part Used- Onion bulbs.

Chemical Constituents: It contains approximately 70% eugenol, carvacrol and eugenol-methyl-ether.

Medicinal Action and Uses: These compounds possess antidiabetic, antibiotic, hypocholesterolaemic, fibrinolytic and various other biological effects.

Extraction of Plant Material:

The plants were coarsely powdered, weighed and filled in Soxhlet apparatus for extraction. The solvent used was hydroalcholic i.e. 70% ethanol and 30% water. % yield was calculated for each extract after drying.

Preparation of Onion juice:

100 g of onion was taken in 250 ml of distilled water made into juice by using mixy.

Polyherbal Preparation:

Momordica charantia, Eugenia jambolana, Ocimum sanctum extracts were mixed properly in 1:1:1 ratio and volume is make up with onion juice.

Qualitative Chemical Evaluation:

Preliminary phytochemical Screening of dried fruit of *Momordicacharantia,* dried seeds*Eugenia jambolana,* dried leaves of*Ocimum sanctum*,bulbs of *Allium cepa*, indicates the presence of carbohydrates, gum and mucilage, proteins, alkaloids, glycosides, steroids, tannins, saponins, flavonoids, anthraquinones, coumarin, terpenoids and furanoidscompounds.Theresults were tabulated in Table 1.

Pharmacological Studies

Procurement and selection of animals:

Wistar albino rats of either sex weighing between 130–180 gm of either sex were obtained from SV animal house, Bangalore. These animals were used for the acute toxicity and antidiabetic activity. The animals were stabilized for 1 week; they were maintained in standard condition at room temp; 60 ± 5% relative humidity and 12 h light dark cycle. They had been given standard pellet diet and water *ad-libitum*throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output.

Acute Toxicity Study:

The acute toxicity study was carried out in adult female albino rats by "fixed" method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. Fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg/Kg body weight was adopted. The animals were fasted overnight and next day poly herbal preparation (suspended in 0.5 % w/v sodium CMC) were administered orally at dose level 2000 mg/kg. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days.

Selection of doses: For the assessment of antidiabetic activity, two dose level were chosen in such a way that, one dose was approximately one tenth of the maximum dose during acute toxicity studies, and a high dose, which was twice that of one tenth dose (200mg/kg, 400mg/kg).

Grouping of animals:

Group I: Kept as Normal control i.e. neither treated with extract or standard.

Group II: Kept as Negative control i.e. treated with alloxan (125 mg/kg).i.p.

Group III: Treated with standard oral hypoglycemic drug i.e. Glibenclamide (0.5 mg/kg) after 3rd day of treatment with alloxan (125 mg/kg i.p.).

Group IV: Treated orally with 200 mg/kg of polyherbal preparation after 3rd day of treatment with alloxan (125 mg/kg i.p).

Group V: Treated orally with 400 mg/kg of polyherbal preparation after 3rd day of treatment with alloxan (125 mg/kg i.p.).

Preparation of dosing:

The dose of 200 mg/kg of polyherbal preparation was made by dissolving appropriate quantity of extracts in onion juice (suspended in 0.5 % w/v sodium CMC).

Determination of OGTT activity (Oral glucose tolerance test): Test samples were given orally using oral gastric gavages to the fasted animals. The blood glucose concentrations of the animals were measured at the beginning of the study. Then the rats were orally treated with at dose level 3 gm/kg body wt. glucose solution. The BGL measurements were repeated after 30, 60, 120 and 180 min after the initial of the experiment.

Determination of Antidiabetic activity:

Test samples were given orally using oral gastric gavages to the animals once before food daily was given. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 3rd, 7th and 11th day after the initial of the experiment. The inference was made by comparing Blood Glucose Level, Body Weight, Serum Creatinine, Blood Urea, Serum Triglycerides and Serum Total Cholesterol with treated and negative control (alloxan treated). Observations mentioned in Table no: 2, 3.4.

3. Results and discussion

The following are the results of our work Qualitative test shows presence of various biochemical as tabulated in table 1.

Acute Toxicity studies:

Acute Toxicity studies on female rat's shows no mortality at a dose of 2000 mg/kg, during a time period of 14 days (Table 2). The Behavioral, Neurological, Autonomic responses were studied for a time period of 4 hrs of toxicity study. During the study no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe.

Fig 1: Effect of plant extracts on blood glucose level in fasted rats (single dose study)

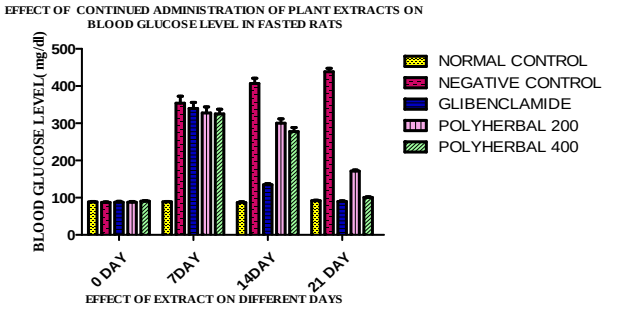

Fig 2: Effect of continued administration of plant extracts on serum glucose level in fasted rats (multi dose study)

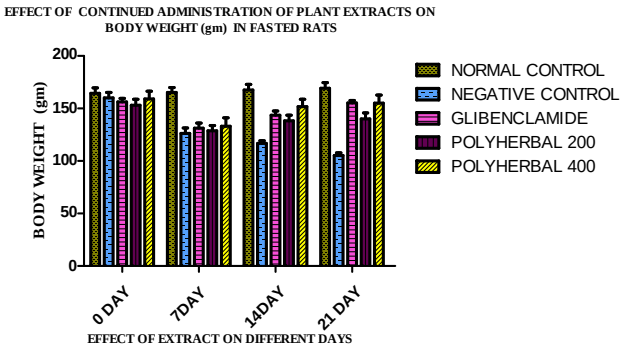

Fig 3: Effect of continued administration of plant extracts on Body weight(gm) in rats (multi dose study)

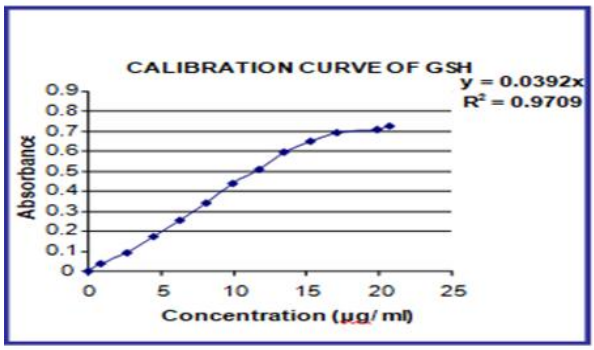

Fig 4: Calibration curve of GSH

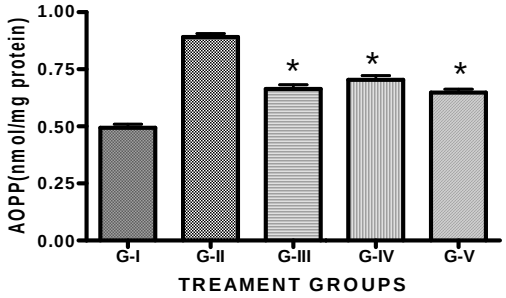

Fig 5: Figure showing levels of AOPP in various, diabetic and diabetic treated rats

4. Conclusions

Momordica charantia, Eugenia jambolana, Ocimum sanctum, and Allium cepa traditionally were used for various diseases and disorders. It is profound that diabetes mellitus is not a single disease, but numerous disease and

symptoms were associated with it.It has been suggested that compounds or extracts having both hypoglycemic and antioxidative properties would be useful antidiabetic agents.The study showed an elevation in GSH level in tissue after extract treatment. This may be again due to the presence of antioxidant compounds in Poly Herbal PreparationFlavonoids and bioflavonoid possess inhibitory effect on aldose reductase enzyme.When rats were tested for OGTT then initially the rats shows a elevation in the blood glucose level for 30 min and then it has started decreasing which may be due to the time taken by the active constituent of Poly Herbal Preparation to reach the systemic circulation and give its effect.The above studies are preliminary study and the exact relationship between chemical constituent and its antidiabetic activity should be done.

Table 1: Preliminary Phytochemical Screening of various extracts

S.No.	Experiment	Polyherbal preparation
1.	Carbohydrates	+
2.	Gum and mucilage	+
3.	Proteins	-
4.	Alkaloids	+
5.	Glycosides	+
6.	Steroids	+
7.	Tannins	+
8.	Saponins	+
9.	Flavanoids	+
10.	Anthraquinones	+
11.	Furanoids	-
12.	Coumarin	-
13.	Terpenoids	+

+ Sign indicates presence whereas – indicates absence of constituents

Table 2: Acute Toxicity Study

S.No.	Treatment (hydroalcholic extract)70:30	Dose (mg/kg)	Number of animals	Mortality			Toxicity Profile
				After 24 Hrs	After 7Days	After 14 Days	
1.	*Polyherbal preparation*	2000	5	0	0	0	Safe

Table 3: Effect in glucose – hyperglycemic animals (Oral glucose tolerance test, OGTT)

Group	Blood Glucose Level (mg/dl)				
	0 min	30 min	60 min	120 min	180 min
Normal	89.00 ± 1.46	129.00 ± 1.60	117.30 ± 2.18	102.70 ± 1.92	94.50 ± 1.38
Positive Control	89.33 ± 2.61	91.50 ± 2.00	88.33 ± 1.14	85.67 ± 0.71	84.17 ± 1.13
Polyherbal preparation (200 mg/kg)	90.7 ± 1.83	129.70 ± 1.56	108.50±2.48*	96.50 ± 2.10*	88.83 ± 1.74*

N=6 * $p < 0.05$, vs. Positive control; Value expressed in mean ±SEM

Table 4: Antidiabetic activity of extracts on BGL (mg/dl)

Group	Regimen	0 DAY	3 DAY	6 DAY	11 DAY
		BGL	BGL	BGL	BGL
G-I	Normal control	88.66 ±1.28	88.80 ±0.94	86.8 ±2.24	92.00±1.15
G-II	Negative control (alloxan)	87.33 ±1.40	354.00 ± 18.84**	406.83 ±14.37**	438.67±9.21**
G-III	Positive control (gilbenclamide)	87.50 ±2.71	339.67 ±16.48**	134.67 ±3.13**	89.66±1.97
G-IV	Drug Treated Polyherbal preparation 200	87.66 ±2.26	327.67 ±16.11**	300±11.93**	171.33± 2.99**
G-V	Drug Treated Polyherbal preparation. 400	90.50 ±1.389	325.33 ±12.48**	277.6 ±11.13**	100.67±1.60

N=6 *p<0.01 **p<0.001vs Negative control ; Value expressed in mean ± SEM

Table 5: Antidiabetic activity of extracts on basis of body wt. as parameter (gm)

Group	Treated	0 DAY	3 DAY	7 DAY	11 DAY
G-I	Normal control	164.43 ±5.20	165.17 ±4.70	167.50 ±5.34	169.33± 5.25
G-II	Negative control (alloxan)	160 ±5.33	126.33 ±5.14	116.67 ±2.62	105.17±2.57
G-III	Positive control (gilbenclamide)	156.17 ±3.351	131.33 ±4.82**	143.50 ±4.06**	155.33±2.24*
G-IV	Drug Treated Polyherbal preparation 200	153 ±5.7	128.67 ±5.11**	138.33 ±5.27**	140±5.81**
G-V	Drug Treated Polyherbal preparation. 400	159 ±7.42	133.1 ±7.9**	151.80 ±6.92	155±7.67

N=6 *p<0.01 **p<0.001vs Negative control Value expressed in mean ± SEM

Table 6: Calibration curve for reduced glutathione (GSH)

S.No.	Volume of Aliquot (ml)	Concentration (µg/ml)	Absorbance (λ_{max} = 410 nm)
1	0	0	0
2	0.3	0.9	0.036
3	0.9	2.7	0.091
4	1.5	4.5	0.171
5	2.1	6.3	0.252
6	2.7	8.1	0.334
7	3.3	9.9	0.433
8	3.9	11.7	0.508
9	4.5	13.5	0.601
10	5.1	15.3	0.649
11	5.7	17.1	0.696
12	6.3	19.9	0.715
13	6.9	20.7	0.728

Table 7:GSH levels in various treatment groups

S.No.	Groups	Sample absorbance	GSH concentration (µg/ml)
1	G-I Normal control	0.692	17.86
2	G-II Negative control	0.238	5.89
3	G-III Positive control	0.386	9.98*
4	G-IV Drug Treated (200mg/kg)	0.314	7.86*
5	G-V Drug Treated (400mg/kg)	0.365	8.78*

n=6 *p<0.01 highly significant with respect to diabetic control

Table 8: Effect of various extracts administration on lipid peroxidation and Advanced Oxidation Protein Products (AOPP)

S.No.	Groups	Sample absorbance	MDA concentration (n mole/ml)	AOPP (nmol/mg protein)
1	G-I Normal control	0.006	1.28	0.48 ± .02
2	G-II Negative control	0.128	23.15	0.86 ± .03
3	G-III Positive control	0.038	6.18*	0.67 ± .03*
4	G-IV Drug Treated (200mg/kg)	0.041	7.36*	0.73 ± .12*
5	G-V Drug Treated (400mg/kg)	0.056	9.68*	0.65 ± .03*

N=6 *p<0.05 *p<0.01 highly significant with respect to diabetic control

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