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Anti-Diabetic Activity of Polyherbal Formulation Containing Ethanolic Extract of Leaves of Azadirachta Indica, Allium Sativum and Annona Squamosa

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Abstract
Azadirachta indica, *Allium sativum* and *Annona squamosa* are well-known plants available throughout India and are commonly used for the treatment of various diseases including diabetes mellitus. The antidiabetic activity of the individual plant parts is well known, but the synergistic or combined effects are unclear. Polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse events. The aim of the present study is to formulate a polyherbal formulation and evaluate its antidiabetic potential in animals. The polyherbal formulation was formulated using the ethanol extracts of the leaves of *Azadirachta indica*, *Allium sativum* and *Annona squamosa*. The oral antidiabetic activity of the polyherbal formulation (200) was screened against alloxan and streptozotocin induced diabetes mellitus in rats. Polyherbal formulation showed significant antidiabetic activity at 200 mg/kg, respectively, and this effect was comparable with that of standard drug. The antidiabetic activity of polyherbal formulation is supported by biochemical and histopathologic analysis.

Key-words: Herbs, Diabetes, Polyherbal formulation

Introduction

Plants are extremely beneficial to humans. Many of them are just utilised for therapeutic purposes. "A medicinal plant is a plant that, in one or more of its organs, contains chemicals that can be utilised for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis," according to the World Health Organization (WHO). Pharmaceutical businesses are in high demand for active compounds from such plants.[1-2]

Diabetes mellitus is one of the most common diseases, affecting over 6% of the global population, and the diabetes dynamics are fast changing in low- and middle-income nations.In 2030, the International Diabetes Federation (IDF) predicts that 80 percent of the world's diabetes population will originate from low- and middle-income countries. According to the International Diabetes Federation's 2011 report, China, India, and the United States of America each have a diabetes population of 90.0, 61.3, and 23.7 million people, respectively, which is expected to rise to 129.7, 101.2, and 29.3 million by 2030. Diabetes is one of the six leading causes of death

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worldwide, as well as a source of numerous systemic problems. Insulin therapy or glucose-lowering medications such as alpha-glucosidase inhibitors, sulfonylureas, and biguanides are used to treat diabetes mellitus. One of the challenges in the treatment of any systemic condition is the formation of an adverse event; as a result, numerous research institutes and pharmaceutical corporations are active in drug development to uncover molecules with strong therapeutic potential and fewer adverse events. In the United States, 10 to 25 percent of patients have an adverse medication reaction, which accounts for 3.4 to 7.0 percent of hospital admissions. [3-4]
Many plants have been demonstrated to be effective in the treatment of various systemic illnesses in traditional medical systems. Many traditional/indigenous medical systems are more effective than modern medical systems, but they suffer from a lack of comprehensive uniformity, which is one of the traditional medical system's major problems. In ancient literature, the concept of polyhedral formulation is well documented. The polyherbal formulation has a greater and longer therapeutic potential than a single herb. As a result, the goal of this study was to create and standardise a polyherbal formulation containing a plant with documented antidiabetic activity, as well as to test its therapeutic effects in rodents.

Material and Method

Animal used: *Swiss albino* mice and Sprague Dawley Rats; **Weight** 30±5 gm (mice) and 200±50 gm (Rat); **Sex-**Either

Route of administration P.O.

Housing Condition

Animals were housed in a group of four in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were further divided in nine groups with six animals in each group.

IAEC Approval

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1283/c/09/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/11/PN-146

Grouping of Animal

Table 1: Grouping of animal

Group No.	Group	Dose	No. of Animals
1	Vehicle		6
2	Diabetic control+(STZ or Alloxan)		6
3	Standard(Glibincamide)+(STZor Alloxan)	600µg/kg	6
4	Azadirachta indica+(STZ or Alloxan)	200 mg/kg	6
5	Allium sativum+(STZ or Alloxan)	200 mg/kg	6

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6	Annona squamosa+(STZ or Alloxan)	200 mg/kg	6
7	Polyherbal+(STZ or Alloxan)	200 mg/kg	6

Acute toxicity study: The acute oral toxicity studies and selection of doses was carried out as per guidelines of Organization for Economic Co-operation and Development (OECD), guidelines 423 Healthy albino mice of either sex weighing (30±5 gm) were used for acute toxicity study to determine LD_{50} of investigating extracts. [5]

Alloxan Induce diabetes: The rats were injected with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg/kg body wt. I.P. After 2 weeks, rats with moderate diabetes having glycosuria tested by blood glucose level ranging from 200_250 mg/dl used for the experiment**.** [6-7]

STZ Induce diabetes: After overnight fasting (deprived of food for 16 h had been allowed free access to water), diabetes was induced in rats by I.P. injection of STZ dissolved in 0.1M cold sodium citrate buffer (pH 4.5) at a dose of 55 mg/kg body weight) The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats. [6-7]

***In-Vivo* hypoglycemic effect:** In vivo hypoglycemic effect of various plant extracts and their polyherbal extract was measured in Sprague Dawley rats (weighing 200-250g) fasted overnight. Rats were divided into 8 groups (n = 6). Group 1 served as normal, Group 2 were serve as control and was given saline (p.o.). Group 3 was treated with Glibincamide (600 µg/kg). Group 4,5,6,7, and 8 was treated with different extract of same dose and to observe the effect on blood glucose level. Blood samples (0.2 ml) were taken at various time points and blood glucose levels were determined using Accu check® Go (Roche Diagnostics, Germany). All the animals were procured from the animal laboratory facility of the institute. They were maintained under standard environmental conditions and housed individually in plastic cages in a controlled environment (22–24 ^{o}C and 12:12 light–dark cycle) with free access to food and water. [6-7]

Biochemical Parameters: Various biochemical parameters such as Serum cholesterol, Serum triglycerides, HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholestrol were investigated as per standard protocol.

Results and Discussion

The acute toxicity studies of ethanolic extract of leaves of *Azadirachta indica, Allium sativum* and *Annona squamosa* were carried out using OECD guidelines 423 and it was found that the drug is safe at the dose of 2000 mg/kg bw.

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Table 2: Acute toxicity study on animals (OECD 423)

Plant	Dose	No. of animals	Death
Azadirachta indica	5 mg/kg	03	00
	50 mg/kg	03	00
	300 mg/kg	03	00
	2000 mg/kg	03	00
Allium sativum	5 mg/kg	03	00
	50 mg/kg	03	00
	300 mg/kg	03	00
	2000 mg/kg	03	00
Annona squamosa	5 mg/kg	03	00
	50 mg/kg	03	00
	300 mg/kg	03	00
	2000 mg/kg	03	00

Hypolipidemic activity is evaluated by changes in serum lipid level (such as total cholesterol, triglycerides, HDL-c etc.) on the experimental animals after administration of the test drugs. The parameters are determined before and after drug administration at regular intervals. Screening of activity with ethanol extracts of polyherbal and alloxan induced hyperlipidemic animal models was employed. These models have been successfully employed for the evaluation of many plant originated drugs. The advantage of this method can be performed easily in normal laboratory conditions.

The initial lipid parameter such as total cholesterol level, triglycerides levels and high density lipoprotein levels were determined. The animals were divided into five groups of five rats each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of alloxan administered at a dose of 150mg/kg, i.p. After 72 hours of alloxan injection, this group received a daily dose of 5% CMC (p.o) for 7 days. The third and fourth group was administered a daily dose of parsely ethanol extract 500mg/kg and 400 mg/kg suspended in 5%CMC, p.o., for 14 days, after inducing hyperlipidemia. Fifth group was administered with the standard glibiclenmide 600 µg/kg, p.o. for 14 days.

In the case of alloxan induced hyperlipidemic model, all animal group were deprived of food over night and blood sample was withdrawn from the plexus of eye vein by retro orbital method and transferred directly in to the centrifuged tube and allowed it to coagulate at a room temperature. Then the blood samples were centrifuged in centrifugation apparatus for 10-15 minutes at the rate of 2000 rpm. After this process, the supernatant clear serum was obtained and transferred carefully with the help of micro pipette into small micro centrifuge tubes for estimation of serum parameters. Serum parameters such as total cholesterol, triglycerides level, HDL were measured by standard reagent kits using auto analyzer

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Table 3: Blood glucose level of alloxan induced rats

GROUP	0 DAY	7 DAY	14 DAY	21 days	28 days
Vehicle	92.55±1.37	94.6±1.44	95.65±1.43	96.083±1.754	96.46±1.265
Diabetic Control	338.66±15.474	353±6.985	359.33±5.955	364.166±6.645	374.166±4.875
Standard	335.66±17.162	298.66±9.231	118.16±9.191	111.6±5.872	103.466±5.196
Azardictica indica	337.88±6.113	321.21±5.626	141.15±6.191	137.316±4.689	132±3.988
Allium sativum	340.26±5.339	316.13±6.993	135.93±5.623	132.4±5.708	128.28±5.769
Anonoma squamosa	339.38±3.657	310.98±4.709	129.85±4.561	126.483±5.117	123.866±4.39
Polyherbal	336.76±6.513	304.21±6.947	122.88±5.032	118.316±3.878	110.7±4.375

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

Table 4: Blood glucose level of STZ induced rats

GROUP	0 Day	7 Day	14 Day	21 Days	28 Days
Vehicle	92.55±1.37	94.6±1.44	95.65±1.43	96.083±1.754	96.46±1.265
Diabetic Control	283.83±1.56	291.6±2.44	297.13±1.47	301.816±4.048	303.766±3.466
Standard	289.65±2.26	208.38±2.15	151.96±4.84	142.783±3.407	134.95±4.881
Azadirachta indica	290.93±4.659	263.58±4.125	233.83±3.929	225.63±4.612	211.68±5.337
Allium sativum	288.6±2.825	246.18±3.576	206.1±2.681	197.183±3.792	186.116±7.360
Annona squamosa	282.58±4.359	229.55±3.530	190.23±4.501	183.116±4.891	174.18±5.046
Polyherbal	285.33±8.402	217.05±7.807	169.76±7.052	158.15±7.760	144.28±7.451

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

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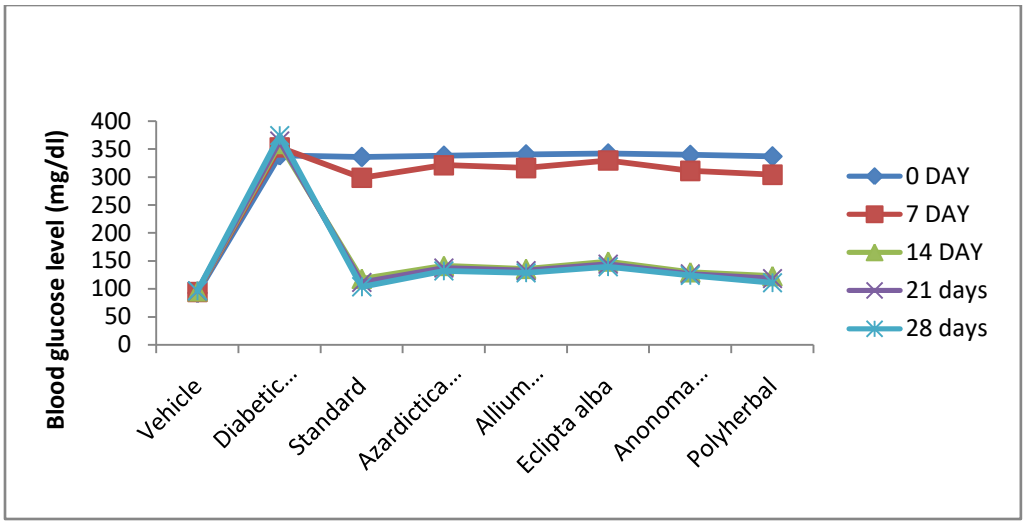
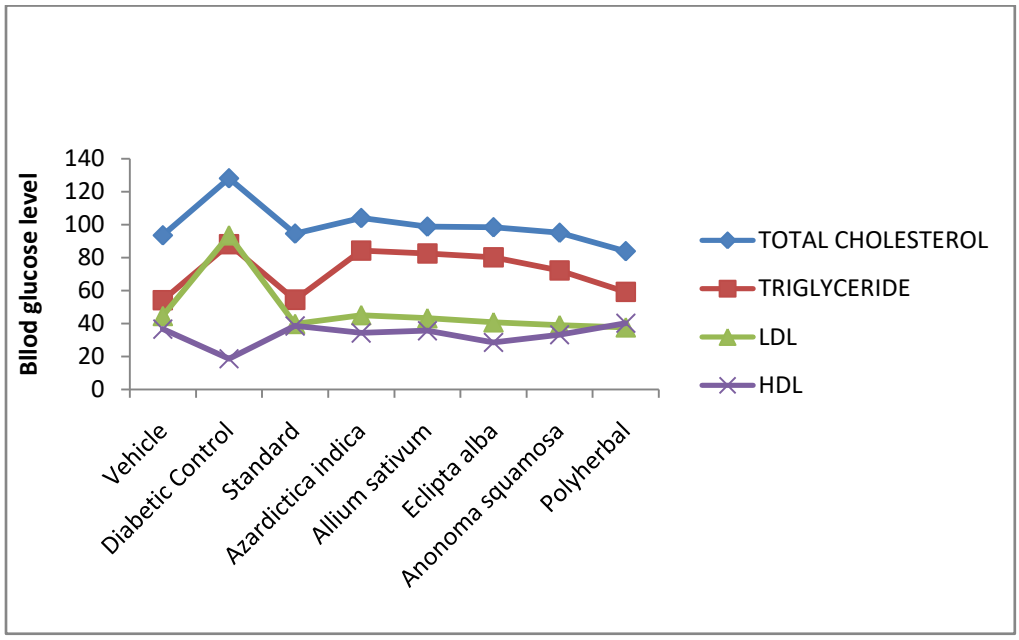


Graph 1: Blood Glucose Level in Alloxan Induced Model



Graph 2: Blood Glucose Level in STZ Induced Mode

Table 5: Lipid profile of Alloxan induced rats

GROUP	TOTAL CHOLESTEROL	TRIGLYCERIDE	LDL	HDL
Vehicle	93.5±3.649	54.26±2.697	44.68±2.895	36.63±2.167
Diabetic Control	128.16±2.968	88.18±2.739	93.683±3.146	18.733±2.841
Standard	94.533±3.849	54.516±2.042	39.85±5.806	38.55±1.635
Azardictica indica	104.016±3.972	84.283±2.763	45.216±2.921	34.3±3.24

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Allium sativum	98.783±3.956	82.53±4.256	43.316±3.112	35.65±2.113
Anonoma squamosa	95.067±3.503	72.283±3.985	39.1±3.394	33.267±3.672
Polyherbal	83.867±3.755	59.233±4.101	37.766±3.662	40.167±5.943

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

Table 6: Total protein in Alloxan induced diabetes

S.No	Group	Serum(g/dl)	Pancrease (mg/gm wet tissue)
1.	Vehicle	9.128±0.837	0.237±0.0081
2.	Diabetic Control	5.355±0.633	0.242±0.0076
3.	Standard	8.341±0.919*	0.235±0.0093
4.	Azadirachta indica	5.921±0.806	0.237±0.0103
5.	Allium sativum	6.768±0.798*	0.235±0.0087
7.	Annona squamosa	7.206±0.776*	0.243±0.0115
8.	Polyherbal	7.725±0.815*	0.239±0.0142

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

Table 7: Lipid profile of STZ induced rats

GROUP	TOTAL CHOLESTEROL	TRIGLYCERIDE	LDL	HDL
Vehicle	93.5±3.649	54.267±2.697	44.683±2.895	36.633±2.167
Diabetic Control	126.416±2.339	88.533±5.376	80.55±5.517	20.05±2.798
Standard	96.066±2.695	56.15±1.452	43.083±2.462	38.966±2.112
Azadirachta indica	116.033±3.396	71.966±3.137	63.016±4.63	26.183±2.983
Allium sativum	105.416±4.69	64.083±3.655	48.816±2.934	31.833±3.573
Annona squamosa	108.4±2.797	67.716±3.674	54.583±3.391	33.6±2.463
Polyherbal	97.85±5.401	59.183±7.778	46.55±7.146	35.667±6.520

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

Table 8: Total Protien in STZ Induced rats

S.No	Group	Serum(g/dl)	Pancreas (mg/gm wet tissue)
1.	Vehicle	7.751±0.383	0.268±0.0111

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2.	Diabetic Control	5.096±0.698	0.261±0.0107
3.	Standard	7.428±0.404*	0.269±0.0087
4.	Azadirachta indica	5.683±0.383	0.260±0.0095
5.	Allium sativum	6.43±0.376*	0.263±0.0085
7.	Annona squamosa	6.781±0.436*	0.265±0.0088
8.	Polyherbal	7.153±0.401*	0.266±0.0070

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

Table 9: Organ weight of alloxan induced rats

S.No	Group	Liver (gm)	Pancrease (gm)
1.	Vehicle	9.035±0.1102	0.6166±0.0074
2.	Diabetic Control	9.992±0.0931	0.8046±0.0090
3.	Standard	9.108±0.1195	0.6232±0.0089
4.	Azadirachta indica	9.708±0.1526	0.6859±0.0097
5.	Allium sativum	9.430±0.1337	0.6625±0.0111
7.	Annona squamosa	9.315±0.1314	0.6506±0.0111
8.	Polyherbal	9.207±0.1302	0.6333±0.0090

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

Table 10: Organ weight of STZ induced rats

S.No	Group	Liver (gm)	Pancrease (gm)
1.	Vehicle	9.072±0.0672	0.6293±0.0075
2.	Diabetic Control	9.871±0.0599	0.793±0.0074
3.	Standard	9.269±0.0740	0.6384±0.0085
4.	Azadirachta indica	9.722±0.1102	0.6798±0.0067
5.	Allium sativum	9.456±0.0975	0.6566±0.0074
7.	Annona squamosa	9.390±0.0909	0.6474±0.0079
8.	Polyherbal	9.328±0.0918	0.6407±0.0092

Values are expressed as Mean ± S.E.M.(n=6).Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Students *t*-test.

Conclusion

Thus, findings demonstrate the antidiabetic effect of the polyherbal formulation at the dose levels of 200 mg/kg. The antidiabetic potential of the polyherbal formulation is comparable with that of glibenclamide, which is evidenced by decreased levels of blood glucose, and other tested biochemical parameters.

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